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Dietary fat, fat subtypes and hepatocellular carcinoma in a large European cohort

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Abbreviations: BMI: body mass index; CI: confidence interval; EPIC: European Prospective Investigation into Cancer and Nutrition; HBV: hepatitis B virus; HCC: hepatocellular cancer; HCV: hepatitis C virus; HR: hazard ratio; IARC: International Agency for Research on Cancer; OR: odds ratio; PLC: primary liver cancer

Additional Supporting Information may be found in the online version of this article.

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The role of amount and type of dietary fat consumption in the etiology of hepatocellular carcinoma (HCC) is poorly understood, despite suggestive biological plausibility. The associations of total fat, fat subtypes and fat sources with HCC incidence were investigated in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, which includes 191 incident HCC cases diagnosed between 1992 and 2010. Diet was assessed by country-specific, validated dietary questionnaires. A single 24-hr diet recall from a cohort subsample was used for measurement error calibration. Hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated from Cox proportional hazard models. Hepatitis B and C viruses (HBV/HCV) status and biomarkers of liver function were assessed separately in a nested case–control subset with available blood samples (HCC = 122). In multivariable calibrated models, there was a statistically significant inverse association between total fat intake and risk of HCC (per 10 g/day, HR = 0.80, 95% CI: 0.65–0.99), which was mainly driven by monounsaturated fats (per 5 g/day, HR = 0.71, 95% CI: 0.55–0.92) rather than polyunsaturated fats (per 5 g/day, HR = 0.92, 95% CI: 0.68–1.25). There was no association between saturated fats (HR = 1.08, 95% CI: 0.88–1.34) and HCC risk. The ratio of polyunsaturated/monounsaturated fats to saturated fats was not significantly associated with HCC risk (per 0.2 point, HR = 0.86, 95% CI: 0.73–1.01). Restriction of analyses to HBV/HCV free participants or adjustment for liver function did not substantially alter the findings. In this large prospective European cohort, higher consumption of monounsaturated fats is associated with lower HCC risk.

What's new?

The rise of hepatocellular carcinoma (HCC) incidence in high- and middle-income countries, where relatively high-fat diets are common, suggests a possible etiological role for dietary fat. In the present study, potential associations between HCC and total fat intake, intake of fat subtypes and intake of fat from different sources were explored with data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Total fat intake, where monounsaturated fats predominated, was inversely associated with HCC risk. By contrast, no risk associations were detected for polyunsaturated or saturated fat intake or fat source.

Epidemiology

Liver cancers, which are usually diagnosed at advanced stages, are the sixth most common cancer and the second leading cause of cancer death worldwide.[1] Hepatocellular carcinoma (HCC), the most common type of liver cancers,[2] is primarily associated with chronic hepatitis B and C virus (HBV/HCV) infections and aflatoxin exposure.[2,3] Other major risk factors

include obesity, type 2 diabetes, tobacco smoking and heavy alcohol drinking.[2–6] A substantial proportion of the steadily increasing incidence of HCC is in high and middle income countries[7] and is more likely to occur in persons without apparent exposure to aflatoxins, or HBV/HCV, suggesting a need for greater emphasis on other modifiable risk factors, particularly those related to diet and lifestyle.[7,8]

Western-type diets are characterized by a relatively higher proportion of fats, but the impact of dietary fat and its main subtypes on HCC risk has not been well explored. Dietary fat contains varying proportions of saturated and unsaturated (mono- and polyunsaturated) fats that are different chemically and are known to alter cell membrane fatty acid composition leading to changes in fluidity and subsequently affecting the cellular responsiveness to external stimuli (*e.g.*, growth factors).[9,10] Furthermore, different fat-subtypes may also be involved in the production of different families of eicosanoid, which can affect cell proliferation, immune response, tumor cell invasion and metastasis.[10] For these reasons, it is important to investigate not only the amount but the type and food source of dietary fat in relation to cancer risks. Furthermore, the liver is a central organ in fat metabolism and processing of dietary fats. After emulsification in the intestinal tract, dietary lipids are absorbed and transported into the liver, where they could have a direct effect on hepatocytes and possibly contribute toward tumor development. A hint about the possible role of dietary fats in liver cancer arises from ecological data from Germany showing a positive correlation between liver cancer mortality in men and higher intake of fats from animal, but not vegetable, sources.[11] Existing observational evidence is limited to a prospective study conducted in the USA showing a positive association with HCC risk for saturated fat,[12] and three case–control studies based on European populations with mixed results.[13–15] However, most of these studies did not consider fat subtypes, and only two[13,15] have considered HBV/HCV infection status as a potential confounder or effect modifier of the association between dietary fat and HCC risk.

The aim of this study was to investigate the associations between intakes of total fat, fat subtypes and fat from different food sources with HCC risk within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, a large geographically and culturally heterogeneous cohort of Europeans, with a nested case–control subset for which measurements of HBV/HCV infection status and liver function biomarkers were conducted.

Methods

Study design

EPIC is a large prospective cohort study designed to investigate the association between diet, lifestyle and environmental factors and the incidence of cancers and other chronic diseases. Detailed information on the study design, rationale and methods of the EPIC study, including assessment of diet and lifestyle factors, has been described previously.[16,17] Briefly, at recruitment (1992–2000), standardized dietary, lifestyle and socio-demographic questionnaires including information on physical activity, education, smoking and medical history data were collected from [mt]520,000 men and women (aged 20–85 years, from 23 centers throughout 10 European countries); anthropometric measurements and blood samples were collected from most participants. Study participants were recruited from the general population residing in a given geographical area, 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).

All cohort members provided written informed consent. Ethical approval for this study was obtained from the IARC ethical review board (Lyon, France) and local participating centers.

Dietary measurement

Diet during the previous year from the date of recruitment into the study was assessed at baseline by validated country-specific dietary questionnaires.[18] Values for daily energy intake, dietary fats (total fat, saturated, monounsaturated and polyunsaturated fats) and fiber intakes were computed based on country-specific food composition tables, which were harmonized across the countries participating in EPIC (EPIC Nutrient DataBase, ENDB).[19] In the case of the dietary fat subtypes, a small proportion of dietary total fat could not be classified and is thus not accounted for [∼7% of total fat or ∼3% of total energy, depending on the country-specific food composition table]. For all subjects, values were also computed for total dietary fat content of specific food groups: red (beef, veal, pork, mutton/lamb, horse and goat) and processed meats, fish and shellfish (fish, crustaceans, molluscs, fish products, and fish in crumbs), added fats and oils (butter, margarine, vegetable oils, and frying fats), dairy products (milk, cheese, and yoghurt) and fats from other sources (vegetables, fruits, legumes, cereals, eggs, poultry, confectionaries, cakes, condiments, sauces and soups). Total dietary fat was also classified as fat of animal, plant or unknown origin based on qualitative information of the predominant origin of the food (>95% animal origin, >95% plant origin or unknown). Additionally, the ratios of polyunsaturated fat to saturated fat (P:S ratio), of monounsaturated fat to saturated fat (M:S ratio), and polyunsaturated and monounsaturated fats to saturated fat [(P+M):S ratio], which indicate the adherence to the recommendation to replace saturated fats with monounsaturated and polyunsaturated fats,[20] were calculated.

In order to improve comparability of dietary data across centers and to partially correct diet–disease associations for random and systematic errors in the dietary questionnaires, a single standardized, computer-assisted 24-hr dietary recall was obtained from an 8% stratified random sample (36,900 participants) for the purposes of calibration.[21,22]

Follow-up for cancer incidence and mortality

Vital status follow-up (98.5% complete) was collected by record linkage with regional and/or national mortality registries in all countries except Germany and Greece, where follow-up was based on active follow-up through study subjects or their next-of-kin. Cancer incidence was determined through record linkage with population-based cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom; complete up to December 2006) or *via* a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries and active follow-up (France, Germany, Greece; complete up to June 2010).

Case ascertainment

HCC was defined as first incident tumor in the liver (C22.0 as per the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death [ICD-10]). For each identified case, the histology, the methods used to diagnose the cancer and α-fetoprotein levels (nested case–control subset only) were reviewed to exclude metastatic cases or other types of liver cancers.

Cohort participants

This analysis includes 477,206 participants (exclusions: 23,818 with prevalent cancer other than non-melanoma skin cancer, 4,380 with incomplete follow-up data/missing information on date of diagnosis, 6,252 with missing dietary/lifestyle information, 9,596 in top or bottom 1% of the ratio of total energy intake/estimated energy requirement and 78 with metastasis in the liver or ineligible histology code). A total of 191 HCC cases were included.

Nested case–control subset

A nested case–control subset of the full cohort was conducted to investigate whether associations between fats intake and HCC risk were independent of chronic HBV/HCV infection. The design has been previously described in detail,[23] and also in the Supporting Information. Briefly, 125 HCC cases with available blood samples at baseline were identified between participants' recruitment and 2006, and matched to two controls. HBV/HCV seropositivity and biomarkers of hepatic injury were measured in 122 HCC cases and 242 matched controls, after excluding participants with missing blood sample or failed laboratory assay ($n = 11$).

Statistical analyses

All nutrient data were adjusted for non-alcohol energy intake by means of the residual method,[24] where we calculated the residuals from center- and sex-specific regression models of nutrient intakes regressed on non-alcohol energy consumption and then rescaled by adding the center- and sex-specific mean intake. We also used the nutrient density method[24] but only in sensitivity analyses. Results for both the density and the residual methods were similar (data not shown).

Dietary intakes were analyzed as both categorical and continuous variables. Categorical variables were presented as sex-specific quartiles based on the distribution of intake across the entire EPIC cohort. For continuous analyses, risk estimates for residual adjusted intakes are presented per 10 g of non-alcohol energy-adjusted intake for total fat, and 5 g for fat subtypes and fat from different food sources. For the M:S, P:S and (P+M):S ratios the risk estimates are presented per 0.2 increment.

Cox proportional hazards models were used to estimate hazard ratios (HR) and their associated 95% confidence intervals (95% CI). Tests and graphs based on Schoenfeld residuals indicated no substantial deviation from the proportional hazards assumption. Age was used as the primary dependent time variable, with entry and exit time defined as the subjects' age at recruitment and age of cancer diagnosis or censoring (age at death, loss to follow-up, end of follow-up or diagnosis of other cancer entities), respectively. For all analyses, both crude and multivariable models were run. All models were stratified by study center (to account for differences in follow-up procedures and questionnaire design), age at recruitment in 1-year categories (to reduce sensitivity to any violations of the proportional hazards assumption) and sex (to allow for different baseline rates). The crude model was adjusted for baseline alcohol intake (g/day), and total non-alcohol energy intake (kcal/day). Multivariable models were additionally adjusted for body mass index (BMI; kg/m^2), smoking status (never, former, current, and not specified), sex-specific physical activity level (inactive, moderately inactive, moderately active, active, and not specified), self-reported diabetes (yes, no, unknown), pattern of lifetime alcohol intake (never drinker, former light drinker, former heavy drinker, light drinker, never heavy drinker, periodically heavy drinker, always heavy drinker, unknown), coffee intake (mL/day) and intake of dietary fiber (g/day). Other factors (height, weight, waist circumference, waist-to-hip ratio, level of education, fruits and vegetables, red and processed meat, fish intake) were tested as potential confounders, but were not included in the final multivariable model for the sake of parsimony, as they did not affect our estimates (change-in-estimate <10%). To test dose–response associations, trend variables were assigned the sex-specific median values for overall quartiles of dietary exposures of interest.

Models were simultaneously adjusted for fat subgroups or sources, so the effect of an independent additive increase in one subgroup of fat (*e.g.,* saturated fat) was estimated while keeping the consumption of the two other fat subgroups constant (*i.e.,* increase in intake of one of the three subgroups, one at a time). Additional models were run to investigate substitution effects for types and sources of fat. In this model, total fat intake was held constant, such that an increase in intake of one of the subgroups is counterbalanced with an

equally divided decrease in intake of the remaining two subgroups.[25,26]

Potential effect modifications of the association between nutrient intakes and cancer risk by important HCC risk factors including sex, age at recruitment, age at diagnosis, years of follow-up, BMI, smoking status, baseline and lifetime alcohol consumption, self-reported diabetes and physical activity were evaluated in separate analyses by including interaction terms formed by the product of modifying variable categories and the value of categories of fat intake. The statistical significance of interactions ($p < 0.05$) was assessed using likelihood ratio tests based on the models with and without the interaction terms.

Sensitivity analyses were conducted excluding (*i*) all participants with a follow-up time less than two years ($n_{cases} = 26$/$n_{non\text{-}cases} = 8{,}078$) in order to rule out possible reverse causation, and (*ii*) self-reported cases of diabetes at recruitment ($n_{cases} = 22$/$n_{non\text{-}cases} = 12{,}496$) due to the potential for modifications in diet after diagnosis of this disease.

Dietary intakes were calibrated by utilizing a multivariable fixed-effects linear model in which 24-hr recall values were regressed on the main dietary questionnaire values for the calibration of sub-sample of the EPIC cohort.[27] The individual predicted values for each of the dietary exposures of interest were computed from the calibration models. Cox proportional hazards models identical to the ones described above were fit with calibrated/predicted values on a continuous scale. The standard error of the calibrated coefficient was estimated by bootstrap sampling with 300 repetitions to take into consideration the uncertainty related to measurement error correction.[28]

Nested case–control study

Conditional logistic regression was used to estimate the associations between cancer risk and fat intakes among all and HBV/HCV negative cases and controls (*n* HBV/HCV—positive cases and controls were 38 and 10, respectively). The statistical analyses for the nested component were based on two conditional logistic models (*a*) crude, which included matching factors with further adjustment for baseline alcohol intake at recruitment (g/day) and total non-alcohol energy intake (kcal/day) and (*b*) multivariable, which was based on the crude model but with additional adjustment for the same confounding variables as described above for the cohort analyses.

Sensitivity analyses were performed including additional adjustment for hepatitis status and liver function score (range from 0 to 6; categorized as 0 = no liver injury, 1–2 = possible minor injury, ≥3 = possible injury). This score summarizes the number of abnormal values for six liver function tests (alanine aminotransferase [ALT] >55 U/L, aspartate aminotransferase [AST] >34 U/L, gamma-glutamyltransferase [GGT] men > 64 U/L/women > 36 U/L, liver-specific alkaline phosphatase [AP] >150 U/L, albumin < 35 g/L, total bilirubin > 20.5 μmol/L; cut-points were provided by the laboratory and were based on assay specifications, Supporting Information Table 1).[29] We also repeated analyses among HBV/HCV-negative participants.

p-Values<0.05 were considered statistically significant. Analyses were performed using SAS version 9.2 (SAS Institute, Inc., NC) and Stata version 11 (StataCorp, College Station, TX).

Results

Cohort study

A total of 5,262,298 person years of follow-up (mean = 11.4 years) were contributed by 142,194 men and 335,012 women between 1992 and 2010. During this period, 191 participants were diagnosed with first incident HCC. The mean intake of total fat varied across countries, with the highest intake (mean = 99.8 g/day) reported in Greece and the lowest (mean = 61.0 g/day) in Norway (Table 1). Participants who developed HCC were more likely to be men, older, current smokers, and to have diabetes and higher baseline BMI compared with participants who did not develop cancer. They also reported higher intake of alcohol, red meat, processed meat and total fat but lower intake of coffee compared to non-cases (Table 2).

Dietary total fat and fat subtypes

The top food sources of dietary total fat were added fats (28%), meat and meat products (16%) and dairy products (20%); of dietary saturated fat—added fats (22%), meat and meat products (16%) and dairy products (33%); of dietary monounsaturated fat—added fats (34%), meat and meat products (18%) and dairy products (15%); and of dietary polyunsaturated fat—added fats (35%), meat and meat products (11%) and dairy products (4%).

Intake of total fat was inversely associated with HCC risk in multivariable model when analyzed continuously (Table 3). The multivariable hazard ratio (HR) per 10 g/day higher total fat intake was 0.88, 95% CI: 0.78–0.98. After calibration, HR remained statistically significant and slightly strengthened the observed association (HR = 0.80, 95% CI: 0.65–0.99).

When examined by fat subtype, monounsaturated fat (37% of total fat), but not polyunsaturated fat (16% of total fat), was associated with lower HCC risk (per 5 g/day, HR = 0.84, 95% CI: 0.74–0.95 before calibration, and HR = 0.71, 95% CI: 0.55–0.92 after calibration; Table 3). Risk estimates for saturated fat (38% of total fat) were elevated but were not statistically significant after adjustment for covariates (per 5 g/day, HR = 1.05, 95% CI: 0.93–1.19 before calibration and HR = 1.08, 95% CI: 0.88–1.34 after calibration). In multivariable substitution models (results not shown), we observed a decreased HCC risk associated with a 5 g increase in monounsaturated fat intake offset by a 5 g decrease in saturated fat intake (HR = 0.80, 95% CI: 0.65–0.99). Also, a 5 g increase in polyunsaturated fat intake offset by a 5 g decrease in saturated fat intake was associated with lower HCC risk

Table 1. Size of the EPIC cohort and numbers of cases for the analyses of fats and liver cancer, by EPIC centre sub-cohort, 1992–2010

			Mean (5th–95th percentiles)			Dietary intake of total fat and fat subtypes (g/day),[1] mean (5th–95th percentiles)			
Country	Cohort size	Total no. of PY	Age at recruitment (years)	No. of yrs of follow-up	No. of HCC cases	Total Fat	Saturated fat	Monounsaturated fat	Polyunsaturated fat
France	67,382	699,339	52.7 (44.2–65.3)	10.5 (4.1–12.0)	3	87.9 (64.8–110.5)	35.7 (23.6–48.8)	29.3 (20.3–39.6)	14.9 (8.5–23.5)
Italy	44,528	500,305	50.5 (37.8–63.2)	11.6 (9.1–14.2)	29	86.9 (64.8–111.1)	30.1 (20.3–41.2)	40.6 (28.2–55.4)	11.1 (7.5–17.1)
Spain	39,995	482,550	49.2 (36.8–62.9)	12.3 (9.5–14.5)	9	84.0 (56.1–118.2)	26.0 (15.0–39.5)	37.3 (21.2–57.9)	13.7 (6.8–27.4)
UK General population	29,503	338,387	57.6 (43.6–73.4)	12.0 (10.1–14.6)	17	77.6 (55.4–99.6)	30.6 (19.5–43.8)	26.1 (17.9–35.1)	14.9 (8.8–23.4)
UK Health conscious	45,880	499,938	43.9 (23.8–70.7)	11.1 (9.2–13.4)	1	70.8 (47.6–93.8)	26.4 (14.9–39.3)	23.3 (14.8–32.1)	15.5 (8.9–25.0)
The Netherlands	36,501	431,230	49.0 (25.6–66.2)	12.2 (10.1–14.6)	4	78.6 (55.1–110.5)	31.7 (21.5–45.0)	24.0 (15.8–35.2)	14.6 (8.2–23.3)
Greece	26,018	247,634	53.1 (33.0–72.4)	9.7 (3.6–13.5)	16	99.8 (79.9–123.5)	28.5 (19.5–39.0)	50.2 (31.2–67.8)	14.3 (8.1–30.9)
Germany	48,569	480,509	50.6 (36.7–63.6)	10.2 (5.5–12.7)	37	80.3 (57.0–107.7)	33.2 (22.2–46.4)	27.8 (19.0–38.3)	13.6 (8.2–21.7)
Sweden	48,672	638,847	52.0 (30.2–68.8)	13.8 (7.6–16.8)	29	81.3 (49.9–119.7)	35.0 (20.8–52.8)	28.4 (17.0–42.5)	11.9 (6.5–20.1)
Denmark	54,989	601,278	56.7 (50.7–64.2)	11.4 (7.6–13.2)	44	82.6 (56.7–109.7)	33.3 (21.2–46.2)	28.6 (18.5–40.0)	12.6 (7.8–19.0)
Norway	35,169	342,279	48.1 (41.6–54.9)	10.0 (10.0–10.1)	2	61.0 (46.6–75.7)	23.8 (17.0–31.6)	19.9 (14.7–25.6)	10.9 (7.3–16.0)
Total	477,206[2]	5,262,298	51.2 (33.4–66.3)	11.4 (6.9–14.8)	191	81.1 (53.6–111.5)	31.0 (18.7–45.6)	30.0 (17.2–50.4)	13.5 (7.6–22.7)

EPIC, European Prospective Investigation into Cancer and Nutrition; PY, person-years; HCC, hepatocellular carcinoma; yrs, years.
[1]All dietary variables were adjusted for non-alcohol energy by the residual method and rescaled by adding the center and sex-specific mean intake. Total fat included saturated, monounsaturated, polyunsaturated fats plus the glycerol moiety.
[2]Exclusions: 23,818 with prevalent cancer other than non-melanoma skin cancer, 4,380 with incomplete follow-up data/missing information on date of diagnosis, 6,252 with missing dietary/lifestyle information, 9,596 in top or bottom 1% of the ratio of total energy intake/estimated energy requirement, and 78 with metastasis in the liver or ineligible histology code.

Table 2. Selected baseline demographic and lifestyle characteristics of cancer cases and non-cases, the EPIC cohort study, 1992–2010

Baseline characteristics	Hepatocellular carcinoma (*N* = 191)	Non-cases (*N* = 476,713)	Total fat			
			Q1 (*N* = 119,302)	Q2 (*N* = 119,301)	Q3 (*N* = 119,302)	Q4 (*N* = 119,301)
Women (*N*, %)	64 (33.5)	334,768 (70.2)	83,753 (70.2)	83,753 (70.2)	83,753 (70.2)	83,753 (70.2)
Age at recruitment (y)	59.6 (6.9)	51.2 (9.9)	50.6 (10.3)	51.0 (10.3)	51.5 (9.9)	51.8 (9.2)
Smoking status (*N*, %)						
Never smoker	55 (28.8)	233,103 (48.9)	59,433 (49.8)	57,853 (48.5)	57,610 (48.3)	58,407 (49.0)
Former smoker	74 (38.7)	106,919 (22.4)	34,978 (29.3)	33,118 (27.8)	30,842 (25.9)	28,171 (23.6)
Current smoker	60 (31.4)	126,967 (26.6)	22,348 (18.7)	26,511 (22.2)	28,887 (24.2)	29,317 (24.6)
No. with diabetes (*N*, %)[1]	22 (11.5)	12,496 (2.6)	2,780 (2.3)	2,690 (2.3)	2,820 (2.4)	4,228 (3.5)
BMI (kg/m^2)	28.0 (4.8)	25.4 (4.3)	25.3 (4.1)	25.4 (4.2)	25.4 (4.3)	25.6 (4.5)
Total physical activity (*N*, %)[2]						
Inactive	18 (9.4)	71,709 (15)	12,647 (10.6)	17,608 (14.8)	20,266 (17.0)	21,243 (17.8)
Moderately inactive	68 (35.6)	142,918 (30)	24,053 (20.2)	32,431 (27.2)	39,877 (33.4)	46,721 (39.2)
Moderately active	78 (40.8)	156,660 (32.9)	33,512 (28.1)	40,817 (34.2)	42,436 (35.6)	40,093 (33.6)
Active	18 (9.4)	39,198 (8.2)	9,111 (7.6)	10,980 (9.2)	10,395 (8.7)	8,757 (7.3)
Lifetime pattern of alcohol intake (*N*, %)[3]						
Never drinkers	8 (4.2)	28,136 (5.9)	5,696 (4.8)	6,072 (5.1)	7,096 (6.0)	9,295 (7.8)
Former light drinkers	12 (6.3)	15,030 (3.2)	3,719 (3.1)	3,894 (3.3)	3,716 (3.1)	3,728 (3.1)
Former heavy drinkers	10 (5.2)	1,979 (0.4)	496 (0.4)	462 (0.4)	506 (0.4)	530 (0.4)
Light drinkers	23 (12.0)	87,806 (18.4)	18,693 (15.7)	21,951 (18.4)	22,791 (19.1)	24,443 (20.5)
Never heavy drinkers	63 (33.0)	184,436 (38.7)	37,931 (31.8)	48,323 (40.5)	50,962 (42.7)	47,402 (39.7)
Periodically heavy drinkers	32 (16.8)	42,408 (8.9)	7,744 (6.5)	10,105 (8.5)	11,644 (9.8)	1,2973 (10.9)
Always heavy drinkers	6 (3.1)	2968 (0.6)	510 (0.4)	598 (0.5)	778 (0.7)	1,091 (0.9)
Daily dietary intake (mean, SD)[4]						
Total non-alcohol energy (kcal)	2,035 (647)	1,991 (590)	1,862 (562)	1,974 (578)	2,045 (591)	2,081 (605)
Total energy (kcal)	2,180 (689)	2,074 (619)	1,926 (588)	2,055 (604)	2,137 (617)	2,178 (636)
Total meat (g)	120.8 (57.8)	99.4 (56.5)	77.2 (48.4)	95.9 (53.1)	108.2 (55.8)	116.5 (60.0)
Red meat (g)	56.3 (40.8)	44.2 (37.3)	29.6 (28.8)	41.0 (34.6)	48.8 (37.6)	52.9 (38.1)
Processed meat (g)	37.9 (31.6)	32.0 (29.2)	26.8 (23.8)	31.2 (28.0)	33.5 (30.9)	35.1 (35.1)
Poultry (g)	20.3 (21.7)	19.7 (20.9)	16.7 (19.7)	18.3 (19.0)	19.7 (18.9)	21.6 (19.8)
Total fish and shellfish (g)	32.3 (24.9)	37.8 (35.6)	42.1 (42.5)	37.1 (36.2)	35.1 (31.1)	37.0 (31.0)
Total fat (g)	87.4 (19.6)	81.1 (17.8)	61.3 (10.1)	75.4 (8.7)	86.0 (9.0)	101.6 (11.9)

Table 2. Selected baseline demographic and lifestyle characteristics of cancer cases and non-cases, the EPIC cohort study, 1992–2010 (Continued)

			Total fat			
Baseline characteristics	Hepatocellular carcinoma (*N* = 191)	Non-cases (*N* = 476,713)	Q1 (*N* = 119,302)	Q2 (*N* = 119,301)	Q3 (*N* = 119,302)	Q4 (*N* = 119,301)
Saturated fat (g)	34.1 (9.1)	31.0 (8.4)	23.6 (5.3)	29.3 (5.5)	33.2 (6.2)	37.9 (8.4)
Monounsaturated fat (g)	33.0 (10.5)	30.0 (10.3)	21.2 (5.2)	26.8 (6.0)	31.8 (7.3)	40.0 (10.7)
Polyunsaturated fat (g)	13.5 (5.3)	13.5 (4.9)	11.0 (3.5)	12.9 (4.0)	14.0 (4.5)	15.9 (5.9)
Coffee (ml)	368.9 (394.2)	384.6 (368.0)	398.4 (345.5)	415.3 (375.2)	396.3 (389.8)	328.4 (353.9)
Total dietary fiber (g)	22.1 (6.0)	22.9 (6.1)	25.0 (6.9)	23.0 (5.8)	22.3 (5.3)	21.4 (5.4)
Alcohol (g)	20.3 (31.6)	11.6 (16.8)	8.8 (15.6)	11.3 (16.4)	12.9 (17.1)	13.4 (17.8)

EPIC, European Prospective Investigation into Cancer and Nutrition; BMI, body mass index; SD, standard deviation; Q, quartile. Missing values were not excluded from percentage calculations; therefore the sum of percent across subgroups may not add up to 100%. The number of non-cases includes only cohort subjects without liver cancer. Categorical variables are presented as numbers and percentages, and continuous variables are presented as mean and standard deviations.
[1]Self-reported data at recruitment. Number of participants with missing data on diabetes status: HCC = 17, non-cases = 39,143.
[2]Total physical activity categories were sex-specific.
[3]No information on past alcohol consumption (defined in the Supporting Information) was available for the following EPIC centres: Naples, Bilthoven, Umeå, Malmö, and Norway.
[4]All dietary variables were adjusted for non-alcohol energy by residual method, except for energy, coffee and alcohol intake.

(HR = 0.86, 95% CI: 0.71–1.05), although not statistically significant.

The results for the P:S ratio were statistically non-significant. There were significant inverse associations between the ratios of M:S and (P+M):S with HCC risk (per 0.2 higher M:S ratio, HR = 0.83, 95% CI: 0.73–0.94; and per 0.2 higher [P+M]:S ratio, HR = 0.89, 95% CI: 0.81–0.97). After calibration, the multivariable association between (P+M):S and HCC risk became statistically non-significant (HR = 0.86, 95% CI: 0.73–1.01) (Table 3).

By food source of total dietary fat

When we examined specific fat sources (Table 4), total fat from animal sources was statistically significantly positively associated with HCC risk (for high *vs.* low quartile, crude HR = 1.88, 95% CI: 1.14–3.12) in crude models including mutual adjustment for fat intake from other sources, but the association was attenuated after multivariable adjustment for main confounders (for high *vs.* low quartile, multivariable HR = 1.45, 95% CI: 0.85–2.47). Total fat from plant sources was not associated with HCC risk (for high *vs.* low quartile, multivariable HR = 0.71, 95% CI: 0.35–1.44; per 5 g/day, multivariable HR = 0.89, 95% CI: 0.82–0.97). No statistically significant associations were observed for total fat intake from red/processed meats, fish or added fats and oils.

Effect modifications and sensitivity analyses

The results did not change substantially after excluding persons with self-reported type 2 diabetes at baseline (cases = 22, non-cases = 12,496), or after exclusion of the first two years of follow-up (data not shown). We did not observe any statistically significant interactions of the dietary exposures and HCC risk by sex, BMI, diabetes, physical activity, smoking status, baseline alcohol intake and dietary fiber consumption (p for interaction > 0.05).

Nested case–control study

A nested case–control component was based on a sub-sample of cases with available biosample and identified in the EPIC cohort before 2006. Cancer cases were diagnosed, on average, 4.95 years (standard deviation = 2.91) after blood collection. Thirty-eight (31.2%) HCC cases and 10 (4.1%) controls were hepatitis B and/or C positive. More than 70% of HCC cases had at least one abnormal liver function test (Supporting Information Table 1). The findings among HBV/HCV-free participants were in line with those observed in the whole cohort, but were not statistically significant because of the small sample size (per 10 g/day of total fat, multivariable OR = 0.74, 95% CI: 0.53–1.03; per 5 g/day, saturated fat, OR = 0.90, 95% CI: 0.65–1.26; monounsaturated fat, OR = 0.80, 95% CI: 0.58–1.08; polyunsaturated fat, OR = 1.00, 95% CI: 0.60–1.66; per 0.2, M:S ratio, OR = 0.83, 95% CI: 0.59–1.17; P:S ratio, OR = 1.16, 95% CI: 0.74–1.83; (P+M):S ratio, OR = 0.95, 95% CI: 0.75–1.20) (Supporting Information Table 2).

Table 3. Hazard ratios and 95% confidence intervals for hepatocellular carcinoma (HCC) by sex-specific quartiles of energy-adjusted[1] dietary intake of total fat and its subtypes, EPIC cohort study, 1992–2010

Dietary intake of fat, fat subtypes and their ratios	HR (95% CI)				P_{trend}	Continuous HR (95%CI)[2]	Calibrated continuous HR (95% CI)[3]
	Q1	Q2	Q3	Q4			
Total fat							
Median for men/women, g/day	74.5/58.0	87.9/70.1	98.8/80.4	112.6/94.1			
Cases/PYs	49/1,315,146	43/1,320,806	45/1,325,602	54/1,300,742			
Crude HR[4]	1.00 (ref.)	0.82 (0.53–1.26)	0.80 (0.51–1.25)	0.97 (0.60–1.57)	0.885	0.95 (0.85–1.06)	0.86 (0.70–1.07)
Multivariable HR[5]	1.00 (ref.)	0.73 (0.47–1.13)	0.69 (0.44–1.10)	0.74 (0.45–1.23)	0.248	0.88 (0.78–0.98)	0.80 (0.65–0.99)
Saturated fat							
Median for men/women, g/day	25.7/21.0	32.2/26.3	37.4/30.9	44.7/38.0			
Cases/PYs	42/1,308,151	42/1,312,227	56/1,321,948	51/1,319,969			
Crude HR[4]	1.00 (ref.)	1.09 (0.70–1.70)	1.53 (0.96–2.44)	1.45 (0.84–2.49)	0.320	1.12 (1.00–1.25)	1.29 (1.05–1.60)
Multivariable HR[5]	1.00 (ref.)	1.02 (0.64–1.61)	1.39 (0.86–2.24)	1.19 (0.67–2.09)	0.878	1.05 (0.93–1.19)	1.08 (0.88–1.34)
Monounsaturated fat							
Median for men/women, g/day	25.3/18.8	31.0/23.5	36.5/28.2	49.1/37.6			
Cases/PYs	50/1,313,921	39/1,317,516	50/1,319,313	52/1,311,546			
Crude HR[4]	1.00 (ref.)	0.60 (0.37–0.97)	0.62 (0.36–1.06)	0.62 (0.32–1.20)	0.288	0.85 (0.75–0.97)	0.63 (0.46–0.85)
Multivariable HR[5]	1.00 (ref.)	0.57 (0.35–0.92)	0.56 (0.33–0.96)	0.53 (0.27–1.01)	0.128	0.84 (0.74–0.95)	0.71 (0.55–0.92)
Polyunsaturated fat							
Median for men/women, g/day	10.1/8.4	12.5/10.6	15.5/13.2	20.9/17.8			
Cases/PYs	65/1,330,337	48/1,306,354	37/1,315,047	41/1,310,558			
Crude HR[4]	1.00 (ref.)	0.78 (0.53–1.16)	0.62 (0.40–0.97)	0.83 (0.53–1.30)	0.279	0.91 (0.77–1.07)	0.88 (0.62–1.25)
Multivariable HR[5]	1.00 (ref.)	0.81 (0.54–1.20)	0.66 (0.42–1.03)	0.86 (0.55–1.35)	0.349	0.90 (0.77–1.05)	0.92 (0.68–1.25)
P:S ratio							
Median for men/women	0.3/0.3	0.4/0.4	0.5/0.5	0.7/0.7			
Cases/PYs	68/1,335,520	50/1,316,714	33/1,305,863	40/1,304,197			
Crude HR[4]	1.00 (ref.)	0.72 (0.50–1.05)	0.51 (0.33–0.78)	0.71 (0.47–1.08)	0.092	0.91 (0.77–1.08)	0.88 (0.65–1.20)
Multivariable HR[5]	1.00 (ref.)	0.78 (0.53–1.13)	0.57 (0.37–0.89)	0.79 (0.51–1.23)	0.267	0.95 (0.80–1.12)	0.95 (0.72–1.26)
M:S ratio							
Median for men/women	0.7/0.7	0.9/0.8	1.0/0.9	1.6/1.4			
Cases/PYs	46/1,330,459	47/1,314,056	53/1,303,273	45/1,314,509			
Crude HR[4]	1.00 (ref.)	0.87 (0.58–1.32)	0.92 (0.61–1.40)	0.51 (0.26–1.01)	0.034	0.81 (0.72–0.92)	0.68 (0.54–0.86)
Multivariable HR[5]	1.00 (ref.)	0.91 (0.60–1.37)	0.93 (0.61–1.42)	0.57 (0.28–1.13)	0.070	0.83 (0.73–0.94)	0.79 (0.63–0.99)

Table 3. Hazard ratios and 95% confidence intervals for hepatocellular carcinoma (HCC) by sex-specific quartiles of energy-adjusted dietary intake of total fat and its subtypes, EPIC cohort study, 1992–2010 (Continued)

Dietary intake of fat, fat subtypes and their ratios	HR (95% CI)				P_{trend}	Continuous HR (95%CI)[2]	Calibrated continuous HR (95% CI)[3]
	Q1	Q2	Q3	Q4			
(P+M):S ratio							
Median for men/women	1.04/1.02	1.27/1.23	1.53/1.46	2.18/1.95			
Cases/PYs	55/1,337,038	48/1,315,381	43/1,301,161	45/1,308,718			
Crude HR[4]	1.00 (ref.)	0.82 (0.55–1.22)	0.72 (0.47–1.10)	0.68 (0.38–1.21)	0.104	0.87 (0.80–0.95)	0.79 (0.67–0.93)
Multivariable HR[5]	1.00 (ref.)	0.88 (0.59–1.31)	0.79 (0.51–1.23)	0.80 (0.44–1.45)	0.293	0.89 (0.81–0.97)	0.86 (0.73–1.01)

EPIC, European Prospective Investigation into Cancer and Nutrition; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval.
[1]All dietary variables were energy-adjusted by residual method.
[2]Risk estimates are per 10 g/day of total fat, 5 g/day for monounsaturated, polyunsaturated and saturated fat, and 0.2 for fat subtype ratios.
[3]Calibrated data were obtained by linear regression models that compared observed nutrient questionnaire measurements with 24-hr dietary recall.
[4]Stratified by age (1-yearearear intervals), sex and center, and adjusted for baseline alcohol intake (g/day; continuous) and non-alcohol total energy intake (kcal/day; continuous).
[5]Additionally adjusted for sex-specific physical activity level (inactive, moderately inactive, moderately active, active, and missing), body mass index (kg/m^2; continuous), smoking status (never, former, current, unknown), lifetime alcohol intake pattern (never drinkers, former light drinker, former heavy drinkers, light drinkers, never heavy drinkers, periodically heavy drinkers, always heavy drinkers, unknown), coffee intake (ml/day; continuous), and intake of dietary fiber (g/day). Fat subtype intakes are mutually adjusted.

Discussion

These findings from a large multicenter prospective cohort study point toward an inverse association between total dietary fat and HCC risk. However, analyses by subgroup of total fat indicate that the observation was mainly driven by strong inverse associations with monounsaturated fats. In addition, no significant association was observed for total dietary fat from either plant or animal sources with HCC risk. Calibration of dietary fat intakes to account for potential measurement error somewhat strengthened the results for total fat and fat subtypes. In a nested case–control subset, restriction of analyses to participants without HBV/HCV infections, or adjustment for liver function parameters, did not appreciably alter the observations.

Few previous studies have investigated the role of dietary fat in liver carcinogenesis, with most evidence coming from three case–control studies[13–15] conducted in Europe, and one prospective cohort from the US.[12] The only prospective evidence to date is limited to the NIH-AARP Diet and Health Study based on an elderly American population, which showed, in contrast to our findings, a strong positive association between saturated fat and liver cancer [hazard ratio $(HR)_{Q5\ vs.\ Q1} = 1.87$, 95% CI: 1.23–2.85], which was attenuated after adjustment for red and white meat ($HR_{Q5\ vs.\ Q1} = 1.51$, 95% CI: 0.96–2.38), indicating that intake of dietary meats explain the observed positive association. Indeed, both saturated and monounsaturated fats from red meat were strongly associated with liver cancer risk in this cohort (per 5% increase in the proportion of total energy, $HR_{saturated} = 1.57$, 95% CI: 1.04–2.37; $HR_{monounsaturated} = 1.47$, 95% CI: 1.05–2.08).[12] This discrepancy could in part be due to differences in predominant dietary sources of these fat subtypes in American *vs.* European populations assessed. For example, the main food sources of monounsaturated fat among US adults are meat and meat products (~22%), whereas in Europe the main food sources are added fats and oils (~35%).[30,31] Furthermore, two studies using the UN Food and Agricultural Organization (FAO) data showed that the US and European populations differ by intakes of fish, a rich source of omega-3 fatty acids, with generally lower intake of fish and higher intake of total vegetable oil in the US compared to Europe, and that overall European countries have healthier dietary patterns, characterized by lower intakes of red and processed meats, and trans and saturated fats.[32,33] We have previously reported that intake of fish is inversely associated with HCC risk in the EPIC cohort[34] ($HR_{Q4\ vs.\ Q1} = 0.63$, 95% CI: 0.39–1.01), whereas in the NIH-AARP study the inverse association was weaker ($HR_{Q5\ vs.\ Q1} = 0.86$, 95% CI: 0.65–1.13).[35] In a hospital-based case–control study from Italy, a strong inverse association was observed with polyunsaturated fat (OR = 0.48, 95%CI: 0.24–0.94 for the highest *vs.* the lowest tertile of intake), and no statistically significant associations were observed with total fat (overall and by source), or with monounsaturated or saturated fats.[13]

Table 4. Hazard ratios and 95% confidence intervals for hepatocellular carcinoma (HCC), by sex-specific quartiles of energy-adjusted intake[1] of total dietary fat from different food sources, EPIC cohort study, 1992–2010

	HR (95% CI)						
Dietary intake of total fat	Q1	Q2	Q3	Q4	P_{trend}	Continuous HR (95% CI)[2]	Calibrated continuous HR (95% CI)[3]
By origin							
Total fat from animals							
Median for men/women, g/day	25.0/20.4	36.3/29.2	45.8/36.8	60.0/48.6			
Cases/PYs	31/1,326,211	43/1,317,330	46/1,315,191	71/1,303,564			
Crude HR[4]	1.00 (ref.)	1.28 (0.79–2.07)	1.32 (0.80–2.18)	1.88 (1.14–3.12)	0.022	1.02 (0.96–1.09)	1.03 (0.91–1.17)
Multivariable HR[5]	1.00 (ref.)	1.18 (0.73–1.92)	1.18 (0.71–1.96)	1.45 (0.85–2.47)	0.294	0.98 (0.92–1.04)	0.93 (0.83–1.04)
Total fat—plant							
Median for men/women, g/day	10.6/9.7	19.4/16.8	31.9/25.3	55.5/40.7			
Cases/PYs	68/1,269,555	41/1,328,293	40/1,346,812	42/1,317,637			
Crude HR[4]	1.00 (ref.)	0.75 (0.49–1.14)	0.92 (0.56–1.51)	0.66 (0.33–1.33)	0.173	0.88 (0.81–0.97)	0.82 (0.64–1.05)
Multivariable HR[5]	1.00 (ref.)	0.82 (0.54–1.24)	1.02 (0.61–1.68)	0.71 (0.35–1.44)	0.252	0.89 (0.82–0.97)	0.81 (0.68–0.97)
By food group							
Total Fat from Red/Processed Meats							
Median for men/women, g/day	6.2/3.7	12.5/8.4	19.5/12.4	30.0/18.6			
Cases/PYs	33/1,312,421	46/1,323,624	60/1,320,406	52/1,305,845			
Crude HR[4]	1.00 (ref.)	1.21 (0.77–1.93)	1.63 (1.00–2.65)	1.31 (0.76–2.26)	0.508	1.03 (0.94–1.14)	1.05 (0.87–1.28)
Multivariable HR[5]	1.00 (ref.)	1.03 (0.65–1.65)	1.25 (0.76–2.07)	0.86 (0.49–1.52)	0.406	0.94 (0.85–1.05)	0.99 (0.85–1.17)
Total Fat from Fish							
Median for men/women, g/day	0.3/0.2	1.0/0.9	2.0/1.8	4.4/4.1			
Cases/PYs	45/1,329,766	43/1,326,783	57/1,313,101	46/1,292,644			
Crude HR[4]	1.00 (ref.)	0.81 (0.53–1.25)	0.96 (0.63–1.48)	0.67 (0.42–1.08)	0.122	0.84 (0.59–1.19)	0.58 (0.26–1.31)
Multivariable HR[5]	1.00 (ref.)	0.84 (0.55–1.29)	1.01 (0.66–1.54)	0.68 (0.42–1.09)	0.113	0.82 (0.59–1.16)	0.77 (0.47–1.28)
Total Fat from Added Fats and Oils							
Median for men/women, g/day	13.1/8.2	22.9/14.4	31.8/21.1	48.3/33.9			

Table 4. Hazard ratios and 95% confidence intervals for hepatocellular carcinoma (HCC), by sex-specific quartiles of energy-adjusted intake of total dietary fat from different food sources, EPIC cohort study, 1992–2010 (Continued)

	HR (95% CI)						
Dietary intake of total fat	Q1	Q2	Q3	Q4	P_{trend}	Continuous HR (95% CI)[2]	Calibrated continuous HR (95% CI)[3]
Cases/PYs	51/1,296,159	42/1,307,976	43/1,337,112	55/1,321,051			
Crude HR[4]	1.00 (ref.)	0.77 (0.51–1.17)	0.77 (0.50–1.20)	0.86 (0.51–1.43)	0.389	0.95 (0.89–1.02)	0.98 (0.85–1.13)
Multivariable HR[5]	1.00 (ref.)	0.73 (0.48–1.11)	0.73 (0.47–1.14)	0.77 (0.46–1.29)	0.223	0.93 (0.87–1.01)	0.93 (0.82–1.05)

EPIC, European Prospective Investigation into Cancer and Nutrition; HR, hazard ratio; CI, confidence interval.
[1]All dietary variables were energy-adjusted by residual method.
[2]Risk estimates for residual adjusted intakes are per 5 g of non-alcohol energy-adjusted intake of fat.
[3]Calibrated data were obtained by linear regression models that compared observed nutrient questionnaire measurements with 24-h dietary recall.
[4]Stratified by age (1-yearearear intervals), sex and center, and adjusted for baseline alcohol intake (g/day; continuous) and non-alcohol total energy intake (kcal/day; continuous).
[5]Additionally adjusted for sex-specific physical activity level (inactive, moderately inactive, moderately active, active, and missing), body mass index (kg/m^2; continuous), smoking status (never, former, current, unknown), self-reported diabetes status (yes, no, unknown), lifetime alcohol intake pattern (never drinkers, former light drinker, former heavy drinkers, light drinkers, never heavy drinkers, periodically heavy drinkers, always heavy drinkers, unknown), coffee intake (ml/d; continuous), and intake of dietary fiber (g/day). Fat intakes from different sources are mutually adjusted (animal fat, vegetable fat, and fat from unknown sources; and fat from processed/red meat, fat from fish, added fats and oils, and other fats).

A case–control study from Greece found a statistically significant positive association of total dietary oils and fats with HCC risk (OR = 1.4, 95% CI: 1.0–1.8 for a quintile increase in average monthly consumption).[14] A more recent hospital-based case–control study also from Greece reported that high consumption of added lipids is associated with a higher risk of developing HCC but this was not statistically significant (OR = 1.14; 95% CI: 0.83–1.55 for a quintile increment in the intake). Also, HCC cases had higher mean daily intakes of saturated and polyunsaturated fats, and a lower intake of monounsaturated fat compared to controls, however the differences were not statistically significant after multivariable adjustment.[15]

The traditional Mediterranean diet characterized by high intake of foods that are rich sources of monounsaturated fat (*e.g.,* olive oil, nuts, fish & shellfish, lean cuts of meat)[36] has been shown to be associated with a significant improvement in health status,[37] and more recently with a lower risk of HCC.[38,39] A pooled study of two hospital-based case–control studies conducted in Italy and Greece found that a high adherence to the traditional Mediterranean diet is associated with lower HCC risk (OR = 0.51, 95% CI: 0.34–0.75).[38] Results from the NIH-AARP cohort also showed that a high alternate Mediterranean Diet Score (aMED) was associated with lower risk of HCC (HR = 0.62; 95% CI: 0.47–0.84), and that both the ratio of (P+M):S (HR = 0.94; 95% CI: 0.91–0.97, comparing $\geq$2.5 *vs.* <2.5), and the ratio of M:S (HR = 0.78; 95% CI: 0.65–0.93, comparing $\geq$ median 1.24 *vs.* <1.24) were associated with lower HCC risk.[39] Our findings are in line with these studies in showing a beneficial effect of higher monounsaturated fat (which is strongly correlated with intake of olive oil in our study, Spearman's correlation coefficient $\rho = 0.40$), M:S and possibly (P+M):S ratios on preventing HCC development.

The exact mechanisms by which different subtypes of fat may contribute to a decreased risk of any cancer, and cancer of the liver in particular, are not well established. Different subtypes of dietary fat may have direct and indirect effects on liver carcinogenesis. For example, polyunsaturated fat was shown to promote hepatic inflammation in mice.[40] Furthermore, diet composition (*e.g.,* high-fat *vs.* low-fat) may affect gut microbiota and subsequent fermentation products that impact the liver.[41] Omega-3 fatty acids, present in fatty fish and fish oil, have been shown to inhibit carcinogenesis through their anti-inflammatory actions, modulation of various transcription factors, alteration of estrogen metabolism and effects on insulin sensitivity and membrane fluidity.[42] Human evidence is limited to a single prospective cohort from Japan showing that high consumption of omega-3 fatty acids is associated with reduced HCC risk.[43] In our study, although an estimate for separate intakes for omega-3 and omega-6 fatty acids is not possible, we have previously shown an inverse HCC risk association with fish consumption, which may be indicative of greater omega-3 monounsaturated fatty acid intake.[34]

Strengths of the present study include its prospective design, which diminishes the potential of differential recall of diet and other important risk factors between cancer cases and non-cases, as well as careful assessment of cancer cases based on tumor morphology, histology and behavior to ensure the inclusion of only first primary tumors. This study was the first to incorporate biomarkers of HBV/HCV infection and liver function into the analysis of a prospective cohort, thus reporting the findings in a hepatitis virus free population. Limitations include the assessment of diet only at baseline, and no data to account for potential dietary changes during follow-up. In addition, it is possible that measurement error from an imprecise dietary measurement instrument may have occurred, but we have used the calibration method to partially address this aspect. Since measurement errors of FFQ and 24-hr recall are likely correlated, the effect estimates observed in our study could possibly underestimate the true associations. Finally, the sample size was relatively small in our nested case–control study, we were unable to observe potentially sex-specific findings due to a low number of women, and no data were available on incidence of type 2 diabetes, prevalence and incidence of cirrhosis and other chronic liver diseases, and on exposure to aflatoxins, although the last is uncommon in Western Europe.[44]

In conclusion, this comprehensive study of a large geographically and culturally heterogeneous cohort of Europeans has shown that a higher intake of monounsaturated fat is associated with a lower risk of HCC among Europeans. No statistically significant associations between polyunsaturated fat and saturated fat intakes and HCC risk were observed.

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Notes

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