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Nutrients intake and the risk of hepatocellular carcinoma in Italy

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ABSTRACT

Although hepatitis C and B viruses and alcohol consumption are the major risk factors for hepatocellular carcinoma (HCC), dietary habits may also be relevant. A hospital-based case-control study was conducted in Italy in 1999–2002, including 185 HCC cases and 412 cancer-free controls. Dietary habits were assessed using a validated food-frequency questionnaire to compute nutrient intakes. Odds ratios (OR) and corresponding confidence intervals (CI) were calculated using the energy-adjusted residual models.

Inverse association emerged for linoleic acid (OR = 0.35 for highest *versus* lowest tertile; 95% CI: 0.18–0.69) and, possibly, β-carotene (OR = 0.48; 95% CI: 0.24–0.93). Among minerals, iron intake was associated with increased HCC risk (OR = 3.00; 95% CI: 1.25–7.23), but the association was considerably reduced when iron from wine was excluded (OR = 1.61; 95% CI: 0.78–3.30).

In conclusion, a diet rich in linoleic acid containing foods (e.g. white meats and fish) and β-carotene was inversely related to HCC risk.

1. Introduction

Hepatitis C (HCV) and hepatitis B virus (HBV) infections and alcohol are the major risk factors recognised for hepatocellular carcinoma (HCC),[1] responsible of approximately 90% of new HCC cases in Europe. Other factors, including diet, may be relevant, but the evidence is still unclear, except for aflatoxin contamination.[2] A number of studies investigated the association of food consumption with HCC risk,[3–5] but those on dietary nutrients were mainly focused on carotenoids, retinoids, and vitamins.[6–8] Only one study from Greece[4] reported null results for a wide range of nutrients, though the sample size was relatively small (97 HCC cases).

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Liver diseases, including HCC, are known complications in patients with hereditary haemochromatosis,[9,10] a disease that causes excessive gastrointestinal absorption of dietary iron. This finding suggested an association between iron intake and HCC risk. However, with the exclusion of a small case-control study,[4] the effect of dietary iron overload on HCC risk in healthy subjects was estimated only among Black Africans,[11] reporting a 3-fold increased risk for elevated iron intake.

To provide further insights into the relationship between HCC risk and several macro- and micronutrients, we analysed data from an Italian case-control study conducted in the context of the Mediterranean diet.

2. Materials and methods

Between January 1999 and July 2002, we conducted a case-control study on HCC and lymphomas in the province of Pordenone, in the Northeast of Italy, and the city of Naples, in the South.[12,13] Cases were patients below 85 years of age with incident HCC, who had not yet received any cancer treatment at study entry. They were admitted at the National Cancer Institute in Aviano, the 'Santa Maria degli Angeli' General Hospital in Pordenone, and the 'Pascale' National Cancer Institute, plus four General Hospitals in Naples. The study originally enrolled 229 HCC cases.[13] However, 44 cases were not questioned on dietary habits,[14] thus leaving 185 cases (median age: 66 years; range: 43–84 years). Histological or cytological confirmation was available from 78% of HCC cases, whereas the remaining HCC cases were diagnosed by ultrasound and/or tomography (12%), and elevated alpha-fetoprotein (9%).

The comparison group included patients aged 40–82 years (median age: 65 years) admitted for a wide spectrum of acute conditions to the same hospitals where HCC cases had been interviewed. Specifically excluded from the control group were patients whose hospital admission was due to diseases related to alcohol and tobacco use (e.g. respiratory diseases, peptic ulcer, lung cancer, head and neck cancer, etc.), hepatitis viruses (e.g. hepatitis, cirrhosis, oesophageal varices, etc.), or chronic diseases that might have resulted in substantial lifestyle modifications (e.g. diabetes, cardio- and cerebro-vascular diseases, etc.). However, co-morbidity for such diseases was not an exclusion criterion. Among 431 subjects included in the control group,[13] only 412 provided comprehensive information on dietary habits; of these, 27% were admitted to the hospital for trauma, 25% for acute surgical conditions, 24% for non-traumatic orthopaedic diseases, 13% for eye diseases, and 11% for other illnesses. Controls were more often females and were younger than HCC cases, as matching was conducted according to the distribution by age and gender of cancer cases in the entire study, which also included lymphomas.[12]

All study participants signed an informed consent form, in agreement with the requirements of the Ethical Committee of the Aviano National Cancer Institute approving the study.

Each case and each control provided a 15-ml blood sample the day the interview took place. Sera were screened for antibodies against HCV (AntiHCV) by means of a third-generation MEIA (AxSYM HCV, version 3.0; Abbott, Wiesbaden, Germany) and for HBV surface antigen (HBsAg) by microparticle enzyme immunoassay (AxSYM HBsAg version 2.0, Abbott Diagnostic Division, Wiesbaden, Germany).[13]

Trained interviewers administered a structured questionnaire to cases and controls during their hospital stay. The questionnaire included information on socio-demographic indicators, tobacco smoking, alcohol drinking, dietary habits, behaviours, and exposures that entailed risk of HCV transmission.

A validated food frequency-questionnaire (FFQ) was employed to assess the subjects' habitual diet during the 2 years before cancer diagnosis or hospital admission for controls. Briefly, the FFQ included 63 foods, food groups or recipes divided into seven sections: (i) milk, hot beverages and sweeteners; (ii) bread, cereals and first courses; (iii) second courses (e.g. meat and other main dishes); (iv) side dishes (i.e. vegetables); (v) fruits; (vi) sweets, desserts and soft drinks; (vii) alcoholic beverages. For vegetables and fruit subject to seasonal variation, consumption in season, and the corresponding duration, was elicited. Serving size was defined in 'natural' units (e.g. 1 teaspoon of sugar, 1 egg) or as an average in the Italian diet. Drinkers were also asked to report any change in alcohol beverage intake, in order to compute the maximal lifetime alcohol intake.[13] The reproducibility and validity of the FFQ were satisfactory.[15,16] Daily intake of energy, macro- and micronutrients were computed using the Italian Food Composition database.[17] In the present study, vitamin supplementation was 6% among cases ($n = 11$) and 2% among controls ($n = 7$), so that it was not considered in the analysis.

Odds ratios (OR), and their corresponding 95% confidence intervals (CI), for increasing levels of nutrient intake compared to the lowest one, were computed using unconditional multiple logistic regression models.[18] The multivariate models included the nutrient under investigation and the following variables as adjustments: age in 5-year categories, study centre, education (<7, 7–11 and 12+ years), seropositivity for HCV and HBV (HBsAg+ and/or AntiHCV+ *versus* HBsAg– and AntiHCV–), drinking habits, maximal lifetime alcohol intake ($\geqslant$21 *versus* <21 drinks/week), and non-alcohol energy intake. Adjustment for energy was made using the residual model;[19] nutrients were entered in the model as tertiles of intake based on the distribution of cases and controls combined. The test for trend was based on the likelihood-ratio test between the models with and without a linear term for each nutrient's tertile.[18]

In order to rule out chance associations due to multiple testing, associations between nutrients and NHL risk were taken into consideration when significance was found for the OR of the highest versus the lowest tertile, the p-value for trend, and the OR for the continuous term.

3. Results

Table 1 shows the distribution of HCC cases and controls according to gender, age, study centre, and other selected risk factors for HCC. Drinking cessation was reported by 51% of cases and 10% of controls, and HCC risk was related to maximal lifetime alcohol intake (OR = 2.38, 95% CI: 1.13–5.01 for $\geqslant$21 drinks/week *versus* abstainers). Nearly 80% of cases and 11% of controls had HBV and/or HCV active infection (coinfec-

Table 1 – Distribution of 185 cases of hepatocellular carcinoma (HCC) and 412 controls, odds ratio (OR) and corresponding 95% confidence intervals (CI)[a] for selected socio-demographic factors, hepatitis viruses infection and total energy, Italy. 1999–2002

	Cases		Controls		OR (95% CI)
	N.	(%)	N.	(%)	
Gender					
Males	149	(80.5)	281	(68.2)	
Females	36	(19.5)	131	(31.8)	
Age (years)					
<54	18	(9.7)	85	(20.6)	
55–64	56	(30.3)	116	(28.2)	
65–74	84	(45.4)	147	(35.7)	
⩾75	27	(14.6)	64	(15.5)	
Centre					
Aviano/Pordenone	61	(33.0)	230	(55.8)	
Naples	124	(67.0)	182	(44.2)	
Place of birth					
North-Centre	52	(28.1)	206	(50.0)	1[d]
South	133	(71.9)	206	(50.0)	1.88 (0.88–4.02)
Education (years)					
<7	126	(67.4)	232	(56.3)	1[d]
7–11	45	(24.4)	93	(22.6)	0.90 (0.57–1.43)
⩾12	14	(8.9)	87	(21.1)	0.30 (0.16–0.58)
χ^2_1 trend					11.29; $p< 0.01$
Drinking habits					
Abstainers	16	(8.7)	63	(15.3)	1[d]
Current	75	(40.5)	307	(74.5)	0.62 (0.31–1.24)
Former	94	(50.8)	42	(10.2)	5.42 (2.59–11.36)
Maximal lifetime alcohol intake (drinks/week)[b]					
<21	64	(34.6)	192	(46.6)	0.97 (0.49–1.91)
⩾21	105	(56.8)	157	(38.1)	2.38 (1.13–5.01)
χ^2_1 trend					10.50; $p<0.01$
Hepatitis viruses					
HBsAg– and AntiHCV–	38	(20.5)	365	(88.6)	1[d]
HBsAg+ and/or AntiHCV+	147	(79.5)	47	(11.4)	30.10 (17.82–50.85)
Total energy intake (kCal/day)[c]					
<1705	32	(17.3)	118	(28.6)	1[d]
1705–<2041	42	(22.7)	107	(26.0)	1.47 (0.83–2.60)
2041–<2389	48	(26.0)	101	(24.5)	1.71 (0.98–2.98)
⩾2389	63	(34.1)	86	(20.9)	2.78 (1.59–4.86)
χ^2_1 trend					13.04; $p < 0.01$

a Estimated from unconditional multiple logistic regression adjusted for gender, age, centre, education, and place of birth when appropriate.
b Former and current drinkers combined.
c Energy from alcohol excluded.
d Reference category.

tion was found in nine cases and two controls), showing a 30-fold increased risk compared to subjects negative to both markers. Total energy intake was associated with increased HCC risk (OR = 2.78, 95% CI: 1.59–4.86 for ⩾2389 *versus* <1705 kCal/day).

The mean daily intake among controls of selected macronutrients, fatty acids, fibre, and cholesterol are reported in Table 2, together with the ORs of HCC according to tertile of intake, and for an increment of intake equal to 1 standard deviation (SD) among controls. Proteins, fat, carbohydrates, saturated fatty acids, and monounsaturated fatty acids were not significantly related to NHL risk. Among specific carbohydrates, sugar (i.e., soluble glucides), but not starch, seemed associated with a decreased HCC risk (OR = 0.46 in the highest *versus* the lowest tertile of intake; 95% CI: 0.23–0.90; p for trend = 0.02). However, no relation emerged when the analysis was restricted to non-added sugars (OR = 1.00 in the highest *versus* the lowest tertile of intake; 95% CI: 0.51–1.86; data not shown). An inverse association was also reported for polyunsaturated fatty acids (OR = 0.48; 95% CI: 0.24–0.94), whose effect was largely due to linoleic acid (OR = 0.35; 95% CI: 0.18–0.69).

Among the examined minerals (Table 3), iron was associated with a 3-fold increased HCC risk (OR = 3.00; 95% CI: 1.25–7.23). However, the resulting risk attenuated (OR = 1.61; 95% CI: 0.78–3.30) when iron from wine was excluded from

Table 2 – Odds ratios (OR) and corresponding 95% confidence intervals (CI)[a] according to intake of selected macronutrients, fatty acids, fibre, and cholesterol among 185 cases of hepatocellular carcinoma (HCC) and 412 controls. Italy, 1999–2002

Nutrient	Mean[b]	(SD)	Tertile, OR (95% CI)			χ^2 for trend	OR (Continuous)[d]
			1[c]	2	3		
Proteins (g)	83.1	(20.8)	1	0.94 (0.50–1.79)	0.91 (0.46–1.81)	$p = 0.79$	0.77 (0.45–1.34)
Animal sources (g)	51.3	(16.7)	1	1.20 (0.63–2.29)	1.51 (0.73–3.09)	$p = 0.27$	0.81 (0.56–1.18)
Vegetal sources (g)	31.7	(9.0)	1	0.63 (0.31–1.28)	1.20 (0.59–2.43)	$p = 0.53$	1.24 (0.75–2.05)
Fat (g)	73.2	(20.0)	1	1.85 (0.95–3.58)	0.93 (0.46–1.86)	$p = 0.90$	0.92 (0.59–1.43)
Animal sources (g)	37.2	(15.4)	1	1.45 (0.73–2.86)	1.18 (0.60–2.35)	$p = 0.64$	0.98 (0.68–1.41)
Vegetal sources (g)	36.0	(10.7)	1	1.65 (0.87–3.16)	1.19 (0.61–2.33)	$p = 0.57$	0.94 (0.68–1.30)
Carbohydrates (g)	272.7	(80.5)	1	1.17 (0.60–2.31)	0.84 (0.42–1.71)	$p = 0.62$	1.19 (0.65–2.18)
Sugar (g)	87.4	(31.3)	1	0.57 (0.30–1.09)	0.46 (0.23–0.90)	$p = 0.02$	0.81 (0.58–1.12)
Starch (g)	185.2	(63.7)	1	1.58 (0.78–3.19)	1.70 (0.81–3.54)	$p = 0.17$	1.38 (0.87–2.21)
Saturated fatty acids (g)	23.7	(8.6)	1	1.13 (0.58–2.21)	1.09 (0.55–2.16)	$p = 0.79$	0.98 (0.67–1.44)
Monounsaturated fatty acids (g)	33.9	(9.5)	1	1.41 (0.75–2.64)	1.21 (0.61–2.39)	$p = 0.55$	1.21 (0.83–1.77)
Oleic acid (g)	31.9	(9.1)	1	1.30 (0.69–2.45)	1.28 (0.65–2.52)	$p = 0.47$	1.21 (0.83–1.75)
Polyunsaturated fatty acids (g)	11.4	(4.3)	1	1.78 (0.94–3.37)	0.48 (0.24–0.94)	$p = 0.05$	0.60 (0.40–0.88)
Linoleic acid (g)	9.6	(3.8)	1	1.18 (0.63–2.20)	0.35 (0.18–0.69)	$p < 0.01$	0.58 (0.39–0.86)
Linolenic acid (g)	1.3	(0.5)	1	1.09 (0.58–2.04)	0.70 (0.37–1.34)	$p = 0.32$	0.75 (0.52–1.09)
Other (g)	0.5	(0.2)	1	0.64 (0.33–1.24)	1.00 (0.52–1.93)	$p = 0.97$	1.06 (0.77–1.44)
Fibre (g)	22.9	(7.0)	1	0.63 (0.31–1.27)	0.78 (0.37–1.65)	$p = 0.57$	0.90 (0.61–1.32)
Cholesterol (mg)	294.8	(121.2)	1	0.81 (0.41–1.57)	0.69 (0.36–1.33)	$p = 0.27$	0.83 (0.57–1.21)

a Estimated from unconditional multiple logistic regression adjusted for gender, age, centre, education, place of birth, hepatitis viruses (HBsAg+ and/or AntiHCV+ *versus* HBsAg– and AntiHCV–), drinking habits (Abstainers, Former, Current), maximal lifetime alcohol intake ($\geqslant 21$ *versus* <21 drinks/week), and energy intake (kCal, energy from alcohol excluded).
b Mean and standard deviation (SD) among controls, per day.
c Reference category.
d OR relative to an increase of 1 standard deviation among controls.

the analysis. None of the other considered vitamins, except thiamine (OR = 2.19; 95% CI: 1.11–4.34), were associated to HCC risk, as well as glutathione (Table 3). Among carotenoids, only β-carotene showed a possible inverse relation with HCC risk (OR = 0.48; 95% CI: 0.24–0.93).

Nutrients resulting in association with HCC risk were further examined in separate strata of hepatitis virus infection (Table 4). No modifying effect of HBV and HCV infection emerged for both polyunsaturated fatty acids and linoleic acid. Conversely, the detrimental effect of high iron intakes seemed restricted to subjects negative to both HBsAg and AntiHCV tests (OR = 3.76; 95% CI: 1.29–10.94).

4. Discussion

The present study reports a direct association between iron intake and HCC risk, notably among individuals who were not infected with HBV and HCV. Although this relationship was weakened when the contribution of wine to total iron load was excluded, the association was biologically plausible. Our finding is also supported by a recent analysis of the present data focusing on food groups,[14] which reported increased HCC risk for elevated consumption of high-iron containing food (i.e., bread, red meats).

Iron is essential in cell proliferation and growth, but excesses may be deleterious. Liver is the major recipient of excessive iron; therefore, hepatotoxicity is the most common finding in patients with iron overload. Excessive intake of dietary iron has been associated to a 3-fold increased HCC risk in Black Africans,[11] where bioavailable iron is mainly ingested through homemade beverages, but not in Europeans.[4] Likewise, patients with hereditary haemochromatosis have an excessive gastrointestinal absorption of dietary iron, resulting in iron accumulation in a number of body tissues, including the liver. In these patients, HCC is 20-fold more frequent than in the general population,[10] and liver diseases account for about 50% of deaths.[9]

Several mechanisms link iron overload to HCC carcinogenesis. In human metabolism, reactive oxygen species (ROS) are generated in iron reduction. Therefore, iron overload is responsible for the overproduction of ROS, which results in tissue damage (through lipid peroxidation) and modification of proteins and DNA molecules.[20,21] Secondly, iron may indirectly contribute to liver toxicity by reducing the antioxidant protections[22] and by suppressing host defences,[20] thus inducing liver fibrogenesis and cirrhosis.[22] The presence of other hepatotoxins, such as alcohol or viruses, may lead to an acceleration of liver disease.[22] Finally, iron could promote unrestricted proliferation of tumour cells.[20] However, it is still unclear whether iron overload was independently associated with cancer risk or whether it acts indirectly by inducing chronic hepatic diseases, such as fibrosis and cirrhosis, which are the strongest predisposing factors of HCC development. In the present study, elevated iron intake was reported among 29 cases with cirrhosis (59% above the median value), as well as in two cirrhotic controls.

Table 3 – Odds ratios (OR) and corresponding 95% confidence intervals (CI)[a] according to intake of selected micronutrients and minerals among 185 cases of hepatocellular carcinoma (HCC) and 412 controls. Italy, 1999–2002

	Mean[b]	(SD)	Tertile, OR (95% CI)			χ^2 for trend	OR (Continuous)[d]
			1[c]	2	3		
Minerals							
Calcium (g)	0.8	(0.4)	1	0.64 (0.34–1.24)	1.05 (0.52–2.12)	$p = 0.97$	0.92 (0.64–1.33)
Sodium (g)	2.1	(0.7)	1	0.83 (0.43–1.62)	1.02 (0.52–2.05)	$p = 0.91$	1.06 (0.66–1.70)
Potassium (g)	3.5	(0.8)	1	0.78 (0.40–1.52)	0.91 (0.47–1.78)	$p = 0.80$	1.24 (0.81–1.92)
Phosphorus (g)	1.4	(0.4)	1	1.20 (0.61–2.35)	1.47 (0.70–3.11)	$p = 0.31$	1.18 (0.72–1.93)
Iron (mg)	13.9	(3.7)	1	1.64 (0.78–3.47)	3.00 (1.25–7.23)	$p = 0.01$	1.99 (1.37–2.90)
Zinc (mg)	10.9	(2.9)	1	0.79 (0.40–1.55)	1.64 (0.79–3.40)	$p = 0.21$	1.09 (0.66–1.80)
Water soluble vitamins							
Thiamine (mg)	0.9	(0.2)	1	0.99 (0.50–1.95)	2.19 (1.11–4.34)	$p = 0.02$	1.21 (0.76–1.95)
Riboflavin (mg)	1.5	(0.5)	1	1.49 (0.76–2.93)	1.65 (0.84–3.27)	$p = 0.15$	1.13 (0.77–1.67)
Vitamin C (mg)	135.1	(58.1)	1	0.58 (0.30–1.11)	0.80 (0.42–1.52)	$p = 0.42$	1.11 (0.84–1.47)
Vitamin B6 (mg)	2.1	(0.5)	1	1.43 (0.73–2.77)	1.34 (0.69–2.59)	$p = 0.39$	1.20 (0.78–1.86)
Folate (mg)	300.9	(78.7)	1	0.68 (0.35–1.33)	0.91 (0.49–1.71)	$p = 0.78$	1.15 (0.81–1.64)
Niacin (mg)	19.9	(5.1)	1	0.95 (0.40–1.48)	0.77 (0.40–1.48)	$p = 0.44$	0.79 (0.54–1.15)
Fat soluble vitamins, carotenoids, and glutathione							
Retinol (mg)	0.5	(0.7)	1	0.88 (0.42–1.87)	1.50 (0.78–2.88)	$p = 0.17$	1.26 (1.01–1.58)
Carotene (mg)	3.8	(2.0)	1	0.33 (0.17–0.64)	0.56 (0.29–1.08)	$p = 0.07$	0.89 (0.65–1.23)
α-carotene (mg)	0.8	(0.7)	1	1.03 (0.54–1.97)	0.80 (0.41–1.54)	$p = 0.51$	0.86 (0.62–1.20)
β-carotene (mg)	4.0	(2.0)	1	0.55 (0.29–1.04)	0.48 (0.24–0.93)	$p = 0.03$	0.93 (0.69–1.27)
β-criptoxanthin (mg)	0.2	(0.2)	1	0.69 (0.35–1.34)	1.23 (0.64–2.35)	$p = 0.50$	1.11 (0.84–1.45)
Luthein/Zeaxanthin (mg)	0.3	(0.1)	1	1.96 (1.04–3.71)	0.99 (0.49–1.97)	$p = 0.95$	1.07 (0.77–1.48)
Lycopene (mg)	6.8	(2.9)	1	1.44 (0.72–2.85)	1.95 (0.96–3.95)	$p = 0.06$	1.25 (0.93–1.68)
Vitamin D (μg)	2.8	(1.2)	1	0.78 (0.41–1.49)	0.56 (0.28–1.10)	$p = 0.09$	0.89 (0.65–1.22)
Vitamin E (mg)	11.7	(3.0)	1	0.96 (0.52–1.78)	0.77 (0.40–1.49)	$p = 0.45$	0.87 (0.62–1.23)
Glutathione (mg)	54.6	(13.9)	1	0.85 (0.44–1.64)	1.59 (0.82–3.10)	$p = 0.18$	1.46 (1.08–1.98)
Reduced glutathione (mg)	40.0	(10.2)	1	0.78 (0.41–1.50)	1.35 (0.70–2.61)	$p = 0.38$	1.44 (1.06–1.96)

a Estimated from unconditional multiple logistic regression adjusted for gender, age, centre, education, place of birth, hepatitis viruses (HBsAg+ and/or AntiHCV+ *versus* HBsAg– and AntiHCV–), drinking habits (Abstainers, Former, Current), maximal lifetime alcohol intake (⩾21 *versus* <21 drinks/week), and energy intake (kCal, energy from alcohol excluded).
b Mean and standard deviation (SD) among controls, per day.
c Reference category.
d OR relative to an increase of 1 standard deviation among controls.

Table 4 – Odds ratios (OR) and corresponding 95% confidence intervals (CI)[a] according to selected nutrients and hepatitis viruses infections. Italy, 1999–2002

	HBsAg– and AntiHCV–		HBsAg+ and/or AntiHCV+	
	Ca:Co	OR (95% CI)	Ca:Co	OR (95% CI)
Polyunsaturated fatty acids (g)				
<10.33	20:163	1[b]	95:21	1[b]
⩾10.33	18:202	0.68 (0.32–1.44)	52:26	0.30 (0.12–0.76)
		χ^2 for heterogeneity = 0.90; $p = 0.34$		
Linoleic acid (g)				
<8.60	19:163	1[b]	95:22	1[b]
⩾8.60	19:202	0.76 (0.36–1.62)	52:25	0.31 (0.12–0.79)
		χ^2 for heterogeneity = 1.05; p=0.31		
Iron (mg)				
<13.43	9:165	1[b]	95:30	1[b]
⩾13.43	29:200	3.76 (1.29–10.94)	52:17	0.85 (0.30–2.44)
		χ^2 for heterogeneity = 9.57; $p < 0.01$		

a Estimated from unconditional multiple logistic regression adjusted for gender, age, centre, education, place of birth, drinking habits (Abstainers, Former, Current), maximal lifetime alcohol intake (⩾21 *versus* <21 drinks/week), and energy intake (kCal, energy from alcohol excluded).
b Reference category.

Although the reported association between iron intake and HCC risk was confirmed among subjects negative to both HBsAg and AntiHCV serum markers, the present study failed to identify any relation among subjects with persistent hepatitis infections. However, among the latter, the possible association could be diluted by the elevated baseline risk. Moreover, the estimated association between HCC and iron intake could be partially explained by wine drinking. Indeed, in the Italian diet, wine is the major source of iron[23] and alcohol drinking is directly involved in HCC aetiology.[4,13,14,24] The small number of cases did not allow the estimation of risk excess due to iron among abstainers only, but the risk estimates substantially declined when iron from wine was excluded. Alternatively, iron may be one of the unfavourable components of wine on liver carcinogenesis.

The present study reported a strong inverse association between HCC risk and polyunsaturated fatty acids, in particular linoleic acid. To our knowledge, only a smaller case-control study from Greece[4] investigated the relation between HCC and specific PUFAs, reporting no association. Linoleic acids showed an inhibitory effect on cell proliferation in HCC cell lines.[25] Further, n-3 PUFAs may up-regulate anticancer defence such as natural killer cell cytotoxicity and humoral and T cell responses.[26]

The association between β-carotene and HCC risk is controversial. A case-control study nested in the Shanghai Cohort Study [8] reported decreased HCC risk for subjects with elevated β-carotene concentration in prediagnostic serum, but others did not.[27] Conversely, a meta-analysis on antioxidant supplementation[7] found no favourable effect on HCC risk for β-carotene containing compounds. Likewise, results from a case-control study from Greece did not find any association.[4] Thus, β-carotene may be an indicator of a diet rich in vegetables, which has been inversely related to HCC in studies conducted in Greece[4] and Italy.[3]

An unexpected result from the present study is the inverse association between HCC risk and sugar intake. Indirect evidence form studies on foods reported no association. A case-control study from Greece[4] found no relation between HCC risk and sugars and syrups, nor did a study from Japan[28] investigating the association between cakes and HCC mortality. In the present study, added sugar (i.e., sugar and artificial sweeteners used to sweeten foods and beverages) accounted for more than 25% of sugar intake and they correlated with coffee drinking (ρ-Spearman = 0.43). Coffee has been associated to a reduced HCC risk in several studies[29] including the present one.[30] Thus, the observed inverse association between sugar intake and HCC risk could merely mirror the effect of coffee. The lack of association between non-added sugar and HCC supported this finding.

The number of cases and controls in this study could have affected the study power, and it did not allow finer categorisation of exposure or stratification. Recall bias is possible, since cases may recall their diet differently than healthy controls. However, awareness of any particular dietary hypothesis in HCC aetiology was limited in the Italian population at the time of the study. Moreover, the lack of differences in intake of total energy, alcohol intake, and in the majority of nutrients across different control groups provides reassurance against recall bias, indicating that the associations we found (notably with iron and linoleic acids) might be real.

An additional potential limitation is selection bias of hospital controls, which may differ from the general population in relation to dietary habits. However, by study design, great attention was paid in excluding controls hospitalised for diseases that might have been associated to or had determined special dietary changes. Although these diseases might have been comorbidities at the time of hospitalisation, no bias was found since no heterogeneity emerged when comparing cases to controls with and without these comorbidities. The questionnaire was administered to cases and controls by the same interviewers under similar conditions in a hospital setting, thus minimising information bias. In addition, our findings are strengthened by the nearly complete participation of identified cases and controls and by the use of a detailed and validated FFQ.[15,16]Adjustments for sex, age, centre, education, smoking and drinking habits, and persistent infection with HCV and HBV were made to address potential confounding.

In conclusion, no strong association was observed between proteins, fat, carbohydrates, fibre and HCC risk. The findings of the present study suggest that dietary iron overload is a risk factor for HCC also in the European population. Conversely, a diet rich in polyunsaturated fatty acids and, possibly, β-carotene could reduce HCC risk.

Conflict of interest statement

None declared.

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