

Published in final edited form as:

Eur J Nutr. 2016 February ; 55(1): 7–20. doi:10.1007/s00394-014-0818-5.

Consumption of soft drinks and juices and risk of liver and biliary tract cancers in a European cohort

Magdalena Stepien¹, Talita Duarte-Salles¹, Veronika Fedirko^{1,2}, Antonia Trichopoulou^{3,4}, Pagona Lagiou^{4,5}, Christina Bamia⁴, Kim Overvad⁶, Anne Tjønneland⁷, Louise Hansen⁷, Marie-Christine Boutron-Ruault^{8,9,10}, Guy Fagherazzi^{8,9,10}, Gianluca Severi^{11,12}, Tilman Kühn¹³, Rudolf Kaaks¹³, Krasimira Aleksandrova¹⁴, Heiner Boeing¹⁴, Eleni Klinaki³, Domenico Palli¹⁵, Sara Grioni¹⁶, Salvatore Panico¹⁷, Rosario Tumino¹⁸, Alessio Naccarati¹⁹, H. Bas Bueno-de-Mesquita^{20,21,22}, Petra H Peeters²³, Guri Skeie²⁴, Elisabete Weiderpass^{24,25,26,27}, Christine L Parr²⁸, José Ramón Quirós²⁹, Genevieve Buckland³⁰, Esther Molina-Montes³¹, Pilar Amiano^{32,33}, Maria-Dolores Chirlaque^{31,33}, Eva Ardanaz^{33,34}, Emily Sonestedt³⁵, Ulrika Ericson³⁶, Maria Wennberg³⁷, Lena Maria Nilsson³⁷, Kay-Tee Khaw³⁸, Nick Wareham³⁹, Kathryn E Bradbury⁴⁰, Heather A Ward⁴¹, Isabelle Romieu¹, and Mazda Jenab^{1,*}

¹International Agency for Research on Cancer (IARC-WHO), Lyon, France ²Rollins School of Public Health, Winship Cancer Institute, Emory University, Atlanta, USA ³Hellenic Health Foundation, Athens, Greece ⁴WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology, Medical Statistics, University of Athens Medical School, Athens, Greece ⁵Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA ⁶Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark ⁷Danish Cancer Society Research Center, Copenhagen, Denmark ⁸INSERM, Centre for research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, F-94805, Villejuif, France ⁹University Paris Sud, UMRs 1018, F-94805, Villejuif, France ¹⁰Institut Gustave Roussy, F-94805, Villejuif, France ¹¹Cancer Epidemiology Centre, Cancer Council Victoria, 3053, Melbourne, Australia ¹²Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, 3010, Melbourne, Australia ¹³Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany ¹⁴Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany ¹⁵Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy ¹⁶Epidemiology and Prevention Unit, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milan, Italy ¹⁷Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy ¹⁸Cancer Registry and Histopathology Unit, "Civile M.P. Arezzo" Hospital, Ragusa, Italy ¹⁹Human Genetics Foundation (HuGeF), Molecular and Genetic Epidemiology Unit Torino, Italy ²⁰National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands ²¹Department of Gastroenterology and

*Address correspondence and reprint requests to: Mazda Jenab, PhD, Scientist, Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC-WHO), Lyon, France, Tel: +33-(0)472738082, Fax : +33-(0)472738361, jenabm@iarc.fr.

Conflict of interest:

None of the authors had a conflict of interest.

Hepatology, University Medical Centre, Utrecht, The Netherlands ²²The School of Public Health, Imperial College London, London, United Kingdom ²³Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands ²⁴Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway ²⁵Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden ²⁶Department of Research, Cancer Registry of Norway, Oslo, Norway ²⁷Samfundet Folkhälsan, Helsinki, Finland ²⁸Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway ²⁹Public Health Directorate, Asturias, Spain ³⁰Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain ³¹Department of Epidemiology, Murcia Regional Health Authority, Murcia, Spain ³²Public Health Division of Gipuzkoa, BioDonostia Research Institute, Health Department of Basque Region, San Sebastian ³³CIBER Epidemiology and Public Health CIBERESP, Madrid, Spain ³⁴Navarre Public Health Institute, Pamplona, Spain ³⁵Department of Clinical Sciences – Malmö, Lund University Malmö, Sweden ³⁶Diabetes and Cardiovascular disease, Genetic Epidemiology, Department of Clinical Sciences in Malmö, Lund University Malmö, Sweden ³⁷Public Health and Clinical Medicine, Nutritional Research, and Arctic Research Center, Umeå university, Umeå, Sweden ³⁸School of Clinical Medicine, Clinical Gerontology Unit, University of Cambridge, UK ³⁹MRC Epidemiology Unit, University of Cambridge, UK ⁴⁰Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK ⁴¹Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, UK

Abstract

Purpose—The aim of the study was to assess associations between intake of combined soft drinks (sugar- and artificially-sweetened) and fruit and vegetable juices and the risk of hepatocellular carcinoma (HCC), intrahepatic bile duct (IHBC) and biliary tract cancers (GBTC) using data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort of 477,206 participants from 10 European countries.

Methods—After 11.4 years of follow up 191 HCC, 66 IHBC and 236 GBTC cases were identified. Hazard ratios and 95% confidence intervals (HR; 95%CI) were estimated with Cox regression models with multivariable adjustment (baseline total energy intake, alcohol consumption and intake pattern, body mass index, physical activity, level of educational attainment and self-reported diabetes status).

Results—No risk associations were observed for IHBC or GBTC. Combined soft drinks consumption of >6 servings/week was positively associated with HCC risk: HR=1.83; 95%CI: 1.11-3.02, $p_{\text{trend}}=0.01$ vs. non-consumers. In sub-group analyses available for 91% of the cohort artificially-sweetened soft drinks increased HCC risk by 6% per 1 serving increment (HR=1.06, 95%CI:1.03-1.09, $n_{\text{cases}}=101$), for sugar-sweetened soft drinks this association was null (HR=1.00, 95%CI: 0.95-1.06; $n_{\text{cases}}=127$, $p_{\text{heterogeneity}}=0.07$). Juice consumption was not associated with HCC risk, except at very low intakes (<1serving/week: HR=0.60; 95%CI:0.38-0.95; $p_{\text{trend}}=0.02$ vs. non-consumers).

Conclusions—Daily intake of combined soft drinks is positively associated with HCC, but a differential association between sugar and artificially sweetened cannot be discounted. This study provides some insight into possible associations of HCC with sugary drinks intake. Further exploration in other settings is required.

Keywords

hepatocellular carcinoma; biliary tract cancers; soft drink; fruit and vegetable juice; prospective cohort

Introduction

Primary liver cancers are comprised of hepatocellular cancer (HCC) and cancers of the intrahepatic bile ducts (IHBC) [1]. Together, they are the seventh most common cancer worldwide [2] and the third cause of death from cancer in both sexes [3]. HCC represents the majority of primary liver cancers. Its risk factors include hepatitis B (HBV) and C (HCV) infections, aflatoxin exposure, tobacco smoking and heavy alcohol consumption mediated by liver cirrhosis [4,5]. However obesity, type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) could also be contributing to the rising incidence of HCC [6–8]. A global increase has been also observed for the incidence of IHBC cancers, while for the extrahepatic bile duct (EBD) cancers, which are anatomically related to IHBC, there has been a decreasing trend worldwide [9]. The etiology of IHBC cancer and cancers originating from biliary tract (GBTC), including: EBD, gallbladder (GB) and Ampulla of Vater (AmpV) cancers, is poorly understood. Obesity, diabetes mellitus, history of gallstones or cholecystitis have been proposed as possible risk factors for GBTC [10,8].

Some dietary exposures may affect the development of cancers of the liver and biliary tract. For example, our own data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort show that daily increase of sugar intake by 50g was associated with a significantly higher risk of HCC, and non-significant positive association with IHBC, but not GBTC [11]. Both soft drinks and some juices may contain high levels of sugars and could be related to HCC, IHBC or GBTC development directly or indirectly through associated diseases. Intake of soft drinks and fruit drinks has been linked to obesity, T2D and NAFLD [12–16]. It has also been shown that intake of soft drinks have increased progressively in the recent years. For example, in the United States, intake of sweetened beverages has increased by 60% between 1977 and 2001 [17]. Also an increase in prevalence of HCC and IHBC was observed in some developed countries; the annual incidence of HCC rose by 80% in the last few decades [7]. Therefore we hypothesize that intake of soft drinks and possibly juices could play a role in the development of HCC and maybe IHBC cancer.

To date, there have been no consensus in the literature regarding risk of various cancers and intake of soft drinks and/or juices [18–23], and associations for cancers of the liver and biliary tract have not been well studied. Given the rising consumption of sweetened non-alcoholic beverages and their likely link to several metabolic disorders that play a role in the

development of these cancers, we present here an analysis of soft drinks and fruit and vegetable juices, in association with HCC, IHBC and GBTC in the EPIC cohort.

Subjects and Methods

Study design

EPIC is a large prospective multicentre study that aims to investigate the relationship between nutrition and cancer, as well as other chronic diseases. The rationale, study populations and data collection have been described previously [24]. Over 520,000 participants were enrolled from 23 centres in Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. Between 1992 and 1998, standardised lifestyle and personal history questionnaires, anthropometric data, and blood samples were collected from most participants at recruitment, before disease onset or diagnosis. Blood samples are stored at the International Agency for Research on Cancer (IARC, Lyon, France; -196°C, liquid nitrogen) for all countries except Denmark (-150°C, nitrogen vapour) and Sweden (-80°C freezers) where they are stored locally. All cohort members provided written informed consent. Approval for this study was obtained from the relevant ethical review boards of the participating institutions and from the IARC ethical review board (Lyon, France) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Case ascertainment

Overall, a total of 477,206 participants were included in this study after the following exclusions: 23,818 with prevalent cancer other than non-melanoma skin cancer, 4,380 with incomplete follow up data or missing information on the date of diagnosis, 6,192 with missing dietary information, 60 with missing lifestyle information, and 9,596 those at the top or bottom 1% of the distribution of the ratio of reported energy intake to energy requirement, and 78 with metastasis in the liver or ineligible histology code.

Cancer cases were identified using record linkage with regional cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, UK; up to December 2006) or for France, Germany and Greece by health insurance records, contact with cancer or pathology registries or active follow up (up to June 2010). Cancer cases were defined according to the 10th revision of International Classification of Diseases (ICD10): HCC (C22.0), IHBC (C22.1), GB (C23.9), AmpV (C24.1), EBD (C24.0, C24.8, C24.9). After a mean of 11.4 person years of follow up 191 HCC, 66 IHBC and 236 GBTC (87 GB, 54 AmpV, 95 EBD) cases were identified.

Dietary assessment and categories of intake

At enrolment, dietary intakes during the preceding twelve months were assessed based on validated country-specific dietary questionnaires designed to ensure high compliance and improved measures of local dietary habits [25]. Daily intakes of soft drinks and juices were determined in grams (g). Daily intakes of nutrients, alcohol and energy were calculated using standardised EPIC nutrient database [26]. The group of soft drinks included carbonated/soft/isotonic drinks, and diluted syrups. Further classification of soft drinks into

sugar-sweetened and artificially-sweetened was possible for participants (n=424,123) in all centres except three; Italy (North and Ragusa), Sweden (Umeå). The group of juices comprise fruit, citrus and/or vegetable juices (including fresh and commercial juices, and nectars, with possible addition of sugars up to 20% of the total weight of the finished product [27]), but the classification by commercial and natural juices was not possible.

For the purposes of the present analysis, the intakes of soft drinks and juices were also categorised into servings, defined to reflect current European intake customs. A serving of soft drinks was defined here as 330g, equivalent to a volume of a soft drink can size in Europe (330mL). For juices, one serving was considered as 200g, equivalent to regular glass (200mL) in Europe considered as a standard portion size for juices [28].

Nested case-control study

A nested case-control study of these cancer sites was also conducted as previously described [11]. For each HCC, IHBC, or GBTC case two controls free of cancer (other than non-melanoma skin cancer) were selected from the cohort by incidence density sampling and matched by study centre, sex, age (± 1 year) at the time (± 2 months) and time of the day (± 3 hours) of blood collection, fasting status (<3, 3-6, >6 hours); for women further for menopausal status (pre-, peri-, postmenopausal), use of exogenous hormones (contraceptives or hormone replacement therapy) at blood collection (yes/no). Between the recruitment and 2006 there were 125 HCC cases identified for which blood samples were available for laboratory measurements. After the exclusion of cases and controls for whom laboratory measurements were not available due to missing sample or unsuccessful testing, the analyses included 121 HCC cases and their 241 matched controls. Additionally, the analyses were conducted for: 34 IHBC cases and their 67 controls, and 131 GBTC cases and 259 controls.

HB) and HCV status was assessed by measurement of the level of HBV surface antigen (HBsAg) or antibody to HCV (anti-HCV) with the use of relevant ARCHITECT chemiluminescent microparticle immunoassay (CMIA) (Abbott diagnostics, France). Liver enzymes and other markers of liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), liver-specific alkaline phosphatase (AP), albumin, bilirubin) were measured on the ARCHITECT c Systems™ (Abbott Diagnostics) according to manufacturer instructions. All laboratory analyses were performed by Centre de Biologie Republique Laboratory, Lyon, France.

Statistical analyses

Cohort study—Comparisons of the baseline subject characteristics were done using the t-test for continuous variables and the chi-square test for categorical variables. Sex-, age- and centre- adjusted Pearson partial correlation coefficients were used to assess the correlations between dietary intakes of soft drinks (sugar- and- artificially-sweetened) and juices and confounding factors in controls. Cox proportional hazard models with age as a timescale (age at recruitment and age of censoring or cancer diagnosis as entry and exit time, respectively), were used to calculate Hazard Ratio (HR) and 95% Confidence Intervals (95% CI) in order to estimate the association between soft drinks/juices and intakes and risks of

HCC, IHBC, GBTC by defined categories, and as continuous variables (per number of servings a week) for all cancer and their subtypes.

In categorical analyses for both soft drinks and juices, non-consumers (reference category) were compared to either: (i) tertiles of intake among consumers, or (ii) categories of portions consumed per week: <1 (low consumers), 1-6 (regular consumers), >6 (high consumers). In the centres with available information (all centres excluding Umea, north Italy and Ragusa) analyses were also conducted stratified by sugar- and artificially- sweetened soft drinks. To test for linear trend median values to each category of intake and 0 g/d for non-consumers was assigned and entered into regression models.

Cox proportional hazards models were run as both crude (stratified by sex, age at recruitment in 1 year categories and study centre to account for differences in data collection, and adjusted for non-alcoholic energy intake using the standard method i.e. by adding to the model [29]) and multivariable (additionally adjusted for a priori selected relevant confounders: smoking status and intensity of smoking (Never; Former smoker: quit <10y ago, quit 11-20y ago, quit >20y ago; Current smoker: 1-15 cigarettes/d, 16-25 cigarettes/d, >25 cigarettes/d; other than cigarettes; current/former missing; unknown), alcohol intake at recruitment (g/day, continuous) and lifetime pattern of alcohol intake (never, former light, former heavy, light, never heavy, periodically heavy, always heavy drinkers, unknown); body mass index (BMI; kg/m², continuous), sex-specific physical activity (inactive, moderately active, active and missing), highest level of education attained (as a proxy for socioeconomic status; none/primary, technical/professional, secondary, university or higher) and self-reported diabetes status (yes, no, missing). Potential additional confounders considered but not included in the final model since they did not change the estimates by more than 10% were: waist-to-hip ratio, level of intake of sugar from other sources other than sugar-sweetened beverages, meats, fish, fruit and vegetables. The associations were also studied mutually adjusting both the crude and multivariable models for the other type of studied beverage (i.e. for soft drinks and juices, sugar- and artificially-sweetened soft drinks), as well as other non-alcoholic beverages intake; i.e. coffee and tea, since their intake may affect the intake of the beverages of interest or disease occurrence; however they also did not appreciably modify the estimates and were not considered in the multivariable model. P for heterogeneity between estimates for individual exposures (i.e. soft drinks vs. juices and sugar-sweetened vs. artificially-sweetened soft drinks) was tested. The difference of these associations in relation to HCC was assessed by inspecting the significance of the parameter related to the arithmetic difference of the two exposures in a model that also included their arithmetic sum.

Cubic spline regression models were computed to visualise the shape of association between soft drinks and juices intake and HCC or GBTC risk, controlling for the same confounders as in the multivariable model. The 5 cut points (knots) for the soft drinks and juices intake were determined corresponding to 10th, 25th, 50th, 75th and 90th percentile of intake expressed as number of servings (330mL can, 200mL glass) a week. For soft drinks that had more than 25% non-consumers and therefore 10th and 25th percentile were equal to 0, only 4 knots were assigned at the level of intake: 0.00, 0.19, 1.82 and 4.58 cans/week. For juices 5 knots were fitted at 0.00, 0.03, 0.69, 3.30 and 5.50 glasses a week. For better readability of

the graph the maximum was set as 99th percentile of intakes for soft drinks and juices (17.65 cans and 17.50 glasses a week, respectively).

Nested case-control subset—In the nested case-control study, Odds Ratio (OR) and 95% CI were computed by conditional logistic regression for HCC, IHBC, GBTC combined and their subgroups. For HCC, the OR (95% CI) were also computed for subjects with HBV/HCV negative infection status. Two analysis models were run for continuous intake per serving of soft drinks and juices: (i) conditioned on the matching factors only and adjusted for non-alcoholic energy intake (crude model) and (ii) multivariable adjustment for the same confounders as described for the cohort analyses. Because liver function may be altered in liver disease [30], the analyses per serving a week were also adjusted for liver function score (score: 0-6), based on the abnormal levels of liver function test (laboratory cut-offs: ALT>55 U/L, AST>34U/L, GGT>64UL-men and >36U/L-women, AP>150U/L, albumin<35g/L, total bilirubin>20.5 µmol/L). Interaction between soft drinks and juices intake and liver function score categories (no damage 0, possible damage 1-6), as well as BMI categories (BMI: <25 kg/m² normal weight, 25-30 kg/m² overweight, 30 kg/m² obese) was also studied. Where the interaction appeared significant, multivariable logistic regression additionally adjusted for matching criteria was run for the individual subgroups within the categories for the cancer risk and intake of soft drinks and juices.

Sensitivity analyses and effect modification—In sensitivity analyses, the analyses were repeated excluding: (i) cases diagnosed prior to 2 years of follow up in order to exclude for potential reverse-causation, (ii) participants with self-reported diabetes at baseline due to possible diet modifications, and (iii) consumers with extreme intakes (the highest percentile).

Interaction between soft drinks and juices intake and sex, BMI and alcohol intake patterns was studied to consider potential effect modification. The statistical significance of associations was based on likelihood ratio tests on the models with and without interaction terms. All statistical analyses were performed with SAS 9.2 and considered statistically significant if $p < 0.05$.

Results

Participants and their lifestyle and diet

Participants who developed HCC were mainly men, older, physically active, less educated, and were more likely to have prevalent diabetes and gallstones, to be current smokers, and to be former or current heavy drinkers than the non-cases. They also had higher BMI and waist-to-hip ratio. IHBC cases were equally distributed between the sexes and older than the non-cases and a higher proportion of them reported gallstones. Participants who developed GBTC were mostly women, less educated and were more likely to have diabetes and gallstones at baseline as compared to non-cases (Table 1).

Both daily consumers of soft drinks and juices were characterised by less healthy dietary pattern than non-consumers (higher consumption of sugar and confectionary, cakes and biscuits, and lower intake of legumes, fruits and vegetables, fish and shellfish), which was

reflected in their higher intake of sugar (30%), fat (5-6%) and energy (10%) (**Online Resource 1**). Self-reported diabetic subjects were more likely to consume daily artificially than sugar-sweetened soft drinks (5.5 vs. 1.9%, respectively). In comparison more non-diabetics consumed sugar-sweetened (2.8%) than artificially-sweetened soft drinks (1.6%). Similar trend was observed for BMI categories; 4% of obese subjects consumed daily artificially-sweetened drinks vs. 1.6% of those with normal weight. For sugar sweetened drinks the proportion was distributed equally between the BMI groups at the level of 3%. Intake of soft drinks and juices positively correlated with dietary sugar ($r=0.28$ and 0.34) and energy ($r=0.10$ and 0.10). Similar coefficients were observed for sugar-sweetened group of soft drinks ($r_{\text{sugar}}=0.33$ and $r_{\text{energy}}=0.10$), but no correlation with these variables existed for artificially-sweetened drinks. No correlation was observed between any type of the beverages and BMI, waist to hip ratio, physical activity level and alcohol intake at recruitment.

Soft drink intake and the risk of HCC

Compared to non-consumers the highest tertile of soft drinks consumers showed a borderline significant higher risk of HCC after adjustment for confounders (HR=1.46, 95% CI: 0.99-2.16; $p_{\text{trend}}=0.01$), and no significant associations were observed for the 1st and the 2nd tertile of consumers (Table 2).

Consumption of more than six (6 x 330mL) cans per week of soft drinks was significantly associated with higher risk of HCC after adjustment for confounders, as compared to non-consumers (HR=1.83, 95% CI: 1.11-3.02; $p_{\text{trend}}=0.01$); no significant associations were observed for lower intakes (Table 2).

In continuous analyses, the increment of 330mL of soft drinks a week was significantly positively associated with the risk of HCC after adjustment for confounders (HR=1.05, 95% CI: 1.02-1.07) (Table 2). Spline regression analyses by an increase of a serving a week showed a linear mostly positive association with HCC that appeared significant for daily and higher consumption of soft drinks (Fig. 1a).

In additional analyses by the type of drinks (sugar-sweetened vs. artificially sweetened), each additional serving of artificially-sweetened soft drink was positively associated with HCC risk (HR=1.06, 95% CI: 1.03-1.09, $n_{\text{cases}}=101$), while for sugar-sweetened soft drinks this association was null (HR= 1.00, 95% CI: 0.95-1.06, $n_{\text{cases}}=127$). The difference between both estimates was borderline significant ($p_{\text{heterogeneity}}=0.07$).

Juice intake and the risk of HCC

Compared to non-consumers the lowest tertile of juice intake was significantly associated with reduced HCC risk in both crude (HR=0.52, 95% CI 0.32-0.92; $p_{\text{trend}}=0.31$) and multivariable models (HR=0.57, 95% CI: 0.35-0.92; $p_{\text{trend}}=0.15$), while no significant association was observed for the 2nd and 3rd tertile of consumers (Table 2).

When considering intake as serving categories in relation to non-consumers, consumption of less than a 200mL glass a week was associated with lower HCC risk in multivariable model (HR=0.60, 95% CI: 0.38-0.95; $p_{\text{trend}}=0.02$) and when only consumers were considered a

positive trend ($p_{\text{trend}}=0.004$) was observed for higher consumption. The highest category of intake was non-significantly positively associated with HCC risk (HR=1.38, 95% CI: 0.80-2.38; $p_{\text{trend}}=0.02$) (Table 2).

In continuous analyses, the increase of intake of one serving (200mL) of juice a week was positively associated with the risk of HCC (HR= 1.03, 95% CI: 1.01-1.06) (Table 2). In spline regression analyses the association for juices was negative and significant only for intakes lower than 1 glass a week (Fig. 1b).

Sensitivity analyses and effect modification for HCC risk and soft drinks and juices intake

Exclusion of persons diagnosed with HCC within the first 2 years from recruitment did not change the findings for either exposure (**data not shown**). When only non-diabetic individuals were studied, the HRs were similar to whole cohort estimates, but weaker, probably due to lower sample size of this sub-cohort. Excluding participants with the 1% highest intakes of soft drinks and juices did not modify the results for juices, but the association for the highest tertile for soft drinks was attenuated (HR=1.35, 95% CI: 0.87-2.10).

No statistically significant interactions were observed between categories of soft drink intake and sex ($p=0.200$), BMI category ($p=0.126$) or alcohol intake pattern ($p=0.912$) nor categories of juices intake and sex ($p=0.568$), BMI category ($p=0.617$) or alcohol intake pattern ($p=0.745$).

Intake of juices and soft drinks and the risk of IHBC, GBTC and its subtypes

The risk of IHBC in an adjusted model per 200mL increase in juice intake a week was higher by 4% (HR=1.04, 95% CI: 1.00-1.08). The increment of a serving of soft drink or juice a week was not significantly associated with GBTC subtypes (Table 3).

No significant associations were observed for tertiles of soft drinks or juices consumers in relation to non-consumers and the risk of GBTC. Also, when the intakes were treated as serving categories of soft drinks compared to non-consumers, no associations were found (**data not shown**). For each additional 330mL of soft drink a week a borderline inverse association was observed with all GBTC combined (HR=0.96, 95% CI: 0.91 -1.00). A mostly negative, although not significant, association was observed based on cubic splines for soft drinks or juice and GBTC risk (**data not shown**).

Nested case-control study

In a nested case-control subset, each additional can of soft drink a week increased the risk of HCC (OR=1.18, 95% CI: 1.04-1.34). These results were maintained in hepatitis-free individuals or after adjustment for hepatitis status or liver function score (Table 4). There was no significant association for soft drinks and the risk of IHBC or GBTC (**data not shown**).

For HCC, an interaction was observed between increase of one portion of soft drink intake and liver function score category ($p=0.028$). Stratified analyses by liver function score category revealed a significantly higher risk of HCC by soft drink intake in the suggested

liver damage subgroup (score 1-6) (OR=1.46, 95% CI: 1.04-2.03), while in the group with no liver damage this association was not significant (OR=1.15, 95% CI: 0.87-1.51) (**data not shown**). There was no interaction between soft drink intake and either BMI (p=0.296) or alcohol intake pattern (p=0.362).

Drinking an additional glass of juice a week was not associated with HCC risk (OR=1.00, 95% CI: 0.93-1.08) (Table 4). There was no significant association for juices and the risk of IHBC or GBTC (**data not shown**). No interaction was observed between juice intake and liver function score (p=0.862), alcohol intake pattern (p=0.055) or BMI category (p=0.195).

Discussion

There was a positive association between consumption of soft drinks and HCC risk, which was present in continuous analyses and for the highest categories of intake in the cohort, but also in the nested case-control subset after adjustment for hepatitis status and liver function score. In subgroup analyses by soft drink category, per serving increase of artificially-sweetened but not sugar-sweetened soft drinks this association was significant. Each increment of a serving of soft drink was associated with lower overall GBTC risk but no significant associations were observed when the intakes were treated as categories. No significant associations existed in the cohort for regular or high juice consumers and risk of HCC. Compared to non-consumers, an intake of up to one serving of juice per week was associated with an inverse HCC risk, but for each additional serving of juice there was a positive association with HCC and IHBC risk.

Previously reported findings from the EPIC cohort have shown that high sugar intakes are positively significantly associated with HCC risk and not significantly with IHBC, but inversely associated with GBTC [11]. Soft drinks contain 55-130g of total sugar per litre [31]. However, most commercial and some natural fruit juices may also be characterised by high sugar levels, i.e. 3-112g/L [32]. Therefore we hypothesized that intakes of high in sugar beverages may be linked to development of HCC and possibly IHBC. In this study, only soft drinks showed a positive association with HCC, but we could not distinguish between commercial and natural juices. The observed link between intake of soft drinks and HCC could be mediated through some conditions associated with HCC, such as obesity [33,34]. In observational studies positive association for soft drinks intake and obesity is mainly observed for extreme categories of intake [35]. In this study, a positive association with HCC exists only for high soft drinks consumers, but interestingly BMI did not appear as an important confounder for this association; addition of BMI to the model did not considerably modify the risk estimates, and no interaction was observed between BMI categories and intakes of combined soft drinks.

Higher risk of HCC in daily consumers of soft drinks could be also related to adverse effects of their high sugar content on lipid and glucose metabolism [36]. Soft drinks, high in both glucose and fructose, result in a rapid increase of blood glucose and insulin levels at the intermediate level between the responses observed for pure glucose and fructose [37]. Fructose is rapidly taken up by the liver and favours *de novo* lipogenesis which, may lead to hepatic lipid accumulation and finally to NAFLD [38]. Soft drink intake was significantly

associated with an increased risk for NAFLD [39]. Taking into account that nearly three quarters of patients infected with HCV and half of HBV positive patients exhibit liver steatosis, of which 10-20% develop NAFLD [40], we repeated the analysis in a case-control subset excluding hepatitis positive individuals and additionally adjusting for liver function score, an indicator of liver dysfunction. We found that each increment of a portion of soft drink was associated with increased risk of HCC by 20%, independently of hepatitis status. However, observed interaction between liver function score and soft drink intake in relation to HCC risk may indicate that liver damage may play a role in HCC development associated with soft drinks intake.

Interestingly, when we investigated these associations further, after categorising soft drinks into sugar- and artificially sweetened in the subset of centres where this data was available (91% of the cohort), only for the artificially-sweetened soft drinks the association was positive. Similar findings were previously reported in the EPIC cohort and its French sub-cohort for the association between soft drinks and juices and diabetes risk; 350mL increment of artificially-sweetened soft drink had stronger effect on increased risk of developing diabetes than sugar-sweetened soft drink, while juice intake was not associated with diabetes [41,8]. Indeed, a recent study in mice reported an effect of non-caloric artificial sweeteners on intestinal microbiota composition leading to induction of glucose intolerance [42], but the findings require further confirmation in humans. Diabetes could be another important intermediate factor between HCC risk and soft drinks consumption. The intake of soft and fruit drinks is associated with increased risk of T2D [12,35]. This may imply that: i) components other than sugar present in diet/reduced-sugar soft drinks, such as sweetening agent or colorants, could be associated with the risk of HCC; ii) artificially-sweetened beverages, in general considered as healthier since they do not contain sugar, could be more frequently consumed by individuals with some existing underlying disorders, for example diabetes or obesity, and iii) diabetes/obesity might have been a consequence of high intake of sugary drinks in the past. Indeed in our cohort self-reported diabetic subjects or obese individuals consumed daily more frequently artificially- than sugar-sweetened soft drinks.

It can also be hypothesized that the group of high consumers of soft drinks would be characterised by less healthy dietary pattern. Data from an American dietary survey 1999-2002 indicated that soft drinks are more frequently consumed in the fast food dietary cluster, but less often by individuals characterised by a diet high in vegetables [43]. In this study, high consumers of both soft drinks and juices had less healthy dietary intakes and higher alcohol, sugar and energy content of their diet. The adjustment for some food components that may affect risk of liver and biliary track cancers (e.g. intake of meat, fish, fruit and vegetables) did not modify the outcomes, but we cannot rule out a confounding effect of other dietary components.

The nature of the association between juice and HCC risk may also vary according to different thresholds of intake. Juices are considered as healthier dietary choices due to their antioxidant, minerals, vitamins, phytochemicals and fibre content [44], as compared to soft drinks with poor nutritional quality. Fibre and polyphenols are known for their protective role against cancers in different sites [45], including HCC [46,11]. Our results may suggest that at lower consumption the beneficent effect of some juice components is present,

whereas at very high levels of consumption the sugar content of juices may override the potentially protective role of other components of juices. This could be supported by the observation that when only consumers were considered increasing risk of HCC was observed with higher category of intake.

Strengths of the present study include its prospective multicentre design that included a diverse European population with different habits of drinks and juices intakes [47]. Availability of detailed lifestyle and health status information made it possible to control for multiple confounders. Additionally, biochemical measurements of hepatitis status and liver enzymes enabled to control for the key risk factors of HCC. We were able to distinguish between multiple morphologic sites of liver and biliary tract cancers.

The study has some limitations. Liver and biliary tract cancers are relatively rare; a small sample size was available for analysis. The dietary and lifestyle data were collected only at baseline; it is possible that participants modified their dietary intakes during the follow up. To control for potential diet modification, we conducted sensitivity analyses excluding cases identified within the first two years of follow up. We were not able to distinguish between different kinds of juice (e.g. natural juices or nectars with added sugar) as well as the type of sugar or sweetener in the beverages, which made it difficult to assess the effect of added sugar or type of artificial sweetener used on the diet-disease relationship. Given the small study size which is even further reduced in the subgroup analyses, it is possible that these results were obtained by chance. So confirmation from other settings and populations is necessary.

In conclusion our results indicate that high consumption (one or more cans a day) of all combined soft drinks may increase the risk for HCC, but not GBTC. Interestingly, this association was mainly driven by the subgroup of artificially-sweetened soft drinks. A modest consumption of juices may be associated with a lower risk of HCC, but this effect disappears at higher levels of consumption. The findings could be important for public health concerning dietary recommendations in cancer prevention. However, more research is required to determine whether the observations presented here are indeed real, and whether they are related directly to higher sugar intake, higher intake of artificial sweeteners or to other dietary or lifestyle patterns or HCC-associated disease status associated with consumption of soft drinks and juices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors' responsibilities were as follows—ER: is the overall PI of the EPIC study which is jointly coordinated from ICL and IARC; MS, VF and MJ: conceptualized, designed, obtained funding for and carried out the present research; MS: performed the statistical analysis; and MS, VF, and MJ: contributed to the writing of the manuscript and data interpretation. Contributing authors from each collaborating centres provided the original data and biological samples, information on the respective populations, advice on study design/analysis, and interpretation of the results and approval of the final version of the manuscript for publication.

Funding:

This work was supported by the French National Cancer Institute (L'Institut National du Cancer; INCA) (grant number 2009-139). The coordination of EPIC is financially supported by the European Commission (DG-SANCO); and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer; Institut Gustave Roussy; Mutuelle Générale de l'Éducation Nationale; and Institut National de la Santé et de la Recherche Médicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ); and Federal Ministry of Education and Research (Germany); Stavros Niarchos Foundation; Hellenic Health Foundation; and Ministry of Health and Social Solidarity (Greece); Italian Association for Research on Cancer (AIRC); National Research Council; and AIRE-ONLUS Ragusa, AVIS Ragusa, Sicilian Government (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS); Netherlands Cancer Registry (NKR); LK Research Funds; Dutch Prevention Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund (WCRF); and Statistics Netherlands (the Netherlands); European Research Council (ERC) (grant number ERC-2009-AdG 232997) and Nordforsk; and Nordic Center of Excellence Programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS); Regional Governments of Andalucía, Asturias, Basque Country, Murcia (No. 6236) and Navarra; and ISCIII RETIC (RD06/0020) and the Catalan Institute of Oncology. (Spain); Swedish Cancer Society; Swedish Scientific Council; and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK; Medical Research Council; Stroke Association; British Heart Foundation; Department of Health; Food Standards Agency; and Wellcome Trust (UK).

Reagents for the hepatitis infection determinations were kindly provided by Abbott Diagnostics Division, Lyon, France. The funding sources had no influence on the design of the study; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the paper for publication.

Abbreviations

HCC	Hepatocellular carcinoma
IHBC	intrahepatic bile duct
HBV	hepatitis B
HCV	hepatitis C
T2D	type 2 diabetes
NAFLD	non-alcoholic fatty liver disease
GBTC	biliary tract cancer
EBD	extrahepatic bile duct cancer
GB	gallbladder
AmpV	Ampulla of Vater
EPIC	European Prospective Investigation into Cancer and Nutrition
ALT	alanine aminotransferase
AST	aspartate aminotransferase
GGT	gamma-glutamyl transferase
AP	liver-specific alkaline phosphatase
BMI	body mass index

References

1. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO Classification of Tumours of the Digestive System. Fourth Edition. Vol. 3. IARC; 2010.
2. IARC. [Accessed 27 August 2014] Estimated incidence, mortality and 5-year prevalence. 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
3. WHO. [Accessed 27 August 2014] Cancer. 2014. <http://www.who.int/mediacentre/factsheets/fs297/en/>
4. Chuang SC, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer letters*. 2009; 286(1):9–14. DOI: 10.1016/j.canlet.2008.10.040 [PubMed: 19091458]
5. Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, Pischon T, Nothlings U, Overvad K, Tjonneland A, Outzen M, et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. *Journal of the National Cancer Institute*. 2011; 103(22):1686–1695. DOI: 10.1093/jnci/djr395 [PubMed: 22021666]
6. Blonski W, Kotlyar DS, Forde KA. Non-viral causes of hepatocellular carcinoma. *World journal of gastroenterology : WJG*. 2010; 16(29):3603–3615. [PubMed: 20677332]
7. Gomaa AI, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World journal of gastroenterology : WJG*. 2008; 14(27):4300–4308. [PubMed: 18666317]
8. Schlesinger S, Aleksandrova K, Pischon T, Jenab M, Fedirko V, Trepo E, Overvad K, Roswall N, Tjonneland A, Boutron-Ruault MC, Fagherazzi G, et al. Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2013; 24(9):2449–2455. DOI: 10.1093/annonc/mdt204
9. Patel T. Worldwide trends in mortality from biliary tract malignancies. *BMC cancer*. 2002; 2:10. [PubMed: 11991810]
10. Ren HB, Yu T, Liu C, Li YQ. Diabetes mellitus and increased risk of biliary tract cancer: systematic review and meta-analysis. *Cancer causes & control : CCC*. 2011; 22(6):837–847. DOI: 10.1007/s10552-011-9754-3 [PubMed: 21424210]
11. Fedirko V, Lukanova A, Bamia C, Trichopolou A, Trepo E, Nothlings U, Schlesinger S, Aleksandrova K, Boffetta P, Tjonneland A, Johnsen NF, et al. Glycemic index, glycemic load, dietary carbohydrate, and dietary fiber intake and risk of liver and biliary tract cancers in Western Europeans. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2013; 24(2):543–553. DOI: 10.1093/annonc/mds434
12. Montonen J, Jarvinen R, Knekt P, Heliovaara M, Reunanen A. Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. *The Journal of nutrition*. 2007; 137(6):1447–1454. [PubMed: 17513405]
13. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, Grosovski M. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie*. 2008; 22(10):811–816. [PubMed: 18925303]
14. Abid A, Taha O, Nseir W, Farah R, Grosovski M, Assy N. Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome. *Journal of hepatology*. 2009; 51(5): 918–924. DOI: 10.1016/j.jhep.2009.05.033 [PubMed: 19765850]
15. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes care*. 2010; 33(11): 2477–2483. DOI: 10.2337/dc10-1079 [PubMed: 20693348]
16. Hu FB, Malik VS. Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. *Physiology & behavior*. 2010; 100(1):47–54. DOI: 10.1016/j.physbeh.2010.01.036 [PubMed: 20138901]
17. Nielsen SJ, P B. Changes in beverage intake between 1977 and 2001. *Am J Prev Med*. 2004 Oct; 27(3):205–10. [PubMed: 15450632]

18. Schernhammer ES, Hu FB, Giovannucci E, Michaud DS, Colditz GA, Stampfer MJ, Fuchs CS. Sugar-sweetened soft drink consumption and risk of pancreatic cancer in two prospective cohorts. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005; 14(9): 2098–2105. DOI: 10.1158/1055-9965.EPI-05-0059
19. Bao Y, Stolzenberg-Solomon R, Jiao L, Silverman DT, Subar AF, Park Y, Leitzmann MF, Hollenbeck A, Schatzkin A, Michaud DS. Added sugar and sugar-sweetened foods and beverages and the risk of pancreatic cancer in the National Institutes of Health-AARP Diet and Health Study. *The American journal of clinical nutrition*. 2008; 88(2):431–440. [PubMed: 18689380]
20. Genkinger JM, Li R, Spiegelman D, Anderson KE, Albanes D, Bergkvist L, Bernstein L, Black A, van den Brandt PA, English DR, Freudenheim JL, et al. Coffee, tea, and sugar-sweetened carbonated soft drink intake and pancreatic cancer risk: a pooled analysis of 14 cohort studies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2012; 21(2):305–318. DOI: 10.1158/1055-9965.EPI-11-0945-T
21. Zhang X, Albanes D, Beeson WL, van den Brandt PA, Buring JE, Flood A, Freudenheim JL, Giovannucci EL, Goldbohm RA, Jaceldo-Siegl K, Jacobs EJ, et al. Risk of colon cancer and coffee, tea, and sugar-sweetened soft drink intake: pooled analysis of prospective cohort studies. *Journal of the National Cancer Institute*. 2010; 102(11):771–783. DOI: 10.1093/jnci/djq107 [PubMed: 20453203]
22. Ren JS, Freedman ND, Kamangar F, Dawsey SM, Hollenbeck AR, Schatzkin A, Abnet CC. Tea, coffee, carbonated soft drinks and upper gastrointestinal tract cancer risk in a large United States prospective cohort study. *Eur J Cancer*. 2010; 46(10):1873–1881. DOI: 10.1016/j.ejca.2010.03.025 [PubMed: 20395127]
23. Lagergren J, Viklund P, Jansson C. Carbonated soft drinks and risk of esophageal adenocarcinoma: a population-based case-control study. *Journal of the National Cancer Institute*. 2006; 98(16): 1158–1161. DOI: 10.1093/jnci/djj310 [PubMed: 16912268]
24. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) : study populations and data collection. *Public health nutrition*. 2002; 5(6B): 1113–1124. DOI: 10.1079/PHN2002394 [PubMed: 12639222]
25. Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods. *Int J Epidemiol*. 1997; 26(Suppl 1):S1–5. [PubMed: 9126528]
26. Slimani N, Deharveng G, Unwin I, Southgate DA, Vignat J, Skeie G, Salvini S, Parpinel M, Moller A, Ireland J, Becker W, et al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *European journal of clinical nutrition*. 2007; 61(9):1037–1056. DOI: 10.1038/sj.ejcn.1602679 [PubMed: 17375121]
27. EFSA. COUNCIL DIRECTIVE 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption. *Official Journal of the European Communities*. 2001
28. Byrne T, Wilmore D. Does growth hormone and glutamine enhance bowel absorption? *Gastroenterology*. 1998; 114(5):1110–1112.
29. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *The American journal of clinical nutrition*. 1997; 65(4 Suppl):1220S–1228S. discussion 1229S–1231S. [PubMed: 9094926]
30. Aragon G, Younossi ZM. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. *Cleveland Clinic journal of medicine*. 2010; 77(3):195–204. DOI: 10.3949/ccjm.77a.09064 [PubMed: 20200170]
31. Ventura EE, Davis JN, Goran MI. Sugar content of popular sweetened beverages based on objective laboratory analysis: focus on fructose content. *Obesity (Silver Spring)*. 2011; 19(4):868–874. DOI: 10.1038/oby.2010.255 [PubMed: 20948525]
32. Densupsoontorn N, Jirapinyo P, Thamonsiri N, Wongarn R, Phosuya P, Tritiprat A, Patraarat S, Pidatcha P, Suwanthol L. Comparison of the nutrient content of fresh fruit juices vs commercial

- fruit juices. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*. 2002; 85(Suppl 2):S732–738. [PubMed: 12403253]
33. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *The American journal of clinical nutrition*. 2013; 98(4):1084–1102. DOI: 10.3945/ajcn.113.058362 [PubMed: 23966427]
 34. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2013; 14(8):606–619. DOI: 10.1111/obr.12040 [PubMed: 23763695]
 35. Malik VS, Popkin BM, Bray GA, Despres JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation*. 2010; 121(11):1356–1364. DOI: 10.1161/CIRCULATIONAHA.109.876185 [PubMed: 20308626]
 36. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA : the journal of the American Medical Association*. 2002; 287(18):2414–2423. [PubMed: 11988062]
 37. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. *The American journal of clinical nutrition*. 2008; 87(5):1194–1203. [PubMed: 18469239]
 38. Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ, Abdelmalek MF. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of hepatology*. 2008; 48(6):993–999. DOI: 10.1016/j.jhep.2008.02.011 [PubMed: 18395287]
 39. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, Oren R. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *Journal of hepatology*. 2007; 47(5):711–717. DOI: 10.1016/j.jhep.2007.06.020 [PubMed: 17850914]
 40. Rafiq N, Y ZM. The Impact of Non-alcoholic Fatty Liver Disease on Chronic Hepatitis B and C. *Practical gastroenterology*. 2008 Jul. (Viral hepatitis, Series 7).
 41. Romaguera D, Norat T, Wark PA, Vergnaud AC, Schulze MB, van Woudenberg GJ, Drogan D, Amiano P, Molina-Montes E, Sanchez MJ, Balkau B, et al. Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. *Diabetologia*. 2013; 56(7):1520–1530. DOI: 10.1007/s00125-013-2899-8 [PubMed: 23620057]
 42. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, Kuperman Y, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. 2014; 514(7521):181–186. DOI: 10.1038/nature13793 [PubMed: 25231862]
 43. Duffey KJ, Popkin BM. Adults with healthier dietary patterns have healthier beverage patterns. *The Journal of nutrition*. 2006; 136(11):2901–2907. [PubMed: 17056820]
 44. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes care*. 2008; 31(7):1311–1317. DOI: 10.2337/dc08-0080 [PubMed: 18390796]
 45. Potter JD, Steinmetz K. Vegetables, fruit and phytoestrogens as preventive agents. *IARC scientific publications*; 1996. 61–90.
 46. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 2012; 50(6):2155–2170. DOI: 10.1016/j.fct.2012.04.002 [PubMed: 22521445]
 47. Cust AE, Skilton MR, van Bakel MM, Halkjaer J, Olsen A, Agnoli C, Psaltopoulou T, Buurma E, Sonestedt E, Chirlaque MD, Rinaldi S, et al. Total dietary carbohydrate, sugar, starch and fibre intakes in the European Prospective Investigation into Cancer and Nutrition. *European journal of clinical nutrition*. 2009; 63(Suppl 4):S37–60. DOI: 10.1038/ejcn.2009.74 [PubMed: 19888280]

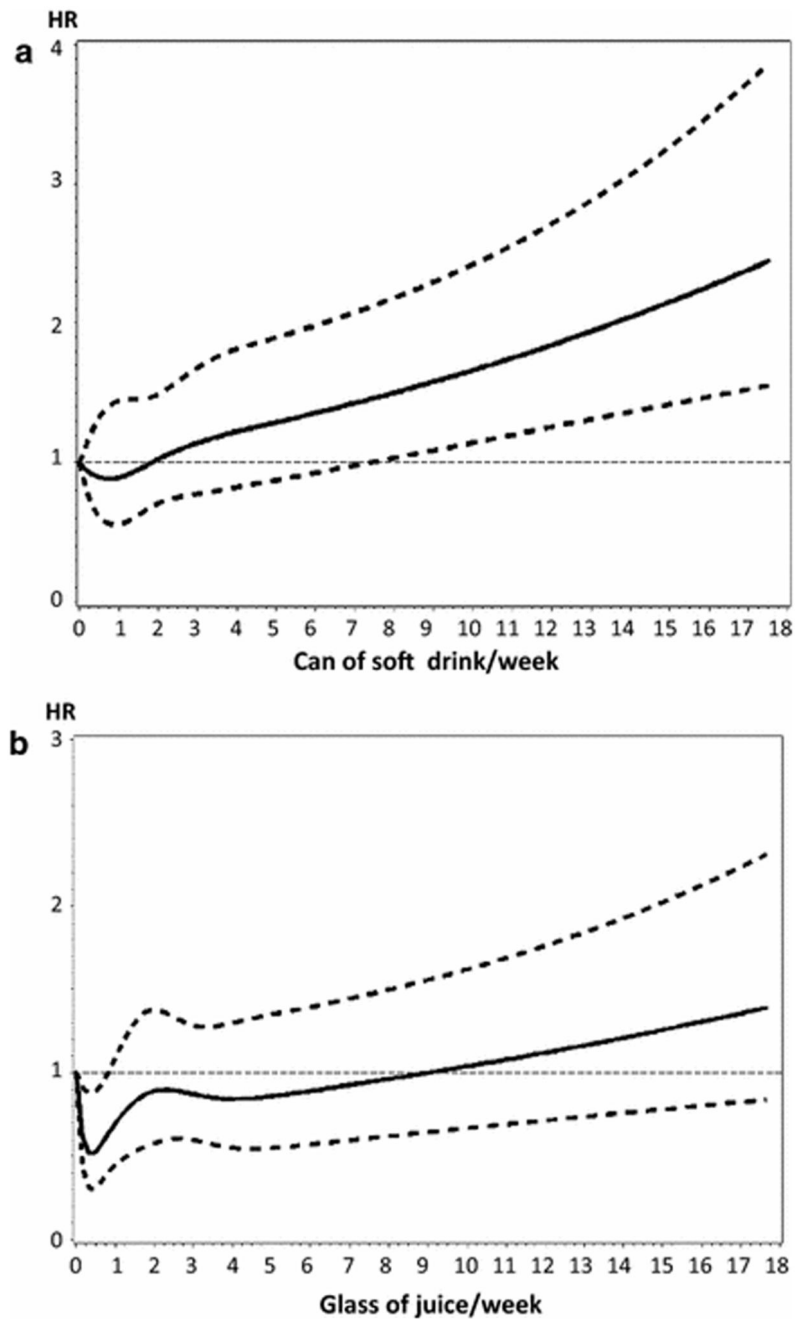


Fig. 1. Spline regression models for the intake of soft drinks (a) and juices (b) in relation hepatocellular carcinoma risk. Reference 0mL/week. Knots correspond to 10th, 25th, 50th, 75th and 90th percentile of intake. The maximum corresponds to the 99th percentile. Solid lines- HR, dashed lines- 95% CI.

Table 1
Baseline characteristics for HCC, IHBC and GBTC cases and non-cases

	HCC	IHBC	GBTC	Non-cases
Sex				
Male [n, (%)]	127 (66.5)	33 (50.0)	89 (37.7)	141945 (29.8)
Female [n, (%)]	64 (33.5)	33 (50.0)	147 (62.3)	334768 (70.2)
Age at recruitment (years) (mean ± SD)	59.6 ± 6.9	59.6 ± 7.7	58.1 ± 8.1	51.2 ± 9.9
BMI (kg/m²) (mean ± SD)	28.0 ± 4.8	27.0 ± 4.2	26.6 ± 4.5	25.4 ± 4.3
Waist-to-Hip Ratio (mean ± SD)	0.94 ± 0.10	0.90 ± 0.10	0.87 ± 0.10	0.84 ± 0.10
Smoking status, duration and intensity^a [n, (%)]				
Never smoker	53 (27.7)	28 (42.4)	110 (46.6)	205157 (43.0)
Current smoker, occasional	14 (7.3)	3 (4.5)	11 (4.7)	40046 (8.4)
Current smoker, 1-15 cigarettes/day	23 (12.0)	6 (9.0)	26 (11.0)	55258 (11.6)
Current smoker, 16-25 cigarettes /day	24 (12.6)	4 (6.1)	17 (7.2)	29822 (6.3)
Current smoker, >25 cigarettes /day	14 (7.3)	1 (1.5)	5 (2.1)	8647 (1.8)
Former smoker, quit ≤ 10 years ago	17 (8.9)	3 (4.5)	15 (6.4)	45552 (9.6)
Former smoker, quit 11-20 years ago	18 (9.4)	9 (13.6)	29 (12.3)	38923 (8.2)
Former smoker, quit > 20 years ago	24 (12.6)	8 (12.1)	15 (6.4)	37566 (7.9)
Highest level of education attained^b [n, (%)]				
None	12(6.3)	3 (4.5)	12 (5.1)	20909 (4.4)
Primary or secondary school	141 (73.8)	47 (71.2)	175(74.2)	325492 (68.3)
University or higher	34 (17.8)	11 (16.7)	41 (17.4)	113406 (23.8)
No. with diabetes at baseline^c [n, (%)]	22 (11.5)	2 (3.0)	16 (6.8)	12478 (2.6)
No. with gallstones at baseline^d [n, (%)]	21 (11.0)	15 (22.7)	30 (12.7)	24473 (5.1)
Physical activity^e (n, %)				
Inactive	18 (9.4)	8 (12.1)	29 (12.3)	71709 (15.0)
Moderately inactive	68 (35.6)	20 (30.3)	76 (32.2)	142918 (30.0)
Moderately active	78 (40.8)	28 (42.4)	92 (39.0)	156660 (32.9)
Active	18 (9.4)	5 (7.6)	22 (9.3)	39198 (8.2)
Alcohol intake lifetime pattern^{f, g} [n, (%)]				
Never drinkers	8 (4.2)	3 (4.5)	12 (5.1)	28136 (5.9)
Former light drinkers	12 (6.3)	6 (9.1)	9 (3.8)	15030 (3.2)
Former heavy drinkers	10 (5.2)	2 (3.0)	3 (1.3)	1979 (0.4)
Light drinkers	23 (12.0)	10 (15.2)	39 (16.5)	87806 (18.4)
Never heavy drinkers	63 (33.0)	25 (37.9)	94 (39.8)	184436 (38.7)
Periodically heavy drinkers	32 (16.8)	9 (13.6)	17 (7.2)	42408 (8.9)
Always heavy drinkers	6 (3.1)	1 (1.5)	2 (0.8)	2968 (0.6)
Alcohol at baseline (g/d)	20.3 ± 31.6	13.5 ± 18.4	11.9 ± 16.9	11.6 ± 16.8
Dietary intakes (mean ± SD)				
Soft drinks (g/d)	129.8 ± 280.3	66.1 ± 155.1	51.7 ± 113.9	76.8 ± 166.3
Juices (g/d)	78.0 ± 150.9	93.9 ± 134.6	58.8 ± 95.7	63.7 ± 108.9
Sugar (g/d)	108.6 ± 51.5	113.4 ± 46.8	99.4 ± 41.3	102.9 ± 43.8

	HCC	IHBC	GBTC	Non-cases
Total energy ^h (kcal/d)	2034.9 ± 647.3	2069.1 ± 649.8	1965.5 ± 595.9	1990.5 ± 590.4

Number of persons with missing information:

^aHCC=4, IHBC=4, EBD=8, non-cases=15742

^bHCC=4, IHBC=5, EBD=8, non-cases=16906

^cself-reported; HCC=15, IHBC=13, EBD=13, non-cases=36823

^dself-reported; HCC=50, IHBC=18, EBD=76, non-cases=145718

^eHCC=9, IHBC=5, EBD=17, non-cases=66228

^fHCC=37, IHBC=10, EBD=60, non-cases=113950

^gSex-specific categories: light drinker (women: 0-3g/d, men: 0-6g/d); heavy drinker (women 30g/d, men 60g/d)

^hTotal energy exempting alcohol

Table 2
HR (95% CI) for HCC by categories of soft drink and juice consumption compared to non-consumers in the EPIC cohort

	PY	Cases	Median intake (5, 95%) (g/day)	Crude Model ^a	Multivariable Model ^b
Soft drinks				Reference	Reference
Non-consumers	2 044 390	78	0.0 (0.0, 0.0)		
Tertile 1	1 058 798	30	6.7 (1.4, 23.0)	0.73 (0.47, 1.15)	0.82 (0.52, 1.29)
Tertile 2	1 097 448	31	50 (28.6, 103.5)	0.84 (0.53, 1.31)	0.94 (0.60, 1.48)
Tertile 3	1 061 657	52	216.8 (114.3, 827.0)	1.47 (1.00, 2.16)	1.46 (0.99, 2.16)
<i>P</i> _{trend}				<0.01	0.01
<1 can ^c /week	1 556 294	44	16.4 (1.4, 42.9)	0.79 (0.53, 1.19)	0.90 (0.60, 1.34)
1-6 cans/week	1 288 584	45	112.5 (50.7, 254.0)	0.98 (0.65, 1.46)	1.05 (0.70, 1.57)
>6 cans/week	373 026	24	500 (295.2, 1155.6)	1.94 (1.19, 3.16)	1.83 (1.11, 3.02)
<i>P</i> _{trend}				<0.01	0.01
Per can/week^e				1.06 (1.03, 1.08)	1.05 (1.02, 1.07)
Juices				Reference	Reference
Non-consumers	1 137 774	51	0.0 (0.0, 0.0)		
Tertile 1	1 391 106	49	6.6 (0.1, 15.7)	0.52 (0.32, 0.92)	0.57 (0.35, 0.92)
Tertile 2	1 396 782	41	42.9 (17.1, 78.6)	0.63 (0.39, 1.02)	0.77 (0.48, 1.25)
Tertile 3	1 336 632	50	142.0 (94.3, 452.6)	0.84 (0.53, 1.33)	0.98 (0.62, 1.55)
<i>P</i> _{trend}				0.31	0.15
<1 glass ^c /week	1 771 163	60	8.4 (0.3, 25.8)	0.54 (0.34, 0.85)	0.60 (0.38, 0.95)
1-6 glasses/week	1 875 327	52	76.8 (35.4, 450.0)	0.62 (0.39, 0.97)	0.75 (0.48, 1.18)
>6 glasses/week	478 030	28	273.8 (179.7, 650.5)	1.24 (0.72, 2.15)	1.38 (0.80, 2.38)
<i>P</i> _{trend}				0.03	0.02
Per glass/week^e				1.03 (1.01, 1.06)	1.03 (1.01, 1.06)

^aCrude Model: Cox proportional hazard model adjusted for non-alcoholic energy intake and stratified by age (1-year intervals), sex and study centre. P for linear trend was computed by assigning median values to each category of consumers and 0 g/d for non-consumers

^bMultivariable Model: additionally adjusted for BMI, sex-specific physical activity, education level, alcohol at recruitment and alcohol intake pattern, smoking intensity, duration and history, diabetes status

^cCan volume 330mL, glass volume 200mL

^dIn reference to non-consumers

^ep=0.57 for heterogeneity for associations for drinks and juices with HCC

Table 3

Hazard ratios and 95% CI for IHBC and GBTC and its subsites^a associated with one serving^b increment per week in the consumption of soft drinks and juices in the EPIC cohort.

	IHBC (N=66) HR (95% CI)	GBTC		All GBTC (N=236) HR (95% CI)
		EBD (N=95) HR (95% CI)	GB (N = 87) HR (95% CI)	
Soft Drinks				
Crude^c	0.99 (0.91, 1.07)	0.95 (0.86, 1.04)	0.91 (0.80, 1.03)	1.02 (0.95, 1.10)
Multivariable^d	0.97 (0.90, 1.06)	0.94 (0.86, 1.03)	0.89 (0.79, 1.01)	1.02 (0.95, 1.10)
Juices				
Crude	1.04 (1.00, 1.08)	1.01 (0.96, 1.06)	0.97 (0.91, 1.04)	0.97 (0.87, 1.08)
Multivariable	1.04 (1.00, 1.08)	1.01 (0.96, 1.06)	0.97 (0.91, 1.04)	0.97 (0.88, 1.08)

^aIHBC, intrahepatic bile duct; GBTC, biliary tract; EBD, extrahepatic bile duct; GB, gallbladder; AmpV, Ampulla of Vater cancers

^bServing for soft drinks corresponds to 330mL and for juices to 200mL.

^cCrude Model adjusted for non-alcoholic energy intake and stratified by age(1-year intervals), sex and study centre.

^dMultivariable Model: additionally adjusted for BMI, sex-specific physical activity, education level, alcohol at recruitment and alcohol intake pattern, smoking intensity, duration and history, diabetes status (IHBC) and gallstones history (GBTC and their subtypes)

Table 4

OR and 95% CI for HCC associated with one serving^a increment per week in the consumption of soft drinks and juices in the nested case-control study within the EPIC cohort.

	Cases	Controls	OR (95% CI)
Soft drinks	121	241	1.21 (1.09, 1.35)
Crude Model ^b			1.18 (1.04, 1.34)
Multivariable Model ^c			1.22 (1.05, 1.40)
Multivariable Model + liver function score			1.19 (1.04, 1.37)
Multivariable Model + Hepatitis status ^d			1.22 (1.04, 1.44)
Multivariable model for Hepatitis free individuals	84	162	1.03 (0.97, 1.09)
Juices	121	241	1.01 (0.93, 1.09)
Crude Model			1.04 (0.95, 1.13)
Multivariable Model			0.99 (0.90, 1.08)
Multivariable Model + liver function score			1.00 (0.90, 1.12)
Multivariable Model+ Hepatitis status			
Multivariable model for Hepatitis free individuals	84	162	

^aServing for soft drinks corresponds to 330mL and for juices to 200mL.

^bCrude Model: matching factors only and adjusted for non-alcoholic energy intake

^cMultivariable Model: crude model additionally adjusted for BMI, sex-specific physical activity, education level, alcohol at recruitment and alcohol intake pattern, smoking intensity, duration and history, diabetes status

^dliver function score (1-6) was calculated according to the cut-off values for: ALT>55 U/L, AST >34U/L, GGT>64UL-men and 36U/L-women, AP>150U/L, albumin<35g/L, total bilirubin >20.5 μmol/L