BJC

Association of serum α-tocopherol, β-carotene, and retinol with liver cancer incidence and chronic liver disease mortality

G Y Lai*[,1,2], S J Weinstein[2], D Albanes[2], P R Taylor[3], J Virtamo[4], K A McGlynn[5] and N D Freedman[2]

[1]Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892, USA; [2]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Room 6E316, Bethesda, MD 20892, USA; [3]Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892, USA; [4]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland and [5]Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892, USA

Background: Micronutrients may influence the development or progression of liver cancer and liver disease. We evaluated the association of serum α-tocopherol, β-carotene, and retinol with incident liver cancer and chronic liver disease (CLD) mortality in a prospective cohort of middle-aged Finnish male smokers.

Methods: Baseline and 3-year follow-up serum were available from 29 046 and 22 805 men, respectively. After 24 years of follow-up, 208 men were diagnosed with liver cancer and 237 died from CLD. Hazards ratios and 95% confidence intervals were calculated for highest vs lowest quartiles from multivariate proportional hazards models.

Results: Higher β-carotene and retinol levels were associated with less liver cancer (β-carotene: 0.35, 0.22–0.55, P-trend <0.0001; retinol: 0.58, 0.39–0.85, P-trend = 0.0009) and CLD mortality (β-carotene: 0.47, 0.30–0.75, P-trend = 0.001; retinol: 0.55, 0.38–0.78, P-trend = 0.0007). α-Tocopherol was associated with CLD mortality (0.63, 0.40–0.99, P-trend = 0.06), but not with liver cancer (1.06, 0.64–1.74, P-trend = 0.77). Participants with higher levels of β-carotene and retinol, but not α-tocopherol, at both baseline and year 3 had lower risk of each outcome than those with lower levels.

Conclusions: Our findings suggest that higher concentrations of β-carotene and retinol are associated with incident liver cancer and CLD. However, such data do not indicate that supplementation should be considered for these diseases.

Liver cancer is the third leading cause of cancer death and the sixth most commonly diagnosed cancer in the world (Ferlay et al, 2010). Most liver cancers are diagnosed among individuals who have chronic liver disease (CLD) that itself is a notable burden. Not only is CLD a leading cause of death worldwide (Williams, 2006; Heron, 2012), but it has also been reported to greatly increase the risk of death from other diseases (Sorensen et al, 2003; Roberts et al,

2005). Noted risk factors for liver cancer and CLD include chronic infection of hepatitis B (HBV) and C viruses (HCV) and aflatoxin ingestion, especially in sub-Saharan African and eastern Asian countries with a high prevalence of liver cancer. Other risk factors include excessive alcohol intake, smoking, obesity, and diabetes. These lifestyle factors, particularly obesity and diabetes, likely contribute to the recent rise in liver cancer and liver disease in

Western populations such as Europe and the United States (Yu and Yuan, 2004; Lim and Kim, 2008; Fan and Farrell, 2009).

Oxidative stress has been hypothesised to contribute to the aetiology of liver cancer and liver disease (Ha *et al*, 2010). For example, both HBV and HCV have been suggested to enrich inflammatory and oxidative conditions in hepatocytes (Marra *et al*, 2011). In addition, obesity and diabetes are also thought to promote inflammation and the formation of reactive oxygen species (Hopps *et al*, 2010; Pitocco *et al*, 2010; Powell *et al*, 2010).

Because of their roles in inhibiting reactive oxygen species, antioxidant micronutrients have been hypothesised to inhibit the development or progression of liver cancer and liver disease (Ha *et al*, 2010; Gambino *et al*, 2011). Antioxidants have been reported to inhibit chemically induced liver cancers in rats and mice (Factor *et al*, 2000; Moreno *et al*, 2002). Micronutrients may also play an important role in disease through other mechanisms. For example, retinol is a key regulator of transcription (Das *et al*, 2014). However, only a few human studies have examined the association of serum micronutrients with liver cancer or chronic liver disease, and most were conducted in Asia where HBV, one of the strongest risk factors for liver cancer and liver disease, is endemic (Knekt *et al*, 1991; Yu *et al*, 1995; Ito *et al*, 2006; Yuan *et al*, 2006). Therefore, we evaluated the association of serum α-tocopherol (a form of vitamin E), β-carotene, and retinol with incident liver cancer and CLD mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, a prospective cohort of Finnish male smokers.

MATERIALS AND METHODS

Participants. Details about the rationale and design of the ATBC Study have been described previously (The ATBC Cancer Prevention Study Group, 1994). In brief, the study was a randomised, double-blind, 2 × 2 factorial, placebo-controlled trial to evaluate the efficacy of daily supplements of vitamin E, in the form of α-tocopherol, and β-carotene in reducing the risk of lung cancer. Incidence of other cancers and cause-specific mortality and other diseases were secondary outcomes. Eligible men who lived in southwestern Finland, were aged 50–69 years, smoked five or more cigarettes per day, and who agreed to participate were enrolled from 1985 to 1988.

A total of 29 133 eligible men were randomly assigned to one of four intervention groups: (1) α-tocopherol (DL-α-tocopheryl acetate, 50 IU per day), (2) β-carotene (20 mg per day), (3) both supplements, (4) or placebo. Although the trial ended on 30 April 1993, the cohort has been followed since that time through national registers for morbidity and mortality end points. Written informed consent was provided by the participants before randomisation. The ATBC Study was approved by the institutional review boards of the National Public Health Institute of Finland and the US National Cancer Institute.

Data collection and laboratory analysis. At baseline, participants provided their medical history, smoking history, alcohol intake, and physical activity. Intake of foods was calculated from a validated food frequency questionnaire in conjunction with a nutrient database (Pietinen *et al*, 1988). An overnight fasting blood sample was drawn and stored at −70 °C. Serum α-tocopherol, β-carotene, and retinol concentrations were obtained via high-performance liquid chromatography for all study participants (Milne and Botnen, 1986). The between-run CVs for each biomarker were 2.2%, 3.6%, and 2.4%, respectively. Total cholesterol was measured using an enzymatic assay (CHOD-PAP method, Boehringer Mannheim, Germany). After 3 years of

follow-up, sera were also collected and measured for α-tocopherol, β-carotene, and retinol.

We excluded 28 men who reported having mild liver cirrhosis at baseline and an additional 59 men who lacked demographic or serum data; our final analytic sample was 29 046 men. Follow-up sera were available from 22 805 men. Within the cohort, data on HBV and HCV status were available from 167 men with incident liver cancers, 212 men who died from chronic liver disease, and 817 controls matched on age (± 5 years) and date of blood draw (± 30 days). The presence of hepatitis B surface antigen (HBsAg) was tested using enzyme immunoassay from Bio-Rad Laboratories (Redmond, WA, USA), and antibodies to hepatitis B core antigen (anti-HBc) and HCV (anti-HCV) were tested using enzyme-linked immunosorbent assays from Ortho-Clinical Diagnostics (Raritan, NJ, USA).

Follow-up and outcome ascertainment. Men diagnosed with incident liver cancer ($n = 208$; ICD-9 = 155 and ICD-10 = C22) were identified through the Finnish Cancer Registry that provided close to 100% case ascertainment (Korhonen *et al*, 2002). Medical records for 80% of the liver cancers were reviewed by a study physician to confirm diagnosis. Data from the remaining 20% of the incident cases were available from the Finnish Cancer Registry only. Follow-up time began at the date of randomisation and continued until the date of cancer diagnosis, death, or 31 December 2009, whichever came first. Men who died from CLD ($n = 237$; ICD-9 = 571 and ICD-10 = K70, K73, or K74) were identified through the Finnish Register of Causes of Death. The underlying cause of ∼ 90% of the CLD deaths was noted to be alcohol-related liver diseases. Follow-up time began at randomisation and continued until date of death or until 31 December 2009, whichever came first. For men who were diagnosed with an incident liver cancer and died from CLD ($n = 8$), we only considered them for the analysis with incident liver cancer and not for CLD mortality.

Statistical analysis. We estimated hazards ratios (HRs) and 95% confidence intervals (CIs) for the association of serum α-tocopherol, β-carotene, and retinol with incident liver cancers or mortality from CLD using Cox proportional hazard regression models with person-years as the underlying time metric. Study participants were placed into quartiles based on the distribution of the serum micronutrients in the cohort (Table 1). We tested for linear trend tests across quartiles of α-tocopherol, β-carotene, and retinol by designating each participant the median level for each quartile and entering that term as a continuous variable in the regression model. We examined the proportional hazards assumption by including the interaction term between serum micronutrients and follow-up time in the model and testing the term via the Wald test. As the assumption failed, we stratified our results by follow-up time and observed similar associations during each time period (Supplementary Table 1). We also evaluated the associations by using logistic regression models and observed similar observations. Therefore, we present results using proportional hazard regression models in this study.

We included the following possible confounders in our multivariable regression models: intervention arm (α-tocopherol, β-carotene, both supplements, or placebo), age at randomisation, body mass index (BMI) (kg m^{-2}), education (greater than elementary school *vs* elementary school or less), marital status (married *vs* divorced, widowed, or never married), smoking duration (years of smoking) and amount (number of cigarettes smoked per day), baseline serum total cholesterol (mmol l^{-1}), history of diabetes (yes *vs* no), and daily intake of coffee (8 oz cups), alcohol (g), fruits (g per 1000 kcal), vegetables (g per 1000 kcal), and total energy (kcal). These variables were evaluated because either a number of known or suspected risk factors for liver cancer and CLD have been reported (Chuang *et al*, 2009;

Table 1. Baseline characteristics of the cohort by quartiles of serum α-tocopherol (mg l^{-1}), β-carotene (μg l^{-1}), and retinol (μg l^{-1})

	α-Tocopherol				β-Carotene				Retinol			
	Q1 <9.75	Q2 9.75–<11.5	Q3 11.5–<13.6	Q4 ≥13.6	Q1 <109	Q2 109–<170	Q3 170–<261	Q4 ≥261	Q1 <500	Q2 500–<576	Q3 576–<662	Q4 ≥662
No. of participants	7247	7245	7287	7267	7143	7275	7340	7288	7229	7250	7291	7276
Age, years[a]	57 (53–62)	57 (53–61)	56 (53–61)	56 (53–60)	56 (53–61)	57 (53–61)	57 (53–61)	57 (53–61)	58 (54–62)	57 (53–61)	56 (53–61)	56 (52–60)
BMI, kg m^{-2} [a]	25.2 (22.8–27.9)	25.8 (23.5–28.3)	26.1 (23.9–28.5)	26.7 (24.6–29.2)	26.5 (23.9–29.5)	26.4 (24.1–28.9)	25.9 (23.7–28.2)	25.3 (23.2–27.5)	25.3 (22.8–27.9)	25.9 (23.5–28.5)	26.1 (24.0–28.6)	26.5 (24.3–29.0)
Elementary school or less, %	84.1	81.5	77.9	72.6	78.9	79.6	79.9	77.5	82.1	80.7	78.4	74.7
Currently married, %	74.7	80.7	82.4	83.1	71.7	81.2	82.9	84.9	78.6	81.5	81.2	79.6
Total no. of cig per day[a]	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–25)	20 (15–20)	20 (15–20)	20 (15–20)	20 (15–20)
Total no. of years smoked[a]	38 (32–43)	37 (31–42)	36 (30–41)	36 (30–41)	37 (32–42)	37 (31–42)	36 (31–42)	36 (30–41)	38 (32–43)	37 (32–42)	36 (30–41)	35 (30–40)
Serum α-tocopherol, mg l^{-1} [a]	8.6 (7.7–9.2)	10.6 (10.2–11.1)	12.4 (11.9–12.9)	15.3 (14.3–17.1)	10.5 (8.6–12.7)	11.3 (9.6–13.3)	11.7 (10.1–13.6)	12.4 (10.7–14.4)	10.7 (9.0–12.5)	11.3 (9.7–13.3)	11.8 (10.1–13.8)	12.3 (10.5–14.6)
Serum β-carotene, μg l^{-1} [a]	130 (82–199)	171 (114–254)	191 (125–284)	197 (129–307)	77 (57–94)	138 (123–154)	208 (187–231)	351 (297–450)	172 (111–264)	180 (116–272)	176 (115–264)	155 (97–240)
Serum retinol, μg l^{-1} [a]	542 (469–628)	565 (494–643)	585 (510–665)	613 (537–704)	588 (503–687)	578 (500–663)	574 (500–653)	568 (498–647)	451 (411–478)	539 (520–558)	615 (595–636)	734 (692–797)
Serum cholesterol, mmol l^{-1} [a]	5.29 (4.74–5.86)	5.93 (5.40–6.49)	6.41 (5.85–7.04)	7.10 (6.43–7.84)	5.78 (5.09–6.52)	6.05 (5.37–6.81)	6.26 (5.58–7.00)	6.52 (5.77–7.29)	5.82 (5.11–6.56)	6.09 (5.42–6.85)	6.26 (5.57–7.03)	6.44 (5.73–7.26)
Alcohol intake (g per day)[a]	12.6 (3.0–28.6)	10.7 (2.3–25.4)	10.6 (2.5–24.4)	10.7 (2.7–24.4)	22.9 (9.1–39.4)	12.5 (3.7–26.3)	8.5 (1.8–22.5)	5.3 (0.9–14.9)	6.7 (1.1–18.8)	8.9 (1.8–22.9)	11.9 (3.5–26.3)	18.4 (6.4–34.4)
History of diabetes mellitus, %	4.0	3.6	3.5	5.9	6.9	4.3	3.2	2.6	5.1	4.0	3.9	4.1
Fruit (g per day)[a]	148 (71–268)	165 (84–284)	180 (94–303)	186 (100–312)	141 (65–263)	169 (85–289)	175 (94–293)	191 (106–316)	165 (82–285)	170 (90–290)	173 (90–295)	173 (87–296)
Vegetable (g per day)[a]	258 (196–337)	277 (214–357)	283 (217–365)	290 (224–371)	251 (189–325)	273 (211–350)	284 (220–363)	302 (233–387)	269 (204–347)	278 (215–360)	279 (217–362)	282 (215–362)
Total energy (kcal per day)[a]	2620 (2167–3154)	2629 (2169–3146)	2607 (2170–3114)	2547 (2124–3064)	2536 (2095–3060)	2609 (2165–3134)	2637 (2181–3148)	2623 (2185–3125)	2578 (2120–3101)	2624 (2180–3124)	2610 (2177–3129)	2593 (2144–3125)
Glucose, mg dl^{-1} [a,b]	98 (93–106)	99 (92–106)	101 (93–107)	98 (93–107)	100 (94–111)	100 (92–109)	99 (92–105)	98 (93–105)	97 (91–105)	99 (93–105)	100 (94–107)	100 (94–108)
Insulin, μU ml^{-1} [a,c]	4.1 (2.6–6.1)	4.2 (2.8–6.4)	4.4 (2.9–7.0)	4.4 (3.0–7.0)	4.7 (3.0–7.7)	4.2 (2.9–6.7)	4.4 (2.8–6.4)	3.8 (2.7–5.4)	4.0 (2.6–6.7)	3.9 (2.6–5.9)	4.9 (3.3–7.2)	4.3 (3.0–6.3)
HBsAg, n, %[d]	0, 0%	1, 0.5%	2, 0.87%	3, 1.52%	1, 0.51%	2, 1.02%	2, 0.93%	1, 0.48%	2, 1.03%	2, 0.94%	1, 0.49%	1, 0.48%
Anti-HBc, n, %[d]	10, 5.26%	15, 7.54%	20, 8.66%	12, 6.09%	18, 9.18%	8, 4.06%	14, 6.54%	17, 8.1%	19, 9.79%	15, 7.08%	12, 5.88%	11, 5.31%
Anti-HCV, n, %[d]	0, 0%	4, 2.01%	1, 0.43%	0, 0%	1, 0.51%	2, 1.02%	2, 0.93%	0, 0%	2, 1.03%	1, 0.47%	0, 0%	2, 0.97%

Abbreviations: Anti-HBc = hepatitis B core antibody; Anti-HCV = hepatitis C antibody; BMI = body mass index; HBsAg = hepatitis B surface antigen.
[a]Presented as medians (interquartile range).
[b]In a subset of 1079 men who did not die of chronic liver disease or were diagnosed with incident liver cancer.
[c]In a subset of 1076 men who did not die of chronic liver disease or were diagnosed with incident liver cancer.
[d]In a subset of 817 men who did not die of chronic liver disease or were diagnosed with incident liver cancer.

Souza *et al*, 2012) or were associated with serum micronutrients. The analyses included mutual adjustment of the serum micronutrients. Risk estimates were similar between models that did and did not mutually adjust for the serum micronutrients, and therefore results from fully and mutually adjusted models are presented. We also conducted lag analyses and evaluated associations after removing the first 2 and 5 years of follow-up, as well as associations in the first 10 years of follow-up and after 10 years of follow-up. In the subset of participants with available information, we investigated whether HBV or HCV status affected associations with serum micronutrients. For this subset, odds ratios (ORs) and 95% CIs were estimated by logistic regression models.

We evaluated possible effect modification by subgroups of intervention group, median age, median BMI, median duration and intensity of smoking, alcohol intake, diabetes, and median serum cholesterol. Effect modification was formally tested by using likelihood ratio tests that compared regression models that did and did not include the cross-product term for each respective biomarker (as a continuous variable to conserve power) and the above categories.

Participants also had their serum micronutrients measured at their third-year follow-up visit. As concentrations of α-tocopherol and β-carotene were substantially higher at the follow-up visit among participants receiving supplementation, we examined repeat measures in those who did not receive supplementation (Figure 1). Thus, when evaluating the association of joint categories of α-tocopherol, both baseline and follow-up, we evaluated the association only in men randomised to the β-carotene and placebo intervention arms, and not the α-tocopherol or the α-tocopherol + β-carotene intervention arm. Similarly, for β-carotene, we evaluated the association in those randomised only to the α-tocopherol and placebo arms. For retinol, we evaluated all men who provided blood at the 3-year follow-up. We classified participants to be low or high at baseline and follow-up based on the median level at each time point. For the follow-up measurement, we selected the median among those participants not receiving supplementation.

All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA) and all *P*-values were two sided.

RESULTS

After over 24 years of follow-up (median follow-up time = 18 years), a total of 208 men were diagnosed with liver cancer and 237 men died from CLD from our baseline analytic sample of 29 046 men. Serum α-tocopherol, β-carotene, and retinol were weakly correlated with each other (Spearman's correlation): (a) α-tocopherol and β-carotene: 0.19, $P < 0.0001$; (b) α-tocopherol and retinol: 0.21, $P < 0.0001$; and (c) β-carotene and retinol: -0.02, $P = 0.0001$. Although micronutrient levels did not vary by age or cigarette smoking at baseline, some differences were noted across categories. For example, alcohol intake was inversely associated with β-carotene, but positively associated with retinol. Diabetes was inversely associated with β-carotene. The prevalences of HBsAg and anti-HCV were low ($\leqslant 2\%$) across quartiles of α-tocopherol, β-carotene, and retinol, whereas the prevalence of anti-HBc ranged from 4% to 9% (Table 1).

Serum β-carotene and retinol were inversely associated with liver cancer risk, (β-carotene: quartile 4 (Q4) *vs* quartile 1 (Q1) HR = 0.36, 95% CI = 0.22–0.58, P-trend < 0.0001; retinol: Q4 *vs* Q1 HR = 0.58, 95% CI = 0.39–0.85, P-trend = 0.0009). However, no association was observed for serum α-tocopherol (Q4 *vs* Q1 HR = 1.05, 95% CI = 0.64–1.74, P-trend = 0.77). All three micronutrients were associated inversely with mortality from CLD (α-tocopherol: Q4 *vs* Q1 HR = 0.63, 95% CI = 0.40–0.99, P-trend = 0.06; β-carotene: Q4 *vs* Q1 HR = 0.47, 95% CI = 0.30–0.74, P-trend = 0.001; retinol: Q4 *vs* Q1 HR = 0.54, 95% CI = 0.38–0.77, P-trend = 0.0005) (Table 2), although the test for trend for the association for α-tocopherol and CLD mortality was attenuated and not statistically significant following adjustment for β-carotene and retinol. In lag analyses, results persisted among events occurring after excluding the first 2 and 5 years of follow-up, as well as in cases occurring after 10 years of follow-up (Supplementary Table 1).

Associations between micronutrients and each end point remained similar across many examined strata including intervention group, age, BMI, diabetes, alcohol, smoking use, and cholesterol. Apparent interactions were observed for BMI with
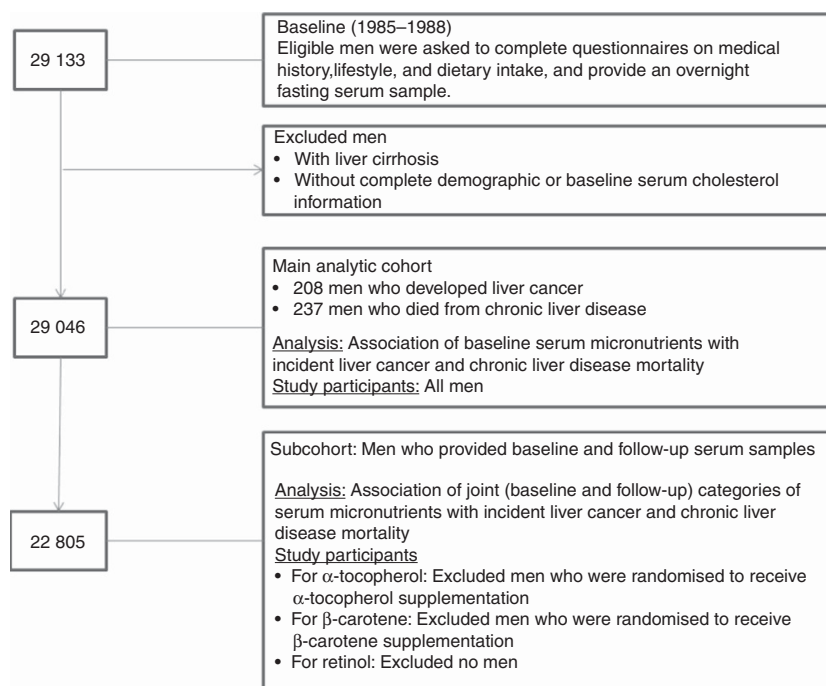

Figure 1. Diagram of analytic sample within ATBC Study.

Table 2. Association of baseline serum α-tocopherol, β-carotene, and retinol with incident liver cancer and chronic liver disease mortality

	Incident liver cancer					Chronic liver disease mortality				
	Q1	Q2	Q3	Q4	*P*-trend	Q1	Q2	Q3	Q4	*P*-trend
Serum α-tocopherol										
No. of cases/all	62/7247	54/7245	52/7287	40/7267		101/7247	47/7245	49/7287	40/7267	
Model 1	1.00	1.01	1.04	0.90	0.72	1.00	0.56	0.63	0.55	0.01
		0.69–1.48	0.69–1.58	0.55–1.48			0.39–0.81	0.43–0.92	0.35–0.86	
Model 2	1.00	1.12	1.22	1.06	0.77	1.00	0.62	0.73	0.63	0.06
		0.76–1.64	0.80–1.85	0.64–1.74			0.43–0.89	0.49–1.07	0.40–0.99	
Serum β-carotene										
No. of cases/all	90/7143	48/7275	44/7340	26/7288		120/7143	50/7275	40/7340	27/7288	
Model 1	1.00	0.57	0.58	0.38	0.0001	1.00	0.60	0.59	0.46	0.0006
		0.40–0.81	0.39–0.85	0.24–0.61			0.43–0.85	0.40–0.86	0.29–0.72	
Model 2	1.00	0.55	0.57	0.36	<0.0001	1.00	0.61	0.60	0.47	0.001
		0.39–0.79	0.38–0.83	0.22–0.58			0.43–0.86	0.41–0.88	0.30–0.74	
Serum retinol										
No. of cases/all	71/7229	54/7250	33/7291	50/7276		77/7229	48/7250	46/7291	66/7276	
Model 1	1.00	0.72	0.44	0.62	0.0026	1.00	0.58	0.51	0.55	0.0006
		0.50–1.03	0.29–0.67	0.42–0.91			0.40–0.84	0.35–0.75	0.38–0.77	
Model 2	1.00	0.71	0.43	0.58	0.0009	1.00	0.59	0.52	0.54	0.0005
		0.50–1.02	0.28–0.65	0.39–0.85			0.43–0.92	0.36–0.76	0.38–0.77	

Model 1: adjusted for Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study intervention arm, age (continuous), body mass index (BMI; continuous), years of smoking (continuous), cigarettes per day (continuous), serum cholesterol (continuous), history of diabetes (yes, no), marital status (currently married, not), education (elementary school or less, higher than elementary school), and daily intake of alcohol (continuous), coffee (continuous), fruits (continuous), vegetables (continuous), and total energy (continuous). Model 2: additionally mutually adjusted for other serum biomarkers. Presented as hazards ratios (HRs) and 95% confidence intervals (CIs).

serum retinol and CLD mortality. Whereas associations were null in men with a BMI $<26 \, \mathrm{kg \, m^{-2}}$ (retinol: Q4 *vs* Q1 HR = 1.11, 95% CI = 0.66–1.85, *P*-trend = 0.76), associations were inverse among men with a BMI of $\geqslant 26 \, \mathrm{kg \, m^{-2}}$ (retinol: Q4 *vs* Q1 HR = 0.28, 95% CI = 0.17–0.46, *P*-trend < 0.0001) (*P*-interaction = 0.01).

Among men with information on HBV and HCV status, adjustment for HBV and HCV had little effect, although some associations in this nested subgroup were attenuated, likely because of smaller sample size (Table 3). Associations were also similar among analyses restricted to HBV- and HCV-negative cases that consisted most of the cases in our cohort (data not shown).

Finally, we investigated associations among participants with measured micronutrient levels at both baseline and at the 3-year follow-up visit. As described previously in the Materials and Methods, these analyses were restricted to participants who did not receive supplementation for that micronutrient. We observed that those in the high/high category of β-carotene and retinol compared with those in the low/low category had a lower risk of incident liver cancer and CLD mortality, although the association for the high/high joint category of β-carotene and incident liver cancer was not statistically significant (Table 4).

DISCUSSION

In this cohort of Finnish male smokers, we observed that higher serum β-carotene and retinol at baseline were inversely associated with incident liver cancer and death from chronic liver disease. Higher α-tocopherol was not associated with incident liver cancer, although a borderline statistically significant reduced risk was observed for CLD death. Adjustment for important risk factors had little effect on the risk estimates and associations persisted with events occurring even 10 years after blood collection. Furthermore, there was a suggestion that consistently higher levels of β-carotene and retinol over time were associated with a reduced risk for incident liver cancer and CLD death.

Our results are generally similar to those observed for liver cancer in previous case–control studies (number of cases ranging from 16 to 84) (Pan *et al*, 1993; Yamamoto *et al*, 1998; Yu *et al*, 1999; Clemente *et al*, 2002). Few prospective studies evaluating the relationship between serum micronutrients and liver cancer are available. Two prospective studies, one based in Taiwan (*n* = 50 cases) and another in China (*n* = 213), observed that men with higher serum retinol had a reduced risk of liver cancer (Yu *et al*, 1995; Yuan *et al*, 2006), whereas a third prospective study based in Japan found that men and women with higher serum α-tocopherol, β-carotene, and retinol had lower risk of liver cancer mortality, although such associations were only statistically significant for β-carotene. However, this study had only a small number of liver cancer deaths (*n* = 12), limiting power (Ito *et al*, 2006). In a Finnish cohort, a nested case–control study found no association for serum α-tocopherol, β-carotene, or retinol with liver cancer. Yet, with only 12 liver cancer cases, that study also had very low power (Knekt *et al*, 1991). Overall, our findings extend previous results to liver cancer occurring in populations with low HBV prevalence.

Epidemiologic studies on the association between serum micronutrients and CLD are scarce. Yet, it is plausible that associations of serum micronutrients with CLD would be similar to that with liver cancer considering the shared risk factors and lengthy period of development for both. We observed inverse associations of β-carotene and retinol with CLD mortality, similar to that with incident liver cancer. In addition, we observed stronger associations between retinol and CLD mortality among those with a BMI of $\geqslant 26 \, \mathrm{kg \, m^{-2}}$ than among participants with a lower BMI.

Table 3. Association of baseline serum α-tocopherol, β-carotene, and retinol with incident liver cancer and chronic liver disease mortality among those with information on hepatitis B and C status

	Incident liver cancer					Chronic liver disease mortality				
	Q1	Q2	Q3	Q4	P-trend	Q1	Q2	Q3	Q4	P-trend
Serum α-tocopherol										
No. of cases/no. of controls	55/190	40/199	39/231	33/197		95/190	40/199	43/231	34/197	
Model 1	1.00	0.85	0.94	0.84	0.64	1.00	0.48	0.53	0.46	0.01
		0.52–1.40	0.55–1.61	0.45–1.57			0.30–0.79	0.32–0.89	0.24–0.86	
Model 2	1.00	0.75	0.90	0.81	0.60	1.00	0.46	0.52	0.45	0.01
		0.45–1.26	0.53–1.54	0.43–1.51			0.28–0.76	0.31–0.87	0.24–0.84	
No. of cases/no. of controls	46/180	30/184	33/209	32/214		89/180	37/184	39/209	31/182	
Model 3	1.00	0.75	0.96	0.89	0.86	1.00	0.47	0.51	0.41	0.007
		0.43–1.30	0.55–1.69	0.47–1.70			0.28–0.79	0.30–0.87	0.21–0.79	
Serum β-carotene										
No. of cases/no. of controls	74/196	39/197	34/214	20/210		107/196	43/197	37/214	25/210	
Model 1	1.00	0.62	0.64	0.46	0.02	1.00	0.61	0.70	0.77	0.41
		0.38–0.99	0.39–1.05	0.25–0.83			0.38–0.96	0.42–1.14	0.44–1.36	
Model 2	1.00	0.63	0.63	0.48	0.02	1.00	0.60	0.69	0.78	0.44
		0.39–1.01	0.38–1.04	0.26–0.88			0.37–0.95	0.42–1.14	0.44–1.38	
No. of cases/no. of controls	59/177	35/189	29/197	18/192		100/177	36/189	36/197	24/192	
Model 3	1.00	0.67	0.65	0.51	0.05	1.00	0.51	0.76	0.83	0.66
		0.41–1.11	0.38–1.13	0.27–0.97			0.31–0.83	0.45–1.27	0.46–1.48	
Serum retinol										
No. of cases/no. of controls	56/194	46/212	23/204	42/207		70/194	45/212	35/204	62/207	
Model 1	1.00	0.70	0.40	0.70	0.07	1.00	0.69	0.52	0.72	0.15
		0.44–1.13	0.23–0.71	0.42–1.17			0.42–1.13	0.31–0.88	0.44–1.18	
Model 2	1.00	0.73	0.43	0.73	0.10	1.00	0.71	0.54	0.72	0.14
		0.45–1.18	0.24–0.76	0.44–1.22			0.43–1.16	0.32–0.91	0.44–1.18	
No. of cases /no. of controls	42/173	40/196	19/191	40/195		61/173	43/196	35/191	57/195	
Model 3	1.00	0.78	0.41	0.81	0.22	1.00	0.70	0.56	0.73	0.20
		0.46–1.30	0.22–0.76	0.47–1.39			0.42–1.18	0.32–0.97	0.44–1.24	

Model 1: adjusted for Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study intervention arm, age (continuous), body mass index (BMI; continuous), years of smoking (continuous), cigarettes per day (continuous), serum cholesterol (continuous), history of diabetes (yes, no), marital status (currently married, not), education (elementary school or less, higher than elementary school), and daily intake of alcohol (continuous), coffee (continuous), fruits (continuous), vegetables (continuous), and total energy (continuous). Model 2: additionally adjusted for hepatitis B surface antigen, hepatitis B antibodies, and hepatitis C antibodies. Model 3: Similar to model 1 but restricted to those who were hepatitis B and C negative. Presented as odds ratio (ORs) and 95% confidence intervals (CIs).

High BMI has been reported to be a risk factor for both liver cancer and liver disease, as reviewed by Marchesini *et al* (2008) and Rui *et al* (2012). However, the mechanism by which obesity may influence the relationship between retinol and CLD is unknown. Such differences may also be due to chance.

Our results are consistent with the hypothesis that antioxidant properties of some micronutrients such as α-tocopherol and β-carotene may contribute to the inhibition of oxidative stress, and thus the inhibition of liver cancer and liver disease, although other mechanisms are possible. Fruits and vegetables are sources of a number of micronutrients such as carotenoids and flavonoids, and numerous *in vivo* studies have reported the antioxidant properties of these micronutrients (Chatterjee *et al*, 2012). However, confounding by some aspect of the diet could influence observed associations, that is, serum micronutrient levels could reflect another aspect of fruit and vegetables or diet that may be related to liver health. We did adjust our models for intake of fruits and vegetables along with total energy and did not observe any appreciable change in the associations. Nevertheless, foods high in certain micronutrients such as β-carotene are also rich in other components and it is possible that associations with serum micronutrient status are actually reflective of another aspect of diet or lifestyle.

The inverse association observed in the epidemiologic literature and the protective effect that micronutrients have had on liver cancers in experimental animal studies (Glauert *et al*, 2010) generated interest that micronutrient supplementation could hinder the initiation and/or progression of liver disease or liver cancer would be an appropriate direction to take. However, a 2011 Cochrane review reported no evidence of supplementation reducing the risk of liver cancer or liver disease (Bjelakovic *et al*, 2011). Most studies in the Cochrane review had relatively small number of participants and short follow-up time and the doses of the supplements varied greatly (e.g., 30–1000 IU/daily for vitamin E, 6–40 mg/daily for β-carotene, and 5000–10 000 IU/daily for vitamin A). A study based in the Linxian General Population Trial, a large trial that examined various combinations of daily supplemental vitamins and minerals in 29 000 men and women over a 5-year period, reported no overall benefit of various combinations – including one with retinol (5000 IU as retinol palmitate) and zinc (22.5 mg as zinc oxide; Factor 'A') and another with α-tocopherol (30 mg), β-carotene (15 mg), and selenium (50 μg as selenium yeast; Factor 'D') – on liver cancer mortality after a total follow-up of ∼18 years. However, the researchers did observe that those <55 years of age who were randomised to Factor A, and men who did not drink alcohol and were

Table 4. Association of joint (baseline and 3-year follow-up) categories of serum α-tocopherol, β-carotene, and retinol with incident liver cancer and chronic liver disease mortality

	Incident liver cancer			Chronic liver disease mortality		
	L/L	L/H or H/L	H/H	L/L	L/H or H/L	H/H
Serum α-tocopherol						
No. of cases/all	35/4484 1.00	13/2253 0.83 0.43–1.59	28/4634 1.00 0.55–1.81	41/4484 1.00	19/2253 0.93 0.53–1.62	21/4634 0.60 0.32–1.10
Serum β-carotene						
No. of cases/all	35/4328 1.00	16/2526 0.84 0.46–1.53	25/4578 0.80 0.45–1.41	55/4328 1.00	17/2526 0.73 0.42–1.29	11/4578 0.32 0.16–0.65
Serum retinol						
No. of cases/all	80/8543 1.00	34/5464 0.63 0.42–0.94	46/8798 0.51 0.35–0.75	58/8543 1.00	48/5464 0.97 0.65–1.43	51/8798 0.57 0.39–0.85

Abbreviations: H = above the median; L = below the median. Median baseline, α-tocopherol: $11.5 \, \text{mg} \, \text{l}^{-1}$; β-carotene: $170 \, \mu\text{g} \, \text{l}^{-1}$; and retinol: $576 \, \mu\text{g} \, \text{l}^{-1}$. Median follow-up, α-tocopherol: $12.4 \, \text{mg} \, \text{l}^{-1}$; β-carotene: $180 \, \mu\text{g} \, \text{l}^{-1}$; and retinol: $590 \, \mu\text{g} \, \text{l}^{-1}$. Adjusted for Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study intervention arm, age (continuous), body mass index (BMI; continuous), years of smoking (continuous), cigarettes per day (continuous), serum cholesterol (continuous), history of diabetes, marital status, education, and daily intake of alcohol, coffee, fruits (energy adjusted), vegetables (energy adjusted), and total energy. Presented as hazards ratios (HRs) and 95% confidence intervals (CIs).

randomised to Factor D, had a reduced risk (Qu *et al*, 2007). Furthermore, we observed no benefit of supplemental α-tocopherol (DL-α-tocopheryl acetate, 50 IU per day) and β-carotene (20 mg per day) on incident liver cancer or CLD in the ATBC cohort (companion work).

Although such observations may appear to conflict with what has been observed in the epidemiologic literature, as well in our study, observational cohorts that evaluate the relationship between serum micronutrients and liver cancer or liver disease address different hypotheses than randomised controlled trials. Oftentimes, randomised controlled trials, such as ATBC, are conducted in the context of assessing the efficacy of supplementation among older individuals who may have already aged past the critical aetiological window where supplementation may have been helpful. In contrast, observational studies utilise markers that may reflect long-term dietary intake and metabolism, perhaps reflecting micronutrient status during the critical window of disease pathogenesis. It is also possible that the serum micronutrients act as proxies for other aspects of diet and lifestyle such that observed associations in our study reflect confounding. For example, eating foods rich in β-carotene may substantially provide many different nutrients, whereas supplementation with β-carotene provides just one. Although we adjusted for known risk factors in the current analysis, other unmeasured or poorly measured confounders are possible.

One particular concern is underlying liver disease, as the liver plays a critical role in micronutrient metabolism. For example, the liver is the primary storage site for retinol. Serum levels of retinol and its primary precursor, β-carotene, may represent hepatic levels of retinol and possibly reflect health status of the liver, although this has been debated (Newsome *et al*, 2000; Ross and Zolfaghari, 2004; Villaca Chaves *et al*, 2008; Arantes Ferreira Peres *et al*, 2013). Low baseline levels of β-carotene and retinol that are a consequence of any underlying liver cancer or CLD could contribute to the associations that we observed in this study.

In addition, local inflammation is thought to play a critical role in the progression of CLD and development of liver cancer (Nikolaou *et al*, 2013). A growing literature has inversely linked systemic levels of inflammation with micronutrient status, possibly by effects on micronutrient metabolism in the liver (Duncan *et al*, 2012; Gashut *et al*, 2013). Whether these data are relevant to our current findings is unclear, especially as the typically measured marker of systemic inflammation, C-reactive protein, is itself metabolised in the liver and may also be affected by underlying liver disease (Pieri *et al*, 2014). It would also be important to measure local inflammation in liver tissue. Yet, this cannot be done in large-scale cohort studies of healthy volunteers, such as the current study. In lag analyses, we observe similar associations even in cases occurring even more than 10 years after our micronutrient measurements, and this offers some evidence that our results are not simply reflective of participants with late-stage underlying liver disease having lower micronutrient levels. Nevertheless, we cannot specifically exclude this possibility and future complementary analyses in clinical populations with extensive data on underlying liver disease are needed.

Our study had a number of strengths, including that micro-nutrients were measured in blood collected before cancer diagnosis that reduces the potential for reverse causality. With 24 years of follow-up, we were able to investigate associations occurring both in the first couple of years after baseline and many years after the start of follow-up. Blood measurements were available for the entire ATBC cohort, and this reduced the possibility of selection bias. In addition, having two separate measurements occurring 3 years apart likely improved our classification of participant micronutrient status. We also considered known risk factors for these diseases including HBV/HCV status, obesity, alcohol consumption and history of diabetes. However, our study also had some limitations. Because the primary purpose of the ATBC Study was to evaluate whether micronutrient supplements could reduce the risk of lung cancer, liver cancer and liver disease are not primary outcomes. In addition, we lacked assessment of undiagnosed chronic liver disease; however, men who reported cirrhosis at baseline were excluded. We performed a number of statistical analyses, and hence multiple comparisons must be considered. Our findings could also be due to chance. Our study was also conducted among Finnish male smokers and hence may not be applicable to

other populations, although our findings are generally similar to those of previous studies performed in Asia that include participants with a distinct spectrum of underlying risk factors.

As micronutrient status likely reflects many aspects of diet and possibly aspects of lifestyle and health, our data do not provide specific support for supplementation by β-carotene or retinol. In particular, decisions about supplementation should only be interpreted within the entire literature and disease burden. We note that data from the current study and that of Beta-Carotene And Retinol Efficacy Trial (CARET) trial suggest that supplementation can cause harm (Virtamo et al, 2003; Goodman et al, 2004).

In summary, we observed that men with higher serum β-carotene and retinol levels had a lower risk of developing liver cancer and dying of chronic liver disease. In addition, men with higher α-tocopherol levels had lower risk of dying of chronic liver disease, although this association was of borderline statistical significance. These results suggest that a diet high in micronutrients may protect against mortality from CLD and the development of liver cancer. However, it is also possible that higher micronutrient status reflects another aspect of diet, lifestyle, or health. As such, our data should not be considered to provide support for micronutrient supplementation in these diseases. Trial Registration number: The ATBC Study was registered with clinicaltrials.gov (NCT00342992).

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Arantes Ferreira Peres W, Villaca Chaves G, Saraiva Goncalves JC, Ramalho A, Moraes Coelho HS (2013) Assessment of the relative dose-response test as indicators of hepatic vitamin a stores in various stages of chronic liver disease. Nutr Clin Pract 28(1): 95–100.

Bjelakovic G, Gluud LL, Nikolova D, Bjelakovic M, Nagorni A, Gluud C (2011) Antioxidant supplements for liver diseases. Cochrane Database Syst Rev CD007749.

Chatterjee M, Roy K, Janarthan M, Das S (2012) Biological activity of carotenoids: its implications in cancer risk and prevention. Curr Pharm Biotechnol 13(1): 180–190.

Chuang SC, La Vecchia C, Boffetta P (2009) Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. Cancer Lett 286(1): 9–14.

Clemente C, Elba S, Buongiorno G, Berloco P, Guerra V, Di Leo A (2002) Serum retinol and risk of hepatocellular carcinoma in patients with child-Pugh class A cirrhosis. Cancer Lett 178(2): 123–129.

Das BC, Thapa P, Karki R, Das S, Mahapatra S, Liu TC, Torregroza I, Wallace DP, Kambhampati S, Van Veldhuizen P, Verma A, Ray SK, Evans T (2014) Retinoic acid signaling pathways in development and diseases. Bioorg Med Chem 22(2): 673–683.

Duncan A, Talwar D, McMillan DC, Stefanowicz F, O'Reilly DS (2012) Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. Am J Clin Nutr 95(1): 64–71.

Factor VM, Laskowska D, Jensen MR, Woitach JT, Popescu NC, Thorgeirsson SS (2000) Vitamin E reduces chromosomal damage and inhibits hepatic tumor formation in a transgenic mouse model. Proc Natl Acad Sci USA 97(5): 2196–2201.

Fan JG, Farrell GC (2009) Prevention of hepatocellular carcinoma in nonviral-related liver diseases. J Gastroenterol Hepatol 24(5): 712–719.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12): 2893–2917.

Gambino R, Musso G, Cassader M (2011) Redox balance in the pathogenesis of nonalcoholic fatty liver disease: mechanisms and therapeutic opportunities. Antioxid Redox Signal 15(5): 1325–1365.

Gashut AR, McMillan DC, Kinsella J, Duncan A, Talwar D (2013) Quantitative data on the magnitude of the systemic inflammatory response and its effect on carotenoids status based on plasma measurements. e-SPEN J 8(5): e193–e199.

Glauert HP, Calfee-Mason K, Stemm DN, Tharappel JC, Spear BT (2010) Dietary antioxidants in the prevention of hepatocarcinogenesis: a review. Mol Nutr Food Res 54(7): 875–896.

Goodman GE, Thornquist MD, Balmes J, Cullen MR, Meyskens Jr FL, Omenn GS, Valanis B, Williams Jr JH (2004) The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. J Natl Cancer Inst 96(23): 1743–1750.

Ha HL, Shin HJ, Feitelson MA, Yu DY (2010) Oxidative stress and antioxidants in hepatic pathogenesis. World J Gastroenterol 16(48): 6035–6043.

Heron M (2012) Deaths: leading causes for 2009. Natl Vital Stat Rep 61(7): 1–96.

Hopps E, Noto D, Caimi G, Averna MR (2010) A novel component of the metabolic syndrome: the oxidative stress. Nutr Metab Cardiovasc Dis 20(1): 72–77.

Ito Y, Suzuki K, Ishii J, Hishida H, Tamakoshi A, Hamajima N, Aoki K (2006) A population-based follow-up study on mortality from cancer or cardiovascular disease and serum carotenoids, retinol and tocopherols in Japanese inhabitants. Asian Pac J Cancer Prev 7(4): 533–546.

Knekt P, Aromaa A, Maatela J, Alfthan G, Aaran RK, Nikkari T, Hakama M, Hakulinen T, Teppo L (1991) Serum micronutrients and risk of cancers of low incidence in Finland. Am J Epidemiol 134(4): 356–361.

Korhonen P, Malila N, Pukkala E, Teppo L, Albanes D, Virtamo J (2002) The Finnish Cancer Registry as follow-up source of a large trial cohort– accuracy and delay. Acta Oncol 41(4): 381–388.

Lim YS, Kim WR (2008) The global impact of hepatic fibrosis and end-stage liver disease. Clin Liver Dis 12(4): 733–746, vii.

Marchesini G, Moscatiello S, Di Domizio S, Forlani G (2008) Obesity-associated liver disease. J Clin Endocrinol Metabol 93(11 Suppl 1): S74–S80.

Marra M, Sordelli IM, Lombardi A, Lamberti M, Tarantino L, Giudice A, Stiuso P, Abbruzzese A, Sperlongano R, Accardo M, Agresti M, Caraglia M, Sperlongano P (2011) Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. J Transl Med 9: 171.

Milne DB, Botnen J (1986) Retinol, alpha-tocopherol, lycopene, and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin Chem 32(5): 874–876.

Moreno FS, T S-Wu, Naves MM, Silveira ER, Oloris SC, da Costa MA, Dagli ML, Ong TP (2002) Inhibitory effects of beta-carotene and vitamin a during the progression phase of hepatocarcinogenesis involve inhibition of cell proliferation but not alterations in DNA methylation. Nutr Cancer 44(1): 80–88.

Newsome PN, Beldon I, Moussa Y, Delahooke TE, Poulopoulos G, Hayes PC, Plevris JN (2000) Low serum retinol levels are associated with hepatocellular carcinoma in patients with chronic liver disease. Aliment Pharmacol Ther 14(10): 1295–1301.

Nikolaou K, Sarris M, Talianidis I (2013) Molecular pathways: the complex roles of inflammation pathways in the development and treatment of liver cancer. Clin Cancer Res 19(11): 2810–2816.

Pan WH, Wang CY, Huang SM, Yeh SY, Lin WG, Lin DI, Liaw YF (1993) Vitamin A, Vitamin E or beta-carotene status and hepatitis B-related hepatocellular carcinoma. Ann Epidemiol 3(3): 217–224.

Pieri G, Agarwal B, Burroughs AK (2014) C-reactive protein and bacterial infection in cirrhosis. Ann Gastroenterol 27(2): 113–120.

Pietinen P, Hartman AM, Haapa E, Rasanen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J, Huttunen JK (1988) Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol* **128**(3): 655–666.

Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, Ghirlanda G (2010) Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud* **7**(1): 15–25.

Powell EE, Jonsson JR, Clouston AD (2010) Metabolic factors and non-alcoholic fatty liver disease as co-factors in other liver diseases. *Dig Dis* **28**(1): 186–191.

Qu CX, Kamangar F, Fan JH, Yu B, Sun XD, Taylor PR, Chen BE, Abnet CC, Qiao YL, Mark SD, Dawsey SM (2007) Chemoprevention of primary liver cancer: a randomized, double-blind trial in Linxian, China. *J Natl Cancer Inst* **99**(16): 1240–1247.

Roberts SE, Goldacre MJ, Yeates D (2005) Trends in mortality after hospital admission for liver cirrhosis in an English population from 1968 to 1999. *Gut* **54**(11): 1615–1621.

Ross AC, Zolfaghari R (2004) Regulation of hepatic retinol metabolism: perspectives from studies on vitamin A status. *J Nutr* **134**(1): 269S–275S.

Rui R, Lou J, Zou L, Zhong R, Wang J, Xia D, Wang Q, Li H, Wu J, Lu X, Li C, Liu L, Xia J, Xu H (2012) Excess body mass index and risk of liver cancer: a nonlinear dose-response meta-analysis of prospective studies. *PLoS One* **7**(9): e44522.

Sorensen HT, Thulstrup AM, Mellemkjar L, Jepsen P, Christensen E, Olsen JH, Vilstrup H (2003) Long-term survival and cause-specific mortality in patients with cirrhosis of the liver: a nationwide cohort study in Denmark. *J Clin Epidemiol* **56**(1): 88–93.

Souza MR, Diniz Mde F, Medeiros-Filho JE, Araujo MS (2012) Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol* **49**(1): 89–96.

The ATBC Cancer Prevention Study Group (1994) The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. *Ann Epidemiol* **4**(1): 1–10.

Villaca Chaves G, Pereira SE, Saboya CJ, Ramalho A (2008) Non-alcoholic fatty liver disease and its relationship with the nutritional status of vitamin A in individuals with class III obesity. *Obes Surg* **18**(4): 378–385.

Virtamo J, Pietinen P, Huttunen JK, Korhonen P, Malila N, Virtanen MJ, Albanes D, Taylor PR, Albert P (2003) Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. *JAMA* **290**(4): 476–485.

Williams R (2006) Global challenges in liver disease. *Hepatology* **44**(3): 521–526.

Yamamoto Y, Yamashita S, Fujisawa A, Kokura S, Yoshikawa T (1998) Oxidative stress in patients with hepatitis, cirrhosis, and hepatoma evaluated by plasma antioxidants. *Biochem Biophys Res Commun* **247**(1): 166–170.

Yu MC, Yuan JM (2004) Environmental factors and risk for hepatocellular carcinoma. *Gastroenterology* **127**(5 Suppl 1): S72–S78.

Yu MW, Chiu YH, Chiang YC, Chen CH, Lee TH, Santella RM, Chern HD, Liaw YF, Chen CJ (1999) Plasma carotenoids, glutathione S-transferase M1 and T1 genetic polymorphisms, and risk of hepatocellular carcinoma: independent and interactive effects. *Am J Epidemiol* **149**(7): 621–629.

Yu MW, Hsieh HH, Pan WH, Yang CS, CH CJ (1995) Vegetable consumption, serum retinol level, and risk of hepatocellular carcinoma. *Cancer Res* **55**(6): 1301–1305.

Yuan JM, Gao YT, Ong CN, Ross RK, Yu MC (2006) Prediagnostic level of serum retinol in relation to reduced risk of hepatocellular carcinoma. *J Natl Cancer Inst* **98**(7): 482–490.

Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)