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Fruit and vegetable consumption in relation to hepatocellular carcinoma in a multi-centre, European cohort study

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Background: Vegetable and/or fruit intakes in association with hepatocellular carcinoma (HCC) risk have been investigated in case–control studies conducted in specific European countries and cohort studies conducted in Asia, with inconclusive results. No multi-centre European cohort has investigated the indicated associations.

Methods: In 486 799 men/women from the European Prospective Investigation into Cancer and nutrition, we identified 201 HCC cases after 11 years median follow-up. We calculated adjusted hazard ratios (HRs) for HCC incidence for sex-specific quintiles and per 100 g d^{-1} increments of vegetable/fruit intakes.

Results: Higher vegetable intake was associated with a statistically significant, monotonic reduction of HCC risk: HR (100 g d^{-1} increment): 0.83; 95% CI: 0.71–0.98. This association was consistent in sensitivity analyses with no apparent heterogeneity across strata of HCC risk factors. Fruit intake was not associated with HCC incidence: HR (100 g d^{-1} increment): 1.01; 95% CI: 0.92–1.11.

Conclusions: Vegetable, but not fruit, intake is associated with lower HCC risk with no evidence for heterogeneity of this association in strata of important HCC risk factors. Mechanistic studies should clarify pathways underlying this association. Given that HCC prognosis is poor and that vegetables are practically universally accessible, our results may be important, especially for those at high risk for the disease.

Hepatocellular carcinoma (HCC) is a leading cause of cancer mortality worldwide, resulting in more than 600 000 deaths annually (Ferlay *et al*, 2013). Apart from the well-established risk factors of chronic infection with hepatitis B (HBV) and hepatitis C (HCV), several modifiable risk factors for HCC have been identified (Trichopoulos *et al*, 2011) but only alcohol (International Agency for Research on Cancer, 1988), obesity (Schlesinger *et al*, 2013) and aflatoxin (International Agency for Research on Cancer, 2002) have been documented as diet related. The role of other dietary exposures is still under investigation (Chuang *et al*, 2009).

Vegetables and fruit are practically universally accessible, and higher intakes have been associated with lower cancer incidence, overall and by several sites (Vainio and Weiderpass, 2006; Soerjomataram *et al*, 2010), although their role in cancer prevention is still inconclusive (World Cancer Research Fund/American Institute for Cancer Research, 2007). Regarding the association of vegetables and fruit with HCC, studies are scarce with conflicting results. Higher intakes of vegetables and a vegetable-based dietary pattern have been associated with lower HCC risk, in case–control studies in Italy (La Vecchia *et al*, 1988; Negri *et al*, 1991; Braga *et al*, 1997; Talamini *et al*, 2006) and Serbia (Kanazir *et al*, 2010) and cohort studies conducted exclusively in Asia (Yu *et al*, 1995; Sauvaget *et al*, 2003; Kurozawa *et al*, 2004; Pham *et al*, 2006; Kurahashi *et al*, 2009; Zhang *et al*, 2013), although in a case–control study in Greece a null association between vegetables and HCC risk was reported (Kuper *et al*, 2000). The association of fruit intake with HCC risk has been investigated in fewer studies and was reported as inverse in Italian case–control studies (La Vecchia *et al*, 1988; Negri *et al*, 1991; Braga *et al*, 1997; Talamini *et al*, 2006), as positive in a cohort study in Japan (Kurahashi *et al*, 2009) and as null in a cohort study in China (Zhang *et al*, 2013). Owing to these conflicted results, in a major review, the evidence regarding the indicated associations was considered inconclusive (World Cancer Research Fund/American Institute for Cancer Research, 2007).

The earlier studies differed by location and background of HCC rates (higher in Asian as compared with European populations), patterns of vegetable/fruit consumption (different in eastern compared with western populations), as well as, in the extent of adjustment for established HCC risk factors, and in the assessment and quantification of vegetable/fruit intakes. Moreover, in most studies, vegetables and fruit were considered independently of each other, although they may act as mutual confounders in their individual associations with HCC risk.

We investigated the associations of vegetable and fruit intakes with HCC risk, in the context of the European Prospective Investigation into Cancer and nutrition (EPIC). This is the first multinational, European-based prospective cohort study undertaken on this topic.

MATERIALS AND METHODS

Participants. EPIC investigates the role of dietary and lifestyle, as well as, biological factors in the aetiology of cancer and other chronic diseases. The EPIC study has been previously described in detail (Riboli *et al*, 2002). More than 500 000 apparently healthy men and women, aged 25–70 years, were recruited between 1992 and 2000 in 23 centres from 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and United Kingdom). The participants are from the general population, except for France (women—members of health insurance plans), Utrecht and Florence (women—breast cancer screening), Naples and Norway (women only), the Oxford cohort (which includes a large proportion of vegetarian volunteers) and subsamples of the Italian and Spanish cohorts (mainly members of blood donor associations).

The study was approved by the Ethical Review Board of the International Agency for Research on Cancer, and by the local Ethics Committees in the participating centres. Participants gave

informed consent before enrolment. Procedures were in line with the Helsinki declaration.

Assessment of diet. Dietary intakes over the previous year were assessed at enrolment with validated centre-specific questionnaires, or food records (Margetts *et al*, 1997), which enquired also about vegetable and fruit consumption (Slimani *et al*, 2002). On the basis of this information, daily intake of vegetables and fruit was estimated in grams (Agudo *et al*, 2002). Legumes and potatoes, as well as vegetable juices are not included in the vegetables food group. Fruit juices, nuts and seeds are not included in the fruit food group. Total energy intake was calculated through the EPIC nutrient database (Slimani *et al*, 2007).

Calibration of the dietary data. A second dietary measurement was taken from an 8% random sample of the cohort (36 994 participants) using a detailed computerised 24-h dietary recall (24HR) method (Slimani *et al*, 2002) to calibrate dietary measurements of foods and food groups including vegetables and fruit intakes across countries (Rosner and Gore, 2001; Ferrari *et al*, 2004).

Assessment of lifestyle, antropometry and medical history. At enrolment, sociodemographic, lifestyle and medical history data were also recorded through standardised questionnaires. Weight, height and waist/hip circumferences were measured for most participants, whereas for a small fraction only self-reported weight and height were available.

Follow-up of study population and case ascertainment. Incident HCC cases (first primary) and vital status were ascertained through record linkage with cancer and death registries in most centres (Riboli *et al*, 2002). In France, Germany and Greece, an ascertainment was done with a combination of methods including health insurance records, pathology registries and active follow-up through mailed questionnaires/telephone interviews (Riboli *et al*, 2002). Incident cancers were subsequently verified through medical records, pathology reports and discharge diagnoses. Cancer incidence was coded according to the International Classification of Diseases-Oncology-2. HCC was defined as C22.0.

Censoring dates for cancer incidence for centres using registry data ranged from December 2004 (Asturias (Spain)) to December 2008 (Turin (Italy), Sweden and Norway). For centres using active follow-up, last contact ranged from July 2005 (France) to June 2010 (Heidelberg (Germany)).

From the initial 521 330 EPIC participants, 23 818 with prevalent cancer, 4383 with insufficient follow-up (e.g., lost from follow-up immediately after recruitment), 78 cases with metastatic cancer or ineligible information on histology, 6192 with no dietary and 60 with no lifestyle questionnaires were excluded. Eventually, 486 799 participants including 201 HCC incident cases were analysed.

Nested case–control study subset. A nested case–control study, using EPIC data as of 2006, including 125 HCC cases and 250 matched controls with available biological samples was conducted. For these subjects, additional laboratory measurements were undertaken regarding established risk factors for HCC (notably serum levels of hepatitis B surface antigen (HBsAg) and antibodies to HCV), which are not available for the whole EPIC cohort. Serum levels of HBsAg and antibodies to HCV (anti-HCV) were determined at the Centre de Biologie République laboratory in Lyon, France. Design and methods of this study have been described elsewhere (Trichopoulos *et al*, 2011). Controls were selected from those alive and free of cancer (except for non-melanoma skin cancer) at the time of diagnosis of the case. Matching criteria included study centre, sex, age at the time of blood collection, date and time of day of blood collection. Women were additionally matched by menopausal status and by use of exogenous hormones.

Statistical analysis. The associations of vegetable intake and fruit intake with HCC risk were investigated through Cox proportional hazard regression. The underlying time variable was age at exit defined as the age of: HCC diagnosis (case); diagnosis of any other incident cancer (censored); death for participants who died without cancer (censored); last follow-up for those alive and with no cancer (censored). Age at enrolment was the entry time. All models were stratified by recruitment age (1-year intervals) and EPIC centre.

Vegetable intake and fruit intake were considered in sex-specific quintiles (categorically, by assigning 0–4 to quintiles Q1–Q5), and as continuous (in $100\,g\,d^{-1}$ increments). The associations of vegetable and fruit intakes with HCC risk were evaluated: (a) by considering vegetable intake and fruit intake separately, (b) by using the sum of vegetables and fruit intakes as a common dietary variable, and (c) by mutually adjusting for vegetable and fruit intakes. Deviations from log linearity of the estimated associations between fruit and vegetable intakes and HCC risk were assessed by comparing models including vegetable and fruit intakes as continuous, with models including both dietary exposures as categorical, using the likelihood ratio test (LRtest).

In all analyses, potential confounders were: sex, self-reported diabetes (categorically: no, yes, unknown), education (categorically: no formal/primary school, technical/secondary school, university/longer, unknown), physical activity (categorically: inactive/moderately inactive, moderately active/active, unknown), smoking (categorically: never, former smoker, current smoker, unknown), ethanol intake (categorically: low: men $<10\,g\,d^{-1}$, women $<5\,g\,d^{-1}$; moderate: men $10-<40\,g\,d^{-1}$, women $5-<20\,g\,d^{-1}$; high: men $\geqslant 40\,g\,d^{-1}$, women $\geqslant 20\,g\,d^{-1}$), body mass index (categorically: $\leqslant 25\,kg\,m^{-2}$, >25 to $<30\,kg\,m^{-2}$, $\geqslant 30\,kg\,m^{-2}$) and energy intake (continuously). In order to minimise residual confounding by smoking/ethanol intake, we further used more detailed variables (but with more missing values), accounting for duration and intensity of smoking and for lifetime alcohol drinking. We also further included in the Cox models meat and coffee intakes to account for residual confounding by these variables.

In the analysis of the nested case–control study, the associations of vegetables and fruit were evaluated through conditional logistic regression adjusting also for HBV/HCV status (positive/negative), as well as, for self-reported diabetes, body mass index, smoking status, ethanol intake, education and energy intake, as previously indicated.

Calibration of the estimated associations. We further re-calculated the log linear associations between vegetable intake and fruit intake with HCC risk after performing linear calibration using data from the second dietary measurement (24HR) study (Rosner and Gore, 2001; Ferrari *et al*, 2004). In brief, the 24HR data were regressed on the intakes of vegetables and fruits, as estimated from the main dietary questionnaire including in the regression models the same potential confounders as in the above-indicated survival models, and further weighted by day of the week and season of the year during which the 24HR was collected. The coefficients from the regression models were used to obtain individual predicted (calibrated) values of vegetable and fruit intakes for all participants. Cox regression models, as specified above, using calibrated vegetable intake and fruit intake as continuous (per $100\,g\,d^{-1}$ increment) were then run in order to obtain the calibrated hazard ratios (HRs). The s.e. of the estimated coefficients (i.e., log of the HRs) were calculated with bootstrap sampling (100 iterations) in the calibration and survival models consecutively and were used to estimate the calibrated 95% CI associated with the calibrated HR (Rosner and Gore, 2001).

Sensitivity analyses. We performed sensitivity analyses by re-calculating the HRs after excluding subjects with missing values on any of the potentially confounding variables that were included in the multivariable models (in order to investigate any influence that missing data on covariates have on the estimated associations). We also restricted the analysis to the 169 histologically confirmed HCC cases. We repeated our analyses after excluding 9596 subjects (10 cases) in the top/bottom 1% of the energy intake distribution (as calculated from the dietary questionnaires and divided by the estimated energy requirement). Finally, we re-estimated the associations between vegetables/fruit intake and HCC risk after excluding the initial 2 years of follow-up, in order to minimise possible effects of pre-existing disease on vegetable and fruit intakes.

Assessing modification of estimated associations across subgroups. Furthermore, we investigated whether the estimated associations differed by sex, diabetes, smoking, ethanol intake and body mass index, by introducing interaction terms between quintiles of vegetable/fruit intakes (ordered) and each of the indicated variables (categorically). The evidence of statistically significant interactions in the multiplicative scale was tested with the LRtest comparing nested models (i.e., with and without the interaction term(s)).

$P<0.05$ was considered statistically significant. Analyses were performed using STATA (Stata Corporation: Stata statistical software, release 11. College Station, TX, USA: Stata Corporation; 2009). Calibration models were run using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

After a median follow-up of 11 years, 1 622 408 and 3 744 060 person-years were accrued from the 145 039 (30%) men and 341 760 (70%) women of the study population, and 133 men and 68 women were diagnosed with HCC.

Table 1 presents the distribution of 486 799 study participants according to sex-specific quintiles of vegetable intake, country of origin and baseline characteristics. Overall median vegetable consumption was 151 g d^{-1} among men and 185 g d^{-1} among women; vegetable intake varied across countries, with the Greeks consuming the highest quantities. Participants with higher vegetable intakes were slightly older and had higher energy intake. Those more educated tended to consume higher quantities of vegetables compared with those less educated as did the never/former smokers compared with current smokers and the moderate/high ethanol consumers compared with no- or low-ethanol consumers. Participants with higher body mass index and/or of lower physical activity consumed higher amounts of vegetables than people with lower body mass index and/or higher physical activity, respectively. Finally, the EPIC subjects who reported to have had diabetes at enrolment, tended to consume more vegetables than the vast majority who did not report diabetes at enrolment.

In Table 2, corresponding distributions as in Table 1 are presented by categories of fruit intake. Median fruit intakes were 156 g d^{-1} for men and 209 g d^{-1} for women. Distribution of participants across categories of fruit intake also varied across countries, with the highest intakes consumed in Greece and Italy. Similarly to vegetable intakes, higher fruit intakes were consumed more frequently: from the slightly older and those with higher energy intakes; from the never/former compared with current smokers; from those with higher compared with lower body mass index; by those with lower compared with higher level of physical activity; and by those who reported to have had diabetes compared with those who did not report diabetes at enrolment. The more educated, however, tended to consume lower quantities of fruit compared with those less educated as did the moderate/high ethanol consumers compared with no- or low-ethanol consumers.

Table 3 shows HRs for HCC incidence by quintiles of vegetable/fruit intakes, separately (models 1 and 2) and after mutual adjustment (model 3). Higher vegetable intake was associated with a monotonic reduction in HCC risk in all models, which reached statistical significance when the association between vegetables and HCC risk was assumed log linear (P-values associated with 100 g d^{-1} increment in vegetable intake for models 1, 2 and 3, respectively, were 0.028, 0.027 and 0.027). Fruit intake, however, was not associated with HCC incidence. No appreciable change in the estimated associations was apparent when duration/intensity of smoking and lifetime alcohol drinking were used as confounders instead of smoking status and ethanol intake at recruitment, or, when meat and coffee intakes were additionally adjusted for.

When the association of vegetable and fruit intakes with HCC risk was assessed by considering the sum of the individual vegetable and fruit intakes, trend results from the fully adjusted HR were, as expected, inverse but not statistically significant (HR for 200 g increment in vegetable and fruit intake 0.90; 95% CI: 0.78–1.05). There was no statistical evidence indicating that log linear models (i.e., using vegetable and fruit intakes as continuous) were of inferior fit as compared with models with no assumption for the type of association of these dietary exposures with HCC risk (i.e., using vegetable and fruit intakes in quintiles, categorically): $\text{LRtest}_{\text{vegetables}}>0.999$, $\text{LRtest}_{\text{fruit}}=0.464$.

Linear calibration enhanced the strength of the indicated associations but also increased the uncertainty of the estimates: HR for 100 g d^{-1} increment in vegetable intake: 0.72; 95% CI: 0.49–1.07; HR for 100 g d^{-1} increment in fruit intake: 0.98; 95% CI: 0.83–1.15.

Sensitivity analyses. Results from sensitivity analyses (using model 3) revealed similar patterns of associations for vegetable intakes with HCC risk, but with larger confidence intervals associated with the respective estimates. More specifically, the HRs associated with 100 g increment in vegetable intake were: 0.83; 95% CI: 0.70–0.99 (P-value 0.033) when subjects without missing values were studied (187 HCC cases/413 666 participants); 0.87; 95% CI: 0.74–1.02 (P-value 0.094) when 9596 subjects (10 HCC cases) in the top/bottom 1% of the energy intake distribution were excluded; 0.90; 95% CI: 0.76–1.06 (P-value 0.210) when analysis was restricted to the 169 histologically confirmed HCC, and; 0.85; 95% CI: 0.71–1.01 (P-value 0.066) when the first 2 years of follow-up were excluded (173 HCC cases/478 436 participants analysed).

The association of fruit intake with HCC risk was essentially null in all sensitivity analyses.

Examination of effect-modifications. In Table 4, evaluation of possible interactions in the multiplicative scale between vegetable and fruit intakes on one hand (per 100 g d^{-1} increments) and certain covariates (in categories as shown in Table 4) on the other hand, among participants without missing data (187 HCC cases/413 666 participants) are shown. The inverse association of vegetable intake with HCC risk was manifested mainly among males, among non-diabetics, among ever smokers, among subjects with moderate/high alcohol intakes and among overweight/obese subjects; nevertheless, the respective interaction terms were far from being statistically significant. The association of fruit intake with HCC risk was in general null in subgroups shown in Table 4, and the respective interaction terms were also far from being statistically significant.

In the analysis of the nested case–control study, the unadjusted, as well as the adjusted for HBV/HCV positivity status associations of vegetables and fruit with HCC risk were in the same direction as in the cohort study but not statistically significant: per 100 g d^{-1} increment the adjusted odds ratios (ORs) were: $\text{OR}_{\text{vegetables}}$ 0.92; 95% CI: 0.66–1.28; OR_{fruit} 0.86; 95% CI: 0.67–1.10 (model with

Table 1. Distribution of baseline characteristics of study participants by quintiles[a] of vegetable intake

	Quintiles of vegetable intake (g d^{-1})				
	Q1	Q2	Q3	Q4	Q5
Median (range): males	56.1 (0–82.5)	104.4 (82.5–126.2)	151.2 (126.2–182.1)	222.0 (182.1–282.0)	387.7 (>282.0)
Median (range): females	76.5 (0–104.9)	129.5 (104.9–155.7)	185.1 (155.7–219.2)	260.7 (219.21–315.70)	403.7 (>315.7)
Country[b] (%)					
France	5.8	10.3	19.1	30.3	34.5
Italy	21.5	23.3	24.6	20.9	9.7
Spain	12.5	13.5	18.5	25.3	30.3
United Kingdom	4.7	10.7	20.9	31.7	32.0
Netherlands	28.0	40.4	23.8	7.2	0.6
Greece	0.5	1.2	3.5	14.1	80.6
Germany	33.7	36.7	21.0	7.1	1.5
Sweden	46.0	21.4	16.9	10.9	4.7
Denmark	20.7	20.5	24.3	23.4	11.2
Norway	36.7	28.8	20.2	10.7	3.6
Age (years): mean (s.d.)	50 (10)	51 (10)	51 (10)	52 (10)	52 (11)
Self-reported diabetes mellitus[b] (%)					
No	19.9	20.0	20.0	20.0	20.0
Yes	14.8	17.1	17.9	20.2	30.0
Educational level[b] (%)					
None/primary	23.6	19.3	18.4	17.9	20.9
Technical/professional/secondary	21.8	21.8	20.6	19.1	16.7
University degree/longer	14.3	18.9	21.1	23.0	22.7
Smoking[b] (%)					
Never	18.4	18.8	19.9	21.2	21.7
Former	18.6	20.8	20.7	20.5	19.4
Current	25.7	22.3	19.5	16.5	16.0
Ethanol intake(g d^{-1})[b] (%)					
Low[c]	23.7	19.8	18.6	18.2	19.8
Moderate[c]	16.0	20.2	21.5	22.0	20.3
High[c]	16.1	20.3	21.6	21.9	20.1
Body mass index[b] (kg m^{-2}) (%)					
⩽25	20.0	20.0	20.4	20.8	18.8
>25–<30	20.3	20.5	20.0	19.3	20.0
⩾30	19.1	18.9	18.5	18.7	24.8
Physical activity index[b] (%)					
Moderately inactive[d]	18.9	18.3	19.7	21.0	22.2
Moderately active[d]	18.2	20.2	20.3	20.7	20.7
Energy intake (kcal d^{-1}): mean (s.d.)	1837.5 (626.7)	2011.4 (621.8)	2103.1 (646.9)	2182.0 (672.5)	2290.3 (731.4)
Total (number)	97 359	97 359	97 361	97 360	97 360

The European Prospective Investigation into Cancer and Nutrition Study.
[a]Sex-specific quintiles in the overall cohort.
[b]Numbers in cells do not always add up to 100% horizontally due to rounding.
[c]Ethanol intake: low: males: <10 g d^{-1}/females: <5 g d^{-1}; moderate: males: 10–<40 g d^{-1}/females: 5–<20 g d^{-1}; high: males: ⩾40 g d^{-1}; females: ⩾20 g d^{-1}.
[d]On the basis of occupational physical activity and physical activity during cycling and sports.

both vegetables and fruit included). No evidence for effect modification of the impact of vegetable and fruit intakes on HCC risk according to HBV/HCV status was observed when the respective interaction terms were included in the model (*P*-interaction of HBV/HCV status: by vegetable intake 0.897; by fruit intake 0.349).

DISCUSSION

In this multi-centre European cohort, we observed a monotonic significant inverse association of intakes of vegetables with HCC incidence, notwithstanding the modest size of HCC cases. Compared with those with minimal consumption of vegetables, and adjusted also for fruit intake, those in the highest quintile of vegetable intake had a lower HCC risk by about 42%, whereas 100 g d^{-1} increment in vegetable intake was associated with a 17% decrease in HCC incidence (*P*-value 0.027). For fruit, no association with HCC risk was evident in any of the analyses undertaken. This is the first prospective cohort study in a multi-centre western population that has investigated the association of vegetable and fruit intakes with HCC risk.

Our results are in line with those reported in most (La Vecchia *et al*, 1988; Negri *et al*, 1991; Braga *et al*, 1997; Talamini *et al*, 2006; Kanazir *et al*, 2010) but not all (Kuper *et al*, 2000), case–control European studies and cohort Asian studies (Yu *et al*, 1995; Sauvaget *et al*, 2003; Kurozawa *et al*, 2004; Pham *et al*, 2006; Kurahashi *et al*, 2009; Zhang *et al*, 2013), with respect to the inverse association of vegetable consumption with HCC risk; however, the indicated inverse associations were not as strong in our study as in the previous case–control European investigations. The strength of the associations estimated in our study were close to the ones reported from the Asian cohort studies (Sauvaget *et al*, 2003; Kurahashi *et al*, 2009), in which associations were quantified in a similar manner to ours (e.g., comparing percentiles of vegetable intake).

Table 2. Distribution of baseline characteristics of study participants by quintiles[a] of fruit intake

	Quintiles of fruit intake (g d^{-1})				
	Q1	Q2	Q3	Q4	Q5
Median (range): males	35.7 (0.0–66.2)	95.2 (66.2–123.3)	156.1 (123.3–197.3)	250.0 (197.3–320.5	435.8 (>320.5)
Median (range): females	62.0 (0.00–101.7)	134.9 (101.7– 171.0)	208.5 (171.0–249.0)	295.8 (249.0–356.4)	461.1 (>356.4)
Country[b] (%)					
France	14.8	18.1	21.0	25.3	20.8
Italy	5.3	9.3	17.6	27.5	40.3
Spain	13.7	10.0	13.7	25.1	37.6
United Kingdom	15.7	18.8	22.7	21.8	21.0
Netherlands	21.5	24.4	22.6	18.5	13.0
Greece	3.7	6.9	13.6	28.1	47.7
Germany	34.1	31.2	19.9	11.0	3.8
Sweden	25.0	25.4	22.9	17.9	8.9
Denmark	28.3	23.2	21.5	14.3	12.8
Norway	36.3	28.3	18.3	10.6	6.5
Age (years): mean (s.d.)	49.7 (9.8)	51.0 (9.9)	51.6 (9.9)	52.1 (10.0)	51.8 (10.0)
Self-reported diabetes mellitus[b] (%)					
No	19.9	19.9	20.0	20.1	20.2
Yes	16.4	19.3	20.2	22.0	22.1
Educational level[b] (%)					
None/primary	19.2	17.4	17.9	20.8	24.7
Technical/professional/secondary	22.1	21.4	20.4	18.9	17.2
University degree/longer	17.8	21.0	21.7	20.7	18.9
Smoking[b] (%)					
Never	16.0	19.1	20.7	21.9	22.3
Former	18.7	20.8	21.0	20.1	19.5
Current	30.3	21.0	17.4	15.8	15.6
Ethanol intake (g d^{-1})[b] (%)					
Low[c]	19.1	19.4	19.5	20.3	21.7
Moderate[c]	19.0	20.8	21.1	20.3	18.8
High[c]	26.0	20.4	19.1	18.0	16.4
Body mass index (kg m^{-2})[b] (%)					
⩽25	21.0	20.9	20.6	19.8	17.7
>25–<30	19.1	19.5	19.8	20.1	21.6
⩾30	18.2	17.9	18.3	20.8	24.8
Physical activity index[b] (%)					
Moderately inactive[d]	18.8	18.9	19.5	21.2	21.7
Moderately active[d]	18.4	19.8	20.9	20.3	20.6
Energy intake (kcal d^{-1}): mean (s.d.)	1880.3 (652.2)	1994.4 (627.2)	2077.2 (642.8)	2152.2 (656.6)	2320.2 (728.2)
Total (number)	97 361	97 358	97 361	97 359	97 360

The European Prospective Investigation into Cancer and Nutrition Study.
[a]Sex-specific quintiles in the overall cohort.
[b]Numbers in cells do not always add up to 100% horizontally due to rounding.
[c]Ethanol intake: low: males: <10 g d^{-1}/females: <5 g d^{-1}; moderate: males: 10–<40 g d^{-1}/females: 5–<20 g d^{-1}; high: males: ⩾40 g d^{-1}; females: ⩾20 g d^{-1}.
[d]On the basis of occupational physical activity and physical activity during cycling and sports.

The role of fruit intake in HCC incidence as investigated in epidemiological studies is inconclusive: an inverse association of fruit intake with HCC risk has been reported mainly from case–control studies in northern Italy, but these associations have either considered the combination of fruit and vegetable intake (Braga *et al*, 1997) or did not reach statistical significance (Talamini *et al*, 2006). Interestingly, the cohort study by Kurahashi *et al* (2009) reported an apparent higher risk of HCC with higher fruit intakes, albeit not statistically significant, whereas an a-posteriori fruit-based dietary pattern was not found to be associated with HCC incidence in the Chinese cohort study of Zhang *et al* (2013). Our analyses did not reveal an association of fruit intake with HCC risk, a finding that was consistent in subgroup and sensitivity analyses. Not surprisingly, when the total intake of fruit and vegetables was considered, an overall association of this combined dietary exposure was not evident due to the null association of fruit with HCC risk.

Several mechanisms could underlie the inverse association between vegetable intake and HCC risk. Vegetable intake has been suggested to be inversely related to chronic liver disease (Cook *et al*, 2014), or diabetes mellitus (Clifton *et al*, 2014). In previous studies, however, the inverse association between vegetable and HCC risk was evident in subjects irrespectively of documented chronic liver disease or HBV/HCV positivity status (Yu *et al*, 1995; Kurahashi *et al*, 2009). We did not have detailed information overall about chronic liver disease: however, when the first 2 years of follow-up were excluded (thus a fraction of cases with underlying liver disease were most likely also excluded), results were practically unchanged. Of note, our results were adjusted for diabetes and there was no evidence for diabetes-by-vegetable interaction. Moreover, in analyses performed in the nested case–control study, adjusting for chronic infection with HBV/HCV did not materially affect the point estimates of the association of vegetable and fruit intake with HCC risk, nor was there a

Table 3. HRs and 95% CIs of HCC incidence according to quintiles[a], as well as by 100 g d^{-1} increments of vegetable and fruit intake in the European Prospective Investigation into Cancer and Nutrition cohort

		Model 1[b,c]		Model 2[b,d]		Model 3[b,e]	
	HCC cases/*N*	HR	95% CI	HR	95% CI	HR	95% CI
Vegetable intake (quintiles)							
Q1	51/97 359	1		1		1	
Q2	47/97 359	0.89	(0.59–1.33)	0.92	(0.61–1.38)	0.92	(0.61–1.38)
Q3	43/97 361	0.84	(0.55–1.28)	0.89	(0.58–1.37)	0.89	(0.58–1.37)
Q4	32/97 360	0.67	(0.42–1.08)	0.71	(0.43–1.15)	0.70	(0.43–1.15)
Q5	28/97 360	0.59	(0.33–1.06)	0.59	(0.32–1.08)	0.58	(0.31–1.07)
Per 100 g d^{-1} increment		0.84	(0.72–0.98)	0.84	(0.72–0.98)	0.83	(0.71–0.98)
Fruit intake (quintiles)							
Q1	43/97 361	1		1		1	
Q2	49/97 358	1.01	(0.67–1.53)	1.10	(0.73–1.67)	1.14	(0.75–1.73)
Q3	31/97 361	0.67	(0.42–1.08)	0.77	(0.48–1.25)	0.81	(0.50–1.32)
Q4	38/97 359	0.81	(0.51–1.30)	0.95	(0.59–1.53)	1.03	(0.63–1.66)
Q5	40/97 360	0.87	(0.53–1.42)	1.03	(0.62–1.71)	1.14	(0.68–1.91)
Per 100 g d^{-1} increment		0.96	(0.88–1.06)	0.99	(0.90–1.08)	1.01	(0.92–1.11)

Abbreviations: CI = confidence interval; HCC = hepatocellular carcinoma; HR = hazard ratio.
[a]Sex-specific quintiles (Q1–Q5) in the overall cohort.
[b]Models 1,2 and 3 are not hierarchical.
[c]Stratified for age at recruitment (in 1-year intervals) and for centre. Adjusted for sex.
[d]Stratified for age at recruitment (in 1-year intervals) and for centre. Adjusted for sex, diabetes mellitus (self-reported at enrolment), highest level of education attainment, body mass index, smoking status, physical activity, ethanol intake at baseline, categorically as in Table 2, and energy intake.
[e]Adjusted as in model 2 but simultaneously including fruit and vegetable intake.

Table 4. HRs and 95% CIs of HCC incidence associated with 100 g d^{-1} increment of vegetable and fruit intake in specific subgroups

		Vegetables		Fruit	
	HCC cases/*N*	HR[a,b]	95% CI	HR[a,b]	95% CI
Sex					
Males	125/134 452	0.76	(0.61–0.93)	1.08	(0.97–1.21)
Females	62/279 214	0.98	(0.75–1.29)	0.92	(0.77–1.09)
P-interaction			*0.874*		*0.300*
Prevalent diabetes					
No	161/401 858	0.80	(0.66–0.96)	1.03	(0.92–1.14)
Yes	26/11 808	1.01	(0.69–1.49)	1.00	(0.75–1.34)
P-interaction			*0.750*		*0.605*
Smoking status					
Never	54/211 466	1.11	(0.85–1.47)	0.88	(0.71–1.08)
Former	63/109 813	0.78	(0.59–1.04)	1.07	(0.92–1.26)
Current	70/92 387	0.79	(0.59–1.06)	1.11	(0.96–1.29)
P-interaction			*0.886*		*0.343*
Ethanol intake					
Low[c]	98/204 811	0.94	(0.77–1.16)	1.00	(0.88–1.14)
Moderate[c]	51/148 036	0.66	(0.44–0.99)	0.96	(0.77–1.20)
High[c]	*38*/60 819	0.69	(0.47–1.01)	1.21	(0.91–1.51)
P-interaction			*0.788*		*0.613*
Body mass index					
⩽25	55/208 442	0.91	(0.69–1.20)	1.10	(0.92–1.33)
>25–<30	79/146 573	0.80	(0.62–1.04)	1.04	(0.91–1.20)
⩾30	53/58 651	0.78	(0.56–1.10)	0.94	(0.76–1.16)
P-interaction			*0.279*		*0.179*

The European Prospective Investigation into Cancer and Nutrition cohort. Abbreviations: CI = confidence interval; HCC = hepatocellular carcinoma; HR = hazard ratio.
[a]Per 100 g d^{-1} increment in vegetable/fruit intakes. Results from analysis of data with no missing values in any covariate (187 HCC cases/413 666 subjects).
[b]Stratified for age at recruitment (in 1-year intervals) and for centre. Adjusted for sex, diabetes mellitus (self-reported at enrolment), highest level of education attainment, body mass index, smoking status, physical activity, ethanol intake at baseline, categorically as in Table 2, and total energy intake. For each subgroup analysis the variable defining subgroups is not included in the model.
[c]Ethanol intake: low: males: <10 g d^{-1}/females: <5 g d^{-1}; moderate: males: 10–<40 g d^{-1}/females: 5–<20 g d^{-1}; high: males: >40 g d^{-1}; females: >20 g d^{-1}.

statistically significant interaction between virus carrier state and vegetable/fruit intakes with respect to HCC risk.

The apparent protective effect of vegetables on HCC risk may be mediated through a joint activity of the antioxidant effects of a wide range of nutritional constituents present in vegetables such as carotenoids, retinoids, ascorbic acid and a-tocopherol (Sporn and Roberts, 1983; Moreno *et al*, 2002; Sano *et al*, 2005). Indeed, in the Taiwan cohort study (Yu *et al*, 1995) an inverse association of HCC

with serum retinol levels was observed, which complemented the apparent inverse association of vegetable intake with HCC risk found in the same study, whereas in the study by Kurahashi *et al* (2009) an inverse association between a- and b-carotene and HCC risk was also shown. Another possible pathway for the protective role of vegetables on HCC risk may be through their flavonoid content, which has been suggested by studies *in vitro* to have antitumour effect in some hepatocarcinoma cell lines (Hwang *et al*, 2011; Mansoor *et al*, 2011). In a previous EPIC study, the association of dietary intakes of flavonoids with HCC risk was investigated (Zamora-Ros *et al*, 2013), and inverse associations with total flavonoid intake (in specific, with flavanol intake) were observed. In a recent EPIC study, Fedirko *et al* (2013) observed an inverse association between dietary fibre from vegetables and HCC risk which, again, may at least partly explain our results. Interestingly, in the study of Fedirko *et al* (2013) fibre from fruit was not associated with HCC risk. We were not able to investigate the mediating effect of antioxidant intakes or total antioxidant capacity on the estimated associations, because this information is not available in our data set.

Regarding fruit, in the case–control studies in which inverse associations with HCC risk were observed, these were attributed mainly to the antioxidant properties of fruit polyphenols. Given the inconclusive collective evidence in the literature, further studies are needed to affirm any role of fruit on HCC risk before exploring any underlying mechanisms.

Strengths of our study are its prospective design, its multicentre coverage allowing investigation of a variable range of fruit and vegetable intakes across European countries, the use of validated questionnaires and common 24HRs, the relatively long follow-up, the common identification criteria of incident HCC and the available information about potential confounders.

A limitation of our study is the unavoidable modest number of HCC outcomes, owing to the low incidence of the disease in most European countries. Errors in reporting vegetable and fruit consumptions cannot be ruled out, but this is more likely to result in random misclassification of the dietary exposures, thus resulting in underestimation of true associations. Heterogeneity in the estimated associations may exist across countries, but we tried to account for this by stratifying for centre and calibrating our estimates using the 24 h recall data. Bias in the estimated associations could have been introduced if cases had modified their vegetable/fruit consumption during the prediagnostic period of their disease, but excluding cases diagnosed in the first 2 years of follow-up did not alter the results. We did not further inspect whether the apparent association between vegetable intake and HCC risk was driven by a specific subgroup of vegetables with similar features (e.g., cruciferous vegetables) because (a) such analysis would be possibly underpowered due to the small number of HCC cases and the small underlying magnitude of the hypothesised associations and (b) there is no current evidence to favour one or another subgroup of vegetables as more/less responsible for the apparent association.

In conclusion, in a large prospective study across European countries, we observed a lower HCC risk associated with higher vegetable but not fruit intakes. Further epidemiological and mechanistic studies should better clarify the pathways underlying this association. There was no evidence of heterogeneity of the estimated associations across strata defined by established HCC risk factors. Given that vegetables are practically universally accessible, and that the prognosis of HCC is poor, our results, taken together with current evidence, may prompt for dietary intervention studies on HCC incidence among those who are at high risk for the disease.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

None of the funding sources had any role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript, and decision to submit the manuscript for publication.

AUTHOR CONTRIBUTIONS

All named authors have read the manuscript, have agreed to the submission and have participated in the study to a sufficient extent to be named as authors.

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