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## **FOOD GROUPS AND RISK OF HEPATOCELLULAR CARCINOMA: A MULTICENTER CASE-CONTROL STUDY IN ITALY**

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# Introduction

Hepatocellular carcinoma (HCC) is a frequent malignancy in many regions of the world. Since the last few decades, increasing incidence and mortality have been reported in the United States, Japan, and in several European countries, including Italy. Infection with hepatitis B virus and (HBV) and hepatitis C virus (HCV) and heavy alcohol drinking are the major risk factors for HCC. However, other factors, including diet, may be relevant, but the evidence is still unclear, except for aflatoxin contamination.

Inverse relations were observed with consumption of fish, but other did not confirm this finding<sup>6</sup>.

To provide further insights on the relation between food and risk of HCC, we analysed data derived from a multi-centre Italian case-control study.

# Material and Methods (I)

Data derived from a case-control study conducted in the Pordenone, Northeast of Italy, and in Naples, South of Italy, to assess the association of HBV and HCV with HCC and HCC.

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 to the National Cancer Institute, Aviano, General Hospital, Pordenone, the National Cancer Institute, and four General Hospitals, Naples.

A total of 261 HCC cases were identified. Histological or cytological confirmation was available for 78.2% of HCC cases, and for the remaining ones the diagnosis was based on ultrasound, tomography, and elevated alpha-fetoprotein levels.

# Material and Methods (II)

Controls were patients aged below 85 years admitted, to the same hospitals where HCC cases had been interviewed for a wide spectrum of acute conditions. 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.) or hepatitis viruses (e.g., hepatitis, cirrhosis, oesophageal varices, etc.), were specifically excluded from the control group, as were those hospitalised for any chronic diseases that might have lead to substantial lifestyle modifications (e.g., diabetes, cardio- and cerebro-vascular diseases, etc.).

All study participants signed an informed consent form, according to the recommendations of the Ethical Committee of the National Cancer Institute.

# Material and Methods (III)

The same interview-based, structured questionnaire and coding manual were used in each study centre. Centrally trained and supervised interviewers identified and questioned patients in hospitals.

A validated food frequency questionnaire (FFQ) was employed to assess subjects' habitual diet and to estimate their total energy intake two years before cancer diagnosis or, for the controls, hospital admission.

The FFQ included information on age, education and other socio-demographic characteristics, anthropometrics measures, tobacco smoking, history of selected diseases, lifestyle behaviours, and exposures that entailed risk of HCV transmission.

The dietary questionnaire included 63 foods, food groups or recipes divided into 7 sections: (i) (milk, yoghurt, coffee, tea, sugar and artificial sweeteners; (ii) bread and cereals (first courses); (iii) meat and foods used as meat substitutes (second courses); (iv) vegetables (side dishes); (v) fruits; (vi) sweets, desserts and soft drinks; (vii) alcoholic beverages.

# Material and Methods (IV)

The FFQ allowed estimation of intake of total energy using the Italian food-composition database<sup>31</sup>. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were computed by unconditional multiple logistic regression, including: age (in quinquennia), sex, center, years of education (<7, 7-11, ≥12 years), place of birth (North-Center and South), seropositivity for HCV (HBsAg- and antiHCV- vs. HBsAg+ and /or antiHCV+), drinking habits (Abstainer, Former, Current), maximal life time alcohol intake (<21 vs. ≥21 drinks/week), and total energy intake (Kcal/day, in continuous). Adjustment for energy was made using the residual model<sup>33</sup>. Food groups were entered in the model as quartile 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 linear terms for each variable of interest.

# Results (I)

An inverse relation was found with educational level (OR for  $\geq 12$  vs.  $\leq 7$  years was 0.31, 95% CI: 0.17-0.59). Compared to never drinkers, former drinkers seemed to be at higher risk than current ones (ORs was 5.20 and 0.60, respectively). Maximal lifetime alcohol intake  $\geq 21$  drinks/week (former and current drinkers combined) was positively related to HCC risk (OR=2.28; 95% CI: 1.08-4.80). Eighty percent of HCC cases, compared to 11% of controls, were positive for markers of HBV and HCV infections (HBsAg+ and /or antiHCV+, OR=30.36; 95% CI: 17.98-51.25). An increased was found for the highest ( $\geq 2650$  Kcal/day) *versus* lowest quartile ( $< 1868$  Kcal/day) of total energy intake (OR=2.34; 95% CI: 1.34-4.11).

TABLE I – DISTRIBUTION OF 185 CASES OF HEPATOCELLULAR CARCINOMA AND 412 CONTROLS AND CORRESPONDING ODDS RATIO (OR) AND 95% CONFIDENCE INTERVALS (CI) FOR SOCIODEMOGRAPHIC FACTORS AND SELECTED VARIABLES (ITALY, 1999–2002)

	Cases		Controls		OR (95% CI) <sup>1</sup>	OR (95% CI) <sup>2</sup>
	N	%	N	%		
Gender						
Males	149	(80.5)	281	(68.2)	1 <sup>3</sup>	
Females	36	(19.5)	131	(31.8)	0.52 (0.34–0.79)	
Age (years)						
<54	18	(9.7)	85	(20.6)	1 <sup>3</sup>	
55–64	56	(30.3)	116	(28.2)	2.28 (1.25–4.16)	
65–74	84	(45.4)	147	(35.7)	2.70 (1.52–4.80)	
≥75	27	(14.6)	64	(15.5)	1.99 (1.01–3.93)	
Centre						
Aviano/Pordenone	61	(33.0)	230	(55.8)	1 <sup>3</sup>	
Naples	124	(67.0)	182	(44.2)	2.57 (1.79–3.70)	
Place of birth						
North-Centre	52	(28.1)	206	(50.0)	1 <sup>3</sup>	1 <sup>3</sup>
South	133	(71.9)	206	(50.0)	2.56 (1.76–3.72)	1.88 (0.88–4.02)
Education (years)						
<7	126	(68.1)	232	(56.3)	1 <sup>3</sup>	1 <sup>3</sup>
7–11	45	(24.3)	93	(22.6)	0.89 (0.59–1.35)	0.90 (0.57–1.43)
≥12	14	(7.6)	87	(21.1)	0.30 (0.17–0.54)	0.30 (0.16–0.58)
$\chi^2_1$ trend					13.66; <i>p</i> < 0.01	11.29; <i>p</i> < 0.01
Drinking habits						
Abstainers	16	(8.7)	63	(15.3)	1 <sup>3</sup>	1 <sup>3</sup>
Current drinkers	75	(40.5)	307	(74.5)	0.96 (0.53–1.76)	0.62 (0.31–1.24)
Former drinkers	94	(50.8)	42	(10.2)	8.81 (4.56–17.02)	5.42 (2.59–11.36)
Maximal lifetime alcohol intake (drinks/week) <sup>4</sup>						
<21	64	(34.6)	192	(46.6)	1.31 (0.71–2.43)	0.97 (0.49–1.91)
≥21	105	(56.8)	157	(38.1)	2.63 (1.44–4.81)	2.38 (1.13–5.01)
$\chi^2_1$ trend					16.69; <i>p</i> < 0.01	10.50; <i>p</i> < 0.01
Hepatitis B virus infection						
HBsAg–	156	(84.3)	408	(99.0)	1 <sup>3</sup>	1 <sup>3</sup>
HBsAg+	29	(15.7)	4	(1.0)	18.96 (6.56–54.81)	17.20 (5.40–54.76)
Hepatitis C virus infection						
AntiHCV–	58	(31.4)	367	(89.1)	1 <sup>3</sup>	1 <sup>3</sup>
AntiHCV+	127	(68.7)	45	(10.9)	17.86 (11.52–27.68)	18.42 (11.20–30.29)
Total energy intake (Kcal)						
<1868	37	(20.0)	113	(27.4)	1 <sup>3</sup>	1 <sup>3</sup>
1868 to <2193	49	(26.5)	100	(24.3)	1.49 (0.90–2.48)	1.59 (0.92–2.77)
2193 to <2650	37	(20.0)	112	(27.2)	1.01 (0.60–1.71)	1.01 (0.57–1.79)
≥2650	62	(33.5)	87	(21.1)	2.18 (1.33–3.57)	2.37 (1.35–4.17)
$\chi^2_1$ trend					6.34; <i>p</i> = 0.01	5.53; <i>p</i> = 0.02

<sup>1</sup>Estimated from unadjusted unconditional logistic regression.–<sup>2</sup>Estimated from unconditional logistic regression adjusted for gender, age, centre, place of birth, and education, when appropriate.–<sup>3</sup>Reference category.–<sup>4</sup>Former and current drinkers combined.

# Results (II)

The upper limits of intake in quartiles of food groups and the corresponding multivariate ORs and relative 95% CI is showed in tab 2. A significant trend in decreasing risks was found for intake of milk and yoghurt (OR for highest vs. lowest quartile =0.28, 95% CI: 0.13-0.61), white meats (OR=0.44, 95% CI: 0.20-0.95), eggs (OR=0.31, 95% CI: 0.14-0.69), and fruits (OR=0.48, 95% CI: 0.22-1.05). Intakes of bread, pasta/rice, soup, read meats, pork and processed meat, fish, cheese, vegetables, potatoes, and dessert were not significantly related to HCC risk.

When separate analysis of food groups associated to HCC risk was conducted across strata of age (<65 *versus* ≥65 years), no differences emerged for highest *versus* lowest consumption of milk and yoghurt (ORs: 0.20 and 0.30, respectively), eggs (ORs: 0.27 and 0.26, respectively).

TABLE II – ODDS RATIO (OR) AND CORRESPONDING 95% CONFIDENCE INTERVAL (CI)<sup>1</sup> OF HEPATOCELLULAR CARCINOMA BY QUARTILE OF INTAKE OF SELECTED FOOD GROUPS (185 CASES AND 412 CONTROLS; ITALY, 1999–2002)

Food groups (servings unit/week)	Energy-adjusted quartile of intake				$\chi^2_1$ trend, <i>p</i> value
	I	II	III	IV	
Milk and yoghurt (125 ml)					
Upper limits	5.25	8.75	12.25		
OR (95% CI)	1 <sup>2</sup>	0.44 (0.21–0.92)	0.40 (0.18–0.86)	0.28 (0.13–0.61)	<0.01
Bread (slices/50 g)					
Upper limits	14.00	21.25	30.50		
OR (95% CI)	1 <sup>2</sup>	1.67 (0.75–3.74)	1.07 (0.48–2.41)	1.64 (0.72–3.74)	0.43
Pasta/Rice (80 g)					
Upper limits	3.00	3.75	5.25		
OR (95% CI)	1 <sup>2</sup>	1.57 (0.70–3.48)	2.47 (1.09–5.63)	2.06 (0.85–4.99)	0.06
Soup (250 g)					
Upper limits	1.00	2.00	3.00		
OR (95% CI)	1 <sup>2</sup>	2.23 (1.01–4.91)	1.85 (0.85–4.03)	0.87 (0.41–1.87)	0.61
White meats (200 g)					
Upper limits	1.00	2.00	3.00		
OR (95% CI)	1 <sup>2</sup>	1.53 (0.73–3.21)	0.66 (0.31–1.38)	0.44 (0.20–0.95)	0.01
Red meats (150 g)					
Upper limits	1.50	2.25	3.00		
OR (95% CI)	1 <sup>2</sup>	1.54 (0.70–3.39)	0.87 (0.37–2.04)	2.07 (0.88–4.82)	0.23
Pork/Processed meat (150 g)					
Upper limits	1.25	2.00	3.00		
OR (95% CI)	1 <sup>2</sup>	0.60 (0.28–1.27)	0.96 (0.46–2.02)	0.83 (0.40–1.70)	0.86
Fish (150 g)					
Upper limits	3.50	5.00	6.50		
OR (95% CI)	1 <sup>2</sup>	0.69 (0.33–1.46)	0.96 (0.46–1.98)	1.12 (0.52–2.40)	0.68
Cheese (80 g)					
Upper limits	2.25	3.25	5.25		
OR (95% CI)	1 <sup>2</sup>	0.78 (0.37–1.66)	1.43 (0.66–3.08)	1.31 (0.58–2.96)	0.31
Eggs (piece)					
Upper limits	0.50	1.00	2.00		
OR (95% CI)	1 <sup>2</sup>	0.64 (0.30–1.34)	0.52 (0.25–1.08)	0.31 (0.14–0.69)	<0.01
Vegetables (100 g/150 g)					
Upper limits	10.00	14.00	19.50		
OR (95% CI)	1 <sup>2</sup>	0.79 (0.38–1.63)	0.68 (0.31–1.49)	0.72 (0.31–1.64)	0.38
Potatoes (200 g)					
Upper limits	0.50	1.00	2.00		
OR (95% CI)	1 <sup>2</sup>	0.56 (0.25–1.24)	1.30 (0.62–2.73)	1.49 (0.69–3.19)	0.09
Fruits (150 g)					
Upper limits	16.50	25.00	34.00		
OR (95% CI)	1 <sup>2</sup>	0.76 (0.37–1.58)	0.53 (0.24–1.16)	0.48 (0.22–1.05)	0.04
Dessert (slice/50 g)					
Upper limits	1.75	4.00	7.00		
OR (95% CI)	1 <sup>2</sup>	0.79 (0.35–1.80)	0.91 (0.40–2.06)	0.70 (0.31–1.62)	0.50

<sup>1</sup>Estimated from unconditional logistic regression adjusted for gender, age, centre, education, place of birth, drinking habits (Abstainers, Former, Current), maximal lifetime alcohol intake ( $\geq 21$  vs.  $< 21$  drinks/week), hepatitis viruses (HbsAg+ and/or anti-HCV+ vs. HbsAg– and anti-HCV–), and total energy intake (Kcal/day, in continuous). –<sup>2</sup>Reference category.

# Results (III)

The joint effects of milk and yoghurt, white meats, eggs and fruits and being HBsAg+ and/or anti-HCV+ is showed in table 3. For all four the food groups, lowest *versus* highest consumption was significantly associated to HCC risk among both non-chronic and chronic carriers of HBV and/or HCV. In particular, among who were either HBsAg+ and/or anti-HCV+, a 2-fold risk persisted for low consumption and of milk and yoghurt (OR=51.54; 95% CI: 21.48-123.68), white meats (OR=56.56; 95% CI: 24.42-130.99), eggs (OR=48.78; 95% CI: 21.36-111.43), and fruits (OR=43.25; 95% CI: 18.64-100.36).

**TABLE III – ODDS RATIO (OR) AND CORRESPONDING 95% CONFIDENCE INTERVAL (CI)<sup>1</sup> OF HEPATOCELLULAR CARCINOMA BY INTAKE OF SELECTED FOOD GROUPS IN SEPARATE STRATA OF HEPATITIS C AND/OR HEPATITIS B VIRUS INFECTION (185 CASES AND 412 CONTROLS; ITALY, 1999–2002)**

Food groups	Level of food group consumption		$\chi^2$ for heterogeneity, <i>p</i> value
	Low ( $\leq$ median value)	High ( $>$ median value)	
<b>MILK AND YOGHURT</b>			
<i>HBsAg– and AntiHCV–</i>			
Cases:Controls	26:173	12:192	
OR (95% CI)	1 <sup>2</sup>	0.33 (0.14–0.75)	
<i>HBsAg+ and/or AntiHCV+</i>			0.25
Cases:Controls	79:21	68:26	
OR (95% CI)	1 <sup>2</sup>	0.53 (0.22–1.26)	
<b>WHITE MEATS</b>			
<i>HBsAg– and AntiHCV–</i>			
Cases:Controls	23:171	15:194	
OR (95% CI)	1 <sup>2</sup>	0.59 (0.27–1.27)	
<i>HBsAg+ and/or AntiHCV+</i>			0.49
Cases:Controls	87:18	60:29	
OR (95% CI)	1 <sup>2</sup>	0.32 (0.13–0.78)	
<b>EGGS</b>			
<i>HBsAg– and AntiHCV–</i>			
Cases:Controls	25:150	13:215	
OR (95% CI)	1 <sup>2</sup>	0.31 (0.14–0.70)	
<i>HBsAg+ and/or AntiHCV+</i>			0.41
Cases:Controls	98:26	49:21	
OR (95% CI)	1 <sup>2</sup>	0.67 (0.28–1.61)	
<b>FRUITS</b>			
<i>HBsAg– and AntiHCV–</i>			
Cases:Controls	25:176	13:189	
OR (95% CI)	1 <sup>2</sup>	0.53 (0.25–1.16)	
<i>HBsAg+ and/or AntiHCV+</i>			0.79
Cases:Controls	77:21	70:26	
OR (95% CI)	1 <sup>2</sup>	0.64 (0.27–1.51)	

<sup>1</sup>Estimated from unconditional logistic regression adjusted for gender, age, centre, education, place of birth, drinking habits (Abstainers, Former, Current), maximal lifetime alcohol intake ( $\geq 21$  vs. 21 drinks/week), and total energy intake (Kcal, in continuous). –<sup>2</sup>Reference category.

# Discussion (I)

The present study showed a significant inverse association between consumptions of milk and yoghurt, white meats, eggs, and fruits and HCC risk.

In our study, a significant inverse association was also found with white meat consumption, whereas, red meat intake showed a borderline increased risk. Previous results for meat consumption and HCC risk were controversial.

An inverse association of HCC risk with egg consumption was found in our study. This effect remained significant across strata of age (<65 and  $\geq$ 65 years), but only among HBsAg and /or antiHCV positive subjects .

An inverse association of HCC risk with fruit was found in our study. Our data showed that the protective effect of highest *versus* lowest consumption of fruit was consistent across strata of age, HBV, and HCV serological markers.

# Discussion (II)

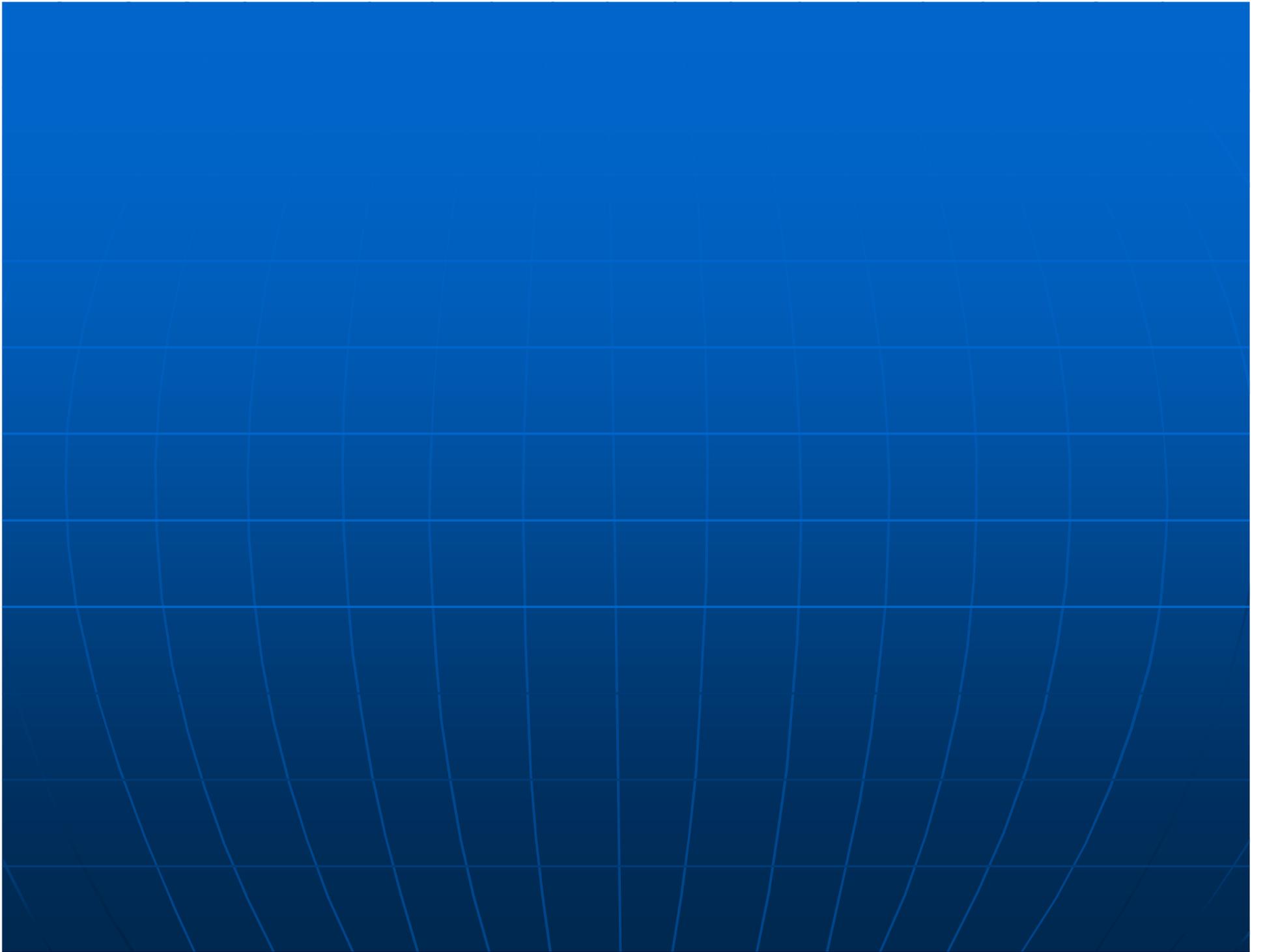
The present study showed a non-significant inverse association with vegetables.

It is possible that dietary habits of hospital controls may have differed from those of the general population; however, by study design, great attention was paid in excluding all diagnoses that might have been associated to or had determined special dietary habits in control subjects.

Moreover, to reduce the possibility of recall bias due to changes in diet related to disease onset, we elicited information on food intake during the two years before the interview. The questionnaire was administered to cases and controls by the same interviewers under similar conditions in a hospital setting, thus minimizing information bias. Adjustments for gender, age, centre, education, place of birth, drinking habits, total energy intake, and HBsAg and/or antiHCV seropositivity were made to address potential confounding.

# Conclusion

Our study indicates that diet has a relevant role in HCC risk, independently from HBV or HCV infection. In addition, our results provide the most important evidence, thus far, of the interaction of chronic HBV- and HCV-infections with dietary habits in the development of HCC, and, hence, may address important indications for diet modification in high-risk subjects.



## Abstract

The role of diet, except for alcohol drinking and aflatoxin contamination, in the etiology of hepatocellular carcinoma (HCC) is unclear.

A hospital-based case-control study was conducted in Italy in 1999-2002, including 185 incident, histologically-confirmed cases of HCC aged 43-84 years. Controls were 412 subjects admitted to hospitals for acute, non-neoplastic diseases unrelated to diet. Dietary habits were assessed using a validated food-frequency questionnaire. Odds ratios (ORs) and the corresponding 95% confidence intervals (CI) were computed using unconditional multiple logistic regression, adjusting for hepatitis B (HBV) and hepatitis C (HCV) virus infection and alcohol drinking.

Energy adjustment was carried out by means of the residual model. A significant inverse association with HCC risk was found for high intakes of milk and yoghurt (OR=0.25; 95% CI: 0.11-0.54), white meats (OR=0.36; 95% CI: 0.17-0.78), eggs (OR=0.30; 95% CI: 0.14-0.66), and fruit (OR=0.43; 95% CI: 0.20-0.93). Interactions between HBV and/or HCV infection and low consumption of milk and yoghurt, white meats, eggs, and fruit were compatible with a multiplicative effect (OR=56.40; OR=56.35; OR=46.14; OR=45.83, respectively). The present study supports the hypothesis of a role of diet in HCC aetiology. Dietary modifications may be indicated in subjects at high-risk for HCC.