Methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC Study) cohort II was launched in 1993–1994 in registered Japanese inhabitants aged 40–69 years at the beginning of the respective baseline survey in six prefectural public health center (PHC) areas ($n = 68\,980$). Details of the study design have been described elsewhere (Tsugane and Sobue, 2001; Inoue *et al.*, 2005). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Japan.

For the present analysis, a total of 19 812 individuals who responded to the questionnaire and provided a blood sample and health checkup data were enrolled.

Baseline survey

A baseline self-administered questionnaire survey on various lifestyle factors was conducted in 1993–1994 (response rate = 82%). A total of 10 ml of blood was also provided voluntarily by 29% of participants during health checkups provided by the local government. The plasma and buffy layer were divided into four tubes holding 1.0 ml each (three tubes for plasma and one for the buffy layer) and stored at −80°C.

Follow-up and identification of hepatocellular carcinoma

Participants were followed from the baseline survey until December 31, 2005. Residence status, including survival, was confirmed through the residential registry. In Japan, resident and death registration are required by law and the registries are believed to be complete. Inspection of the resident registry is legally sanctioned by the resident registration law. The occurrence of HCC was determined by notification from hospitals in the study areas and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 4.8%. This ratio was of satisfactory quality, for this study, on the basis of the international standard (Parkin *et al.*, 2002). The site of origin and histological type were coded using the International Classification of Diseases for Oncology, third edition (ICD-O-3; C22.0) (World Health Organization, 2000). We excluded participants with no data on aminotransferase levels in blood samples taken during their health checkup. Through this procedure, a total of 109 newly diagnosed HCC cases were identified during follow-up as of December 31, 2005.

Laboratory analysis

Serum ALT, AST, and γ-glutamyl transferase (GGT) levels were determined at the baseline health checkup. These items were measured in 23 laboratories in the cohort area, with accuracy control and standardization among the laboratories provided by the Japan Medical Association via its External Quality Control Survey. The upper limit of normal ALT was tentatively defined as 30 IU/l, as used in recent clinical or prospective studies (Kunde *et al.*, 2005; Okanoue *et al.*, 2005).

Plasma samples were screened for anti-HCV using a third-generation immunoassay (Lumipulse II Ortho HCV; Ortho-Clinical Diagnostics K.K., Tokyo, Japan) (Abdel-Hamid *et al.*, 2002) and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology Co., Ltd, Tokyo, Japan). The virus-positive group consisted of individuals positive for either or both anti-HCV and HBsAg.

Statistical analysis

P values for differences between groups were calculated using the χ^2 test or *t*-test. Person–years of follow-up were calculated from the date of baseline survey until the date of diagnosis of HCC, the date of a participant's death, the date of moving from a PHC area, or 31st December 2005, whichever occurred first. Multivariate-adjusted hazard ratios (HR) and corresponding 95% confidence intervals (CI) of HCC were estimated by the Cox proportional hazards model. The estimates were adjusted for the following potential confounding factors incorporated into the model: hepatitis virus positivity (HCV or HBV – positive, negative), age at baseline (40–49, 50–59, 60–69 years), study area (six PHC areas), smoking status (never, past, current), weekly ethanol intake (none, 1–149, 150 g or more), body mass index ($< 23.0\,kg/m^2$, 23.0–24.9, 25.0–26.9, 27.0–29.9, ≥ 30.0), and coffee intake (almost never, 1–4 days/week, almost every day). Statistical analyses were performed using STATA version 9.2 (StataCorp, Texas, USA) (Stata Corporation, 2005).

Results

Of 19 812 participants, 737 were identified with HCV monoinfection (3.7%), 479 with HBV monoinfection (2.4%), and 20 with HCV and HBV coinfection (0.1%). The proportion of participants with an ALT level ≥ 30 IU/l was 35.1% in the virus-positive group, and 11.0% in the virus-negative group. Current smokers and heavy alcohol drinkers tended to have elevated ALT levels regardless of virus-positive or virus-negative status. Further, obese participants without hepatitis virus infection had higher ALT levels (Table 1).

During the 234 016 person–years of follow-up (average follow-up period, 11.8 years) for the 19 812 participants (6920 men and 12 892 women), a total of 109 newly diagnosed cases of HCC (71 men, 38 women) were documented. Of these, 75 participants had HCV monoinfection (68.8%), 10 had HBV monoinfection (9.2%),

Table 1 Baseline characteristics

	Hepatitis virus-positive[c] n=1236			Hepatitis virus-negative n=18576		
	Serum ALT level			Serum ALT level		
	<30 IU/l	≥ 30 IU/l	P[b]	<30 IU/l	≥ 30 IU/l	P[b]
Number	802	434		16528	2048	
Age (years)[a]	58.3 (7.8)	58.2 (7.6)	0.41	57.3 (8.2)	55.4 (7.9)	0.001
Sex, men (%)	39.9	56.5	0.001	32.0	52.1	0.001
Current smoker (%)	24.0	29.8	0.001	15.7	23.5	0.001
Ethanol intake ≥ 150 g/week (%)	14.0	18.8	0.02	13.3	25.4	0.001
Body mass index ≥ 27 kg/m^2 (%)	10.6	13.4	0.30	11.0	26.4	0.001
Coffee intake, daily (%)	8.7	8.2	0.21	9.6	7.5	0.36

ALT, alanine aminotransferase.
[a]Mean (SD).
[b]P for difference.
[c]Anti-hepatitis C virus-positive and/or hepatitis B virus antigen-positive.

two had coinfection with HCV and HBV (1.8%), and 22 had no virus infection (20.9%).

After adjustment for potential confounding risk factors, such as hepatitis virus positivity, sex, age, study area, weekly ethanol intake, body mass index and coffee intake, HCC was found to occur significantly more frequently in participants with serum ALT levels ≥ 30 IU/l (HR: 13.5, 95% CI: 8.0–22.0), serum AST levels ≥ 30 IU/l (HR: 14.3, 95% CI: 8.0–25.8), and serum GGT levels ≥ 60 IU/l (HR: 5.5, 95% CI: 3.5–8.8). ALT and AST levels increased in parallel (correlation coefficient = 0.81). Most cases had both an abnormal ALT level and abnormal AST level, although a few had a normal ALT level and abnormal AST level or the converse. In contrast, the correlation of ALT and GGT was relatively low (correlation coefficient = 0.43). We also observed that the association of incidence of HCC with elevated GGT level was relatively weak compared with that with ALT level after further adjustment for serum ALT (HR: 2.1, 95% CI: 1.3–3.3) (Table 2). On these bases, we restricted further analysis to ALT.

Compared with participants in the normal range of ALT (< 30 IU/l), those with elevated levels (30–69, 70–99, ≤ 100 IU/l) had a significantly higher risk of developing HCC [HR: 10.5 (95% CI: 6.0–18.3), 25.2 (12.7–49.7), and 43.9 (22.7–84.8), respectively] after adjustment for virus positivity, age, sex, study area, smoking status, ethanol intake, body mass index, and coffee intake. We observed positive linear trends in HR according to level of ALT category ($P < 0.001$).

Among virus-positive participants (virus-positive; anti-HCV and/or HBsAg-positive), elevated ALT (30–69, 70–99, 100 IU/l) was significantly associated with the incidence of HCC [HR: 12.0 (95% CI: 5.8–24.9), 25.6 (11.3–58.1), and 37.1 (16.3–84.2), respectively] (Table 3). Cumulative incidence of HCC at 10 years among virus-positive participants was 1.1% for participants with ALT less than 30 IU/l, but 11.0 and 27.2% for those with ALT

Table 2 Hazard ratios (HR) and 95% confidence intervals (CI) of hepatocellular carcinoma by serum liver enzyme level

	Person-years	No. of cases	HR[a]	(95% CI)
Serum ALT level				
<30 IU/l	205509	20	1.0	
≥ 30 IU/l	28507	89	13.5	(8.0–22.0)
Serum AST level				
<30 IU/l	202065	15	1.0	
≥ 30 IU/l	31951	94	14.3	(8.0–25.8)
Serum GGT level				
<60 IU/l	218544	70	1.0	
≥ 60 IU/l	15472	39	5.5[b]	(3.5–8.8)

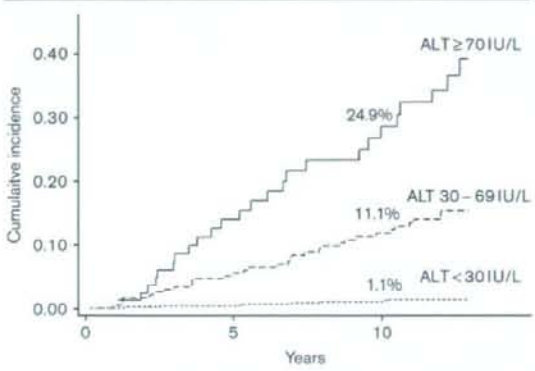
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.
[a]Adjusted for hepatitis virus positivity (negative, positive), sex, years of age at baseline (40–49, 50–59, 60–69 years) and study area (six public health center areas), weekly ethanol intake (none, 1–149, 150 g and more), body mass index (<23.0 kg/m^2, 23.0–24.9, 25.0–26.9, 27.0–29.9, ≥ 30.0), and coffee intake (almost never, 1–4 days/week, almost every day).
[b]HR: 2.1, 95% CI 1.3–3.3 after further adjustment for ALT level.

Table 3 Hazard ratios (HR) and 95% confidence intervals (CI) of hepatocellular carcinoma incidence by serum ALT level

	Person-years	No. of cases	HR	(95% CI)	P for trend
Serum ALT level					
Total[a] (n=19812)					
– 29 IU/l	205509	20	1.0		
30–69 IU/l	25361	48	10.5	(6.0–18.3)	
70–99 IU/l	2087	18	25.2	(12.7–49.7)	
100- IU/l	1059	23	43.9	(22.7–84.8)	0.001
Virus-positive[b] (n=1236)					
– 29 IU/l	9341	10	1.0		
30–69 IU/l	3406	41	12.0	(5.8–24.9)	
70–99 IU/l	571	17	25.6	(11.3–58.1)	
100- IU/l	481	19	37.1	(16.3–84.2)	0.001
HCV[b] (n=757)					
– 29 IU/l	4724	6	1.0		
30–69 IU/l	2543	37	11.4	(4.7–27.2)	
70–99 IU/l	485	16	25.1	(9.8–64.8)	
100- IU/l	425	18	35.0	(13.4–91.4)	0.001
HBV[b] (n=499)					
– 29 IU/l	4744	4	1.0		
30–69 IU/l	933	6	18.5	(3.7–93.1)	
70-IU/l	165	2	35.0	(4.2–293.1)	0.001
Virus-negative[b] (n=18576)					
– 29 IU/l	196167	10	1.0		
30–69 IU/l	21956	7	6.5	(2.2–18.8)	
70- IU/l	2095	5	60.5	(19.5–187.9)	0.001

ALT, alanine aminotransferase; HBV, hepatitis B virus antigen-positive; HCV, anti-hepatitis C virus-positive.
[a]Adjusted for hepatitis virus positivity (negative, positive), sex, years of age at baseline (40–49, 50–59, 60–69 years) and study area (six public health center areas), weekly ethanol intake (none, 1–149, 150 g and more), body mass index (<23.0 kg/m^2, 23.0–24.9, 25.0–26.9, 27.0–29.9, ≥ 30.0), and coffee intake (almost never, 1–4 days/week, almost every day).
[b]Adjusted for sex, years of age at baseline (40–49, 50–59, 60–69 years) and study area (six public health center areas), weekly ethanol intake (none, 1–149, 150 g and more), body mass index (<23.0 kg/m^2, 23.0–24.9, 25.0–26.9, 27.0–29.9, ≥ 30.0), and coffee intake (almost never, 1–4 days/week, almost every day).

Fig. 1



Cumulative incidence of hepatocellular carcinoma (HCC) among hepatitis virus-positive participants by serum alanine aminotransferase (ALT) level. Cumulative incidence of HCC at 10 years was 1.1% for participants with ALT less than 30 IU/l, but 11.0 and 27.2% for those with ALT 30–69 IU/l and ALT ≥ 70 IU/l, respectively. Elevated ALT levels showed a statistically significant association with the risk of HCC compared with normal ALT levels (ALT <30 IU/l) after adjustment for age, sex, study area, smoking status, ethanol intake, body mass index, and coffee intake.

Table 4 Hazard ratios (HR) and 95% confidence intervals (CI) of hepatocellular carcinoma incidence by serum ALT level and virus positivity

	Person-years	No. of cases	HR[a]	(95% CI)	*P* for trend
Serum ALT level					
Virus-negative[b]					
–29 IU/l	196 167	10	1.0		
30-IU/l	24 050	12	9.4	(3.9–22.3)	
Virus-positive					
–29 IU/l	9341	10	15.2	(6.1–37.6)	
30–69 IU/l	3406	41	180.5	(89.4–364.2)	
70-IU/l	1052	36	454.2	(221.5–931.2)	0.001

ALT, alanine aminotransferase.
[a]Adjusted for sex, years of age at baseline (40–49, 50–59, 60–69 years) and study area (six public health center areas), weekly ethanol intake (none, 1–149, 150 g and more), body mass index (<23.0 kg/m^2, 23.0–24.9, 25.0–26.9, 27.0–29.9, ≥ 30.0), and coffee intake (almost never, 1–4 days/week, almost every day).
[b]Neither anti-hepatitis C virus-positive nor hepatitis B virus antigen-positive.

between 30 and 69 IU/l and ALT ≥ 70 IU/l, respectively (Fig. 1). This ALT-dependent increase in risk was identified in subset analyses of participants with HCV (anti-HCV-positive), HBV (HBsAg-positive), and without hepatitis virus infection (virus-negative) (Table 3). Furthermore, virus-positive participants with normal ALT had a higher risk of HCC than virus-negative participants with normal ALT [HR, 15.2 (95% CI: 6.1–37.6)]. Virus-positive participants with elevated ALT (30–69, 70 IU/l) had an extremely elevated risk of HCC [HR, 180.5 (95% CI: 89.4–364.2), and 454.2 (221.5–931.2), respectively] (Table 4).

Discussion

This population-based prospective study in Japan has demonstrated that serum ALT level is concentration-dependently associated with an increased risk of HCC in both virus-positive and virus-negative participants. Compared with virus-negative participants with a normal ALT level (< 30 IU/l), virus-positive participants with an ALT level ≥ 30 IU/l had a greater than 180-fold higher risk of HCC as well as higher cumulative incidence of HCC at 10 years. This finding suggests the need for antiviral therapy in these patients to reduce the risk of HCC. Even virus-negative participants with an ALT level ≥ 30 IU/l and virus-positive participants with an ALT level less than 30 IU/l showed a statistically significant risk of HCC, suggesting the necessity of regular follow-up.

An association between higher levels of ALT or AST and the development of HCC has been hypothesized, but few studies have investigated the link. In a prospective study in Japan, Tanaka *et al.* (2004) investigated the association between serum ALT level and incidence of HCC in 1927 voluntary blood donor individuals positive for anti-HCV and negative for HBsAg. Results showed that elevated serum ALT level at blood donation was positively associated with the risk of HCC; compared with participants at less than 30 IU/l, those at 30–59 IU/l had a 6.2-fold increase in risk, whereas those at more than 60 IU/l had a 9.5-fold increase. This stepwise increase was statistically significant ($P < 0.0001$). Although our cutoff and categorization of ALT were different, our present data also identified a stepwise increase in risk of HCC with increasing ALT in participants with anti-HCV. Further, Suruki *et al.*'s (2006) investigation of risk in a prospective community-based study in a single prefecture in Japan identified 667 anti-HCV-positive participants and 52 cases of HCC at 10-years' follow-up. The risk of HCC was increased four-fold with abnormal (≥ 35 IU/l) compared with normal ALT levels (< 35 IU/l).

The long-term outcome of elevated ALT values in patients with virus infection remains uncertain. A second finding from Suruki *et al.*'s (2006) prospective study was that participants with persistently abnormal ALT over multiple measurements during follow-up had a 19.8-fold risk of HCC compared with those with persistently normal values. Persico *et al.*'s (2000) prospective evaluation of disease progression in 37 HCV-infected patients found that chronic hepatitis with persistently normal ALT serum levels was mild and did not progress with time. Another study, which assessed hepatic fibrosis by liver biopsy in HCV patients with normal ALT levels, showed weaker histological activity and a lower progression rate of fibrosis (Mathurin *et al.*, 1998). Regarding hepatocarcinogenesis, persistently high serum ALT levels were closely associated with a high incidence of HCC in

patients with chronic HCV hepatitis (Tarao *et al.*, 2002), and with multicentric hepatocarcinogenesis in compensated cirrhosis patients with HCV infection in a follow-up study (Hayashi *et al.*, 2000).

As participants with hepatitis virus and a normal ALT range might be less likely to develop HCC, participants with normal ALT have been routinely excluded from clinical trials with interferon therapy. However, several studies have reported marked liver lesions or fibrosis in HCV patients with persistently normal ALT levels (Martinot-Peignoux *et al.*, 2001; Pradat *et al.*, 2002), and such participants in this study had a 15-fold risk of HCC compared with noninfected participants with normal ALT levels. Together, these findings advocate against the routine exclusion of patients with 'normal' ALT levels from therapy. Zeuzem *et al.* (2004) reported that combination therapy with peginterferon α-2a and ribavirin produced comparable sustained virological response rates and safety in patients with elevated ALT activity as in those with normal ALT. Treatment should be appropriate to the results of risk evaluation for items such as age, infectious period, and virus genotype (Bacon, 2002). From our results, the population-attributable fraction (Rockhill *et al.*, 1998) for HCC occurring during the study period attributable to virus-positive participants with normal ALT can be estimated as 8.6%. Notwithstanding the low fraction of total HCC in this population, a decrease in incidence of 8.6% would be achieved if treatment were provided.

Further, participants in our study who were neither anti-HCV-positive nor HBsAg-positive but had elevated ALT levels had a positive association with the incidence of HCC, albeit in a small number of cases. This association remained even after adjustment for alcohol consumption. To our knowledge this finding has not been previously reported.

The role of obesity in these findings also warrants mention. Elevated ALT can result from such etiologies as hepatitis virus infection and alcohol consumption to NAFLD, autoimmune liver disease, and metabolic disease (Yu and Keeffe, 2003). Of these, NAFLD has recently attracted attention because of its close association with obesity, metabolic syndrome, and progression to cryptogenic cirrhosis (Clark and Diehl, 2003; Marchesini *et al.*, 2003; Caldwell *et al.*, 2004). NAFLD is often associated with obesity and is assumed to be a common underlying liver disease in nonviral infection patients with HCC in the United States (Marrero *et al.*, 2002). In our study, it is uncertain whether elevated ALT was caused by NAFLD because no histological evidence was obtained; nevertheless, the association between ALT level and incidence of HCC was identified after adjustment for body mass index, indicating that elevated levels cannot be explained by obesity or NAFLD only, and raising the possibility that unknown factors might have influenced the incidence of HCC. Our study identified a positive association between elevated ALT and the incidence of HCC in participants with HBV infection, as previously reported (Bell *et al.*, 2005), but any interpretation of this finding requires caution owing to the small number of cases.

The strength of this study is its population-based prospective design and low proportion of losses to follow-up (0.1%). Information was collected before the subsequent diagnosis of cancer, thereby avoiding the exposure recall bias inherent to case–control studies. Moreover, the proportion of losses to follow-up during the study period was negligible.

Nonetheless, several obvious limitations can be identified. First, we had no information on the clinical severity of hepatitis, or on the treatment of participants with hepatitis virus infection before and during the study period. Interferon therapy, which decreases the risk of HCC in responding patients with HCV and HBV infection, has been available in Japan for the last decade, and it is possible that some of our infected participants received this treatment. This in turn may have led to the underestimation of HCC occurrence by responders, which would also bias the results toward the null. As most participants were asymptomatic and lived in rural areas, however, most eligible participants would not have received interferon therapy, and the influence of interferon, if present, is considered to be relatively small.

Second, evaluation of a single ALT measurement at baseline might have produced misclassification. Aminotransferase levels fluctuate on a day-to-day basis, with exercise and with liver disease, particularly hepatitis virus infection, and the need for multiple measurement to ensure accuracy is well recognized (Green and Flamm, 2002). Further, without clinical information, participants with severe liver injury in whom damaged hepatocytes are unable to produce ALT might have been classified into the normal group. If such misclassifications were present, however, they are likely to be nondifferential and would in any case lead to an underestimation of results.

Third, differences in the proportion of hepatitis virus positivity or in the dominant type of hepatitis virus may have resulted in geographical variation in blood biochemistry testing or incidence of HCC. We assumed that the association between ALT level and risk of HCC was not influenced by geographical area, but nevertheless adjusted for study area (PHC) on analysis. The small number of HCC cases prevents any conclusive evaluation of this point, but we assume that the influence of geographical area on the risk of HCC is not substantial.

Fourth, the failure to confirm HBV DNA means that occult HBV infection may have been present in noninfected HCC cases. Even after HBsAg clearance, some patients with chronic HBV infection develop HCC (Huo *et al.*, 1998). If present, however, such misclassification would be random, and in any case would bias the results toward the null.

Further, the present participants were restricted to the 28.7% of the study participants of the JPHC Study cohort II group who provided blood samples. More women than men tend to participate in health checkup surveys provided by local governments. Participants often differ from nonparticipants in socioeconomic status and have a more favorable lifestyle profile, such as lower smoking rates, greater participation in physical exercise, and higher intake of green vegetables and fruits, particularly women (Iwasaki *et al.*, 2003), although the influence of these factors on the association between hepatitis virus-related factors and HCC would not be substantial. In addition, the incidence of HCC in this study population was 46.6 cases per 100 000 people during the follow-up period, versus 64.2 cases per 100 000 people in the whole JPHC Study cohort II, suggesting that participants who were already under care for hepatitis infection may have been less willing to attend a health checkup. Together, these considerations mandate the need for caution in interpreting or generalizing these results.

In conclusion, this population-based prospective study in Japan showed that an increase in serum ALT level is positively associated with the incidence of HCC. Risk increased stepwise in an ALT concentration-dependent manner regardless of hepatitis virus infection status. Although any interpretation of these results requires close consideration of these methodological issues, this finding may indicate that ALT level is a good independent determinant of the need for intervention. Clinical application of this finding may contribute to a decrease in HCC-associated mortality.

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Appendix

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Isoflavone consumption and subsequent risk of hepatocellular carcinoma in a population-based prospective cohort of Japanese men and women

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The incidence of hepatocellular carcinoma (HCC) is much higher in men than in women. Several experiment and epidemiological studies have suggested that estrogen might play an inhibitory role in the development of HCC. Because isoflavones have a similar structure as 17β-estradiol and appear to have an anti-estrogenic effect in women and estrogenic effect in men, we hypothesized that the effect of isoflavones on HCC differs by sex. We investigated the association between isoflavones (genistein and daidzein) and soy products and HCC in Japan in a population-based prospective study in 19,998 Japanese (7,215 men and 12,783 women) aged 40–69 years. During 11.8 years of follow-up, 101 subjects (69 men and 32 women) were newly diagnosed with HCC. Case patients were grouped according to consumption of isoflavones and soy products and stratified by hepatitis virus infection. Hazard ratios (HRs) and 95% confidence intervals (CIs) for HCC were calculated by Cox proportional-hazards modeling. In women, genistein and daidzein were dose-dependently associated with an increased risk of HCC, with multivariable HRs for the highest versus lowest tertile of 3.19 (95%CI = 1.13–9.00, p_{trend} = 0.03) and 3.90 (95% CI = 1.30–11.69, p_{trend} = 0.01), respectively. No association between isoflavones and HCC was observed in men. These results persisted when analysis was restricted to subjects positive for either or both hepatitis C and B virus. In conclusion, isoflavone consumption may be associated with an increased risk of HCC in women. Women with hepatitis virus infection may be advised to abstain from isoflavone consumption. Further studies are warranted to confirm these findings.
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Key words: isoflavone; hepatocellular carcinoma (HCC); hepatitis C virus (HCV); hepatitis B virus (HBV); JPHC study

Hepatocellular carcinoma (HCC) is an important disease worldwide. In Japan, HCC ranks as the third- and fourth-leading cause of death from cancer among men and women, respectively.[1] The most important risk factors for the development of HCC in humans are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).[2] However, despite a similar prevalence of chronic HCV or HBV infection in men and women,[3,4] the incidence of HCC is higher in men, with the International Agency for Research on Cancer (IARC) reporting a 2-fold or greater difference.[5] Although this may partly result from differences in exposure to environmental risk factors such as alcohol consumption and cigarette smoking, several human and nonhuman studies point to a possible role of hormonal factors. In laboratory experiments, ovariectomy in mice increased susceptibility to chemically induced hepatocarcinogenesis,[6,7] whereas administration of estrogens inhibited the development of HCC in male mice.[7] Additionally, Naugler *et al.*[8] showed that estrogen-mediated inhibition of interleukin-6 (IL-6) reduced HCC risk in female mice. In an epidemiological study, Yu *et al.* reported that natural menopause at a younger age and ovariectomy during premenopause were associated with an increased risk of HCC.[9] These various observations suggest that sex hormones, especially estrogen, may confer a protective effect against the development of HCC.

Isoflavones are structurally similar to 17β-estradiol and have the ability to bind to estrogen receptors (ERs),[10] suggesting that they may influence the development of HCC. However, to date this possibility has received relatively scant interest.[11–16] Isoflavones act as estrogen agonists and also as antagonists competing for estradiol at the receptor complex.[17] Because physiological levels of estradiol differ substantially between men and women,

Abbreviations: CI, confidence interval; ER, estrogen receptor; HBsAg, hepatitis B virus antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IL, interleukin.

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we speculated that the effects of isoflavones on HCC might differ by sex. Long-term isoflavone consumption in typical daily life appears to have an anti-estrogenic effect in women and an estrogenic effect in men, although several studies have reported that short-term dietary soy showed a weak estrogenic response in the breast in women.[18–20] Indeed, in epidemiological studies, isoflavones have been inversely associated with breast cancer in women[10,21,22] and prostate cancer in men.[10,23,24] Additionally, the inverse association between isoflavones and breast cancer was more pronounced in women with high blood levels of estradiol.[25] On the basis of these, the effects of isoflavones may be dependent on endogenous levels of estradiol, and we hypothesized that their effects on HCC may differ by sex. However, previous epidemiological findings for isoflavones or soy food intake and HCC are inconsistent, and most studies did not analyze by sex,[11,13–16] nor consider HCV or HBV infection status.[11,12,14,15]

Here, we investigated the presence of an association between isoflavone consumption and HCC in Japanese men and women in a large-scale population-based cohort study in Japan, with due consideration for HCV and HBV infection status.

Material and methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC study) Cohort II was initiated in 1993–1994. This cohort consisted of 6 PHC areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa and Osaka) across Japan. The study design has been described in detail previously.[26] The study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. The study population was defined as all residents aged 40–69 years at the start of the respective baseline survey. In the present analysis, we excluded some subjects from the Osaka area for whom different definitions were used. Initially, we defined a population-based cohort of 68,974 subjects (33,888 men and 35,086 women) after the exclusion of ineligible subjects ($n = 103$).

Baseline survey

At baseline, participants completed a self-administered questionnaire that assessed information on personal medical history, smoking and drinking habits, diet and other lifestyle factors. Completed questionnaires were received from 26,850 men (response rate, 79%) and 29,785 women (response rate, 85%). Subjects with a self-reported history of cancer at baseline were excluded from analysis ($n = 1,219$).

Blood collection

Subjects were asked to voluntarily provided 10 mL of blood during health checkups in 1993–1995. Samples were divided into plasma and buffy layers, and preserved at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 20,406 subjects (36%) (7,442 men and 12,964 women) donated blood.

Food frequency questionnaire

The questionnaire asked about the usual consumption of 52 foods, including beverages, during the previous year. We then calculated the consumption of isoflavones (genistein and daidzein) and soy food. Soy food referred to the consumption of *tofu*, *miso* (soybean paste) and *natto* (fermented soybeans), for which the major ingredient is soybean. Standard portion sizes were specified for each food item in 3 amounts: small (50% smaller), medium (same as the standard) and large (50% larger). The frequency of soy food intake was divided into 5 categories: almost never, sometimes, 1 or 2 times per week, 3 or 4 times per week and almost daily. The total consumption of soy food (g/day) was calculated from these responses, whereas isoflavone consumption was calculated using values in a specially developed food composition table for isoflavones in Japanese foods.[27,28] Energy was calculated using the fifth revised edition of the Standard Tables of Food Composition in Japan.[29]

Validity was assessed in subsamples using 14- or 28-day dietary records. Spearman's correlation coefficients between energy-adjusted intake of soy food from the questionnaire and from dietary records for men and women were 0.47 and 0.44, respectively, whereas those for energy-adjusted intake of genistein and daidzein were 0.56 and 0.55 for men, and 0.51 and 0.49 for women, respectively (unpublished data).

Among the 20,406 subjects who responded to the questionnaire and provided a blood sample, 408 who reported extreme total energy intake (upper 1.0% or lower 1.0%) were excluded, leaving 19,998 subjects (7,215 men and 12,783 women) for analysis.

Follow-up and identification of HCC

Subjects were followed from the baseline survey until December 31, 2005. Changes in residence status, including survival, were identified annually through the residential registry in their public health center area. Among study subjects, 1,070 (5.4%) moved out of their study area and 49 (0.2%) were lost to follow-up during the study period.

Incidence data on HCC were identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Death certificates were used as a supplementary information source. In our cancer registry system, 5.9% of cases were based on death certificate only. Cases were coded using the International Classification of Diseases for Oncology, Third Edition (ICD-O-3; C22.0).[30] We identified 101 (69 for men, 32 for women) newly diagnosed cases of HCC during the study period among subjects who had returned the baseline questionnaire and provided blood samples.

Laboratory assays

Plasma samples were screened for anti-HCV using a third-generation immunoassay (Lumipulse II Ortho HCV, Ortho-Clinical Diagnostics K.K., Tokyo, Japan)[31] and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology, Tokyo, Japan). The virus-positive group consisted of subjects positive for either or both anti-HCV and HBsAg.

Statistical analysis

Person-years of follow-up were calculated for each subject from the date of completion of the baseline questionnaire to the date of HCC diagnosis, date of emigration from the study area, or date of death, whichever occurred first; or if none of these occurred, follow-up was through to the end of the study period (December 31, 2005). Subjects who were lost to follow-up were censored at the last confirmed date of presence in the study area. Hazard ratios (HRs) of HCC were calculated by tertiles of isoflavones and soy food consumption, with the lowest consumption category as the reference. HRs and 95% confidence intervals (CIs) were calculated by the Cox proportional hazards model, adjusting for age at baseline survey (5-year age categories) and study area (6 PHC areas) according to the SAS PHREG procedure (Version 9.1; SAS Institute, Cary, NC). For further adjustment, additional possible confounders were incorporated into the model: smoking status (never, former, current); alcohol intake (non- and ex-drinkers, less than weekly, weekly or more [<150 g/week, 150-300 g/week or ≥300 g/week]); intake of coffee (almost never, 1–4 days/week, 1–4cups/day or 5 or more cups/day) and vegetables (continuous); and HCV or HBV infection status (positive or negative). In females, further adjustment was made for menopausal status (yes or no). These variables are either known or suspected risk factors for cancer or were previously associated with the risk of HCC.[32] When covariates were entered into the statistical model, isoflavones, soy food and vegetable intakes were adjusted for total energy intake using the residual method.[33] Vegetable intakes were

TABLE I – SUBJECT CHARACTERISTICS AT BASELINE ACCORDING TO GENISTEIN CONSUMPTION

	Men			Women		
	Genistein consumption			Genistein consumption		
	Low	Middle	High	Low	Middle	High
Age, years ± SD	56.6 ± 8.6	57.5 ± 8.2	59.0 ± 7.6	55.9 ± 8.5	56.2 ± 8.3	57.3 ± 7.9
Current smoker (%)	47.0	41.5	36.9	5.4	3.9	3.0
Regular drinker (yes, %)	64.6	63.6	59.3	12.1	9.0	7.2
Postmenopausal (%)	–	–	–	69.7	70.6	76.9
Coffee, daily (%)	46.6	36.3	31.6	42.4	34.7	27.3
Vegetables (g/day)	49.4 ± 67.1	59.3 ± 65.9	71.9 ± 68.9	55.2 ± 68.2	63.5 ± 68.5	69.6 ± 64.6
Soy food (g/day)	21.7 ± 10.2	49.5 ± 14.4	81.9 ± 18.8	23.1 ± 11.1	49.7 ± 15.5	76.1 ± 17.3
Genistein (mg/day)	6.0 ± 2.7	13.8 ± 3.5	24.2 ± 6.3	6.4 ± 2.9	13.9 ± 3.8	23.2 ± 6.3
Daidzein (mg/day)	3.6 ± 1.6	8.2 ± 2.1	14.5 ± 3.8	3.8 ± 1.7	8.4 ± 2.3	13.9 ± 3.8
Infection status						
HCV(−)/HBV(−)	89.43	90.89	90.61	92.65	93.24	93.22
HCV(−)/HBV(+)	3.29	2.83	2.99	2.00	1.92	2.06
HCV(+)/HBV(−)	7.20	6.24	6.28	5.19	4.76	4.62
HCV(+)/HBV(+)	0.08	0.04	0.12	0.16	0.07	0.09

Values are mean unless otherwise indicated. SD = Standard deviations, HCV = hepatitis C virus, HBV = hepatitis B virus.

TABLE II – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR HEPATOCELLULAR CARCINOMA (HCC) ACCORDING TO ISOFLAVONE AND SOY FOOD CONSUMPTION AMONG JAPANESE MEN AND WOMEN

		No. of cases	Person-years of follow-up	HR (95%CI)[1]	HR (95%CI)[2]
Men (N = 7,215)					
Genistein (mg/day)					
Low	<12.0	26	27,304	1	1
Middle	12.0–19.9	16	27,793	0.66 (0.35–1.23)	0.78 (0.41–1.49)
High	≥20.0	27	27,863	1.07 (0.61–1.89)	1.13 (0.60–2.11)
p_{trend}				0.93	0.80
Daidzein (mg/day)					
Low	<8.0	26	27,310	1	1
Middle	8.0–12.7	17	27,781	0.69 (0.37–1.29)	0.81 (0.43–1.54)
High	≥12.8	26	27,869	1.03 (0.58–1.82)	1.09 (0.58–2.05)
p_{trend}				0.98	0.87
Soy food (g/day)					
Low	<37.6	26	27,318	1	1
Middle	37.6–64.9	16	27,738	0.65 (0.34–1.21)	0.77 (0.40–1.47)
High	≥65.0	27	27,904	1.05 (0.60–1.84)	1.10 (0.59–2.03)
p_{trend}				0.97	0.84
Women (N = 12,783)					
Genistein (mg/day)					
Low	<12.2	6	50,398	1	1
Middle	12.2–19.5	12	51,424	2.36 (0.88–6.32)	2.36 (0.85–6.51)
High	≥19.6	14	51,029	2.86 (1.07–7.64)	3.19 (1.13–9.00)
p_{trend}				0.03	0.03
Daidzein (mg/day)					
Low	<8.1	5	50,402	1	1
Middle	8.1–12.5	13	51,408	3.08 (1.09–8.70)	3.10 (1.07–8.99)
High	≥12.6	14	51,041	3.46 (1.21–9.83)	3.90 (1.30–11.69)
p_{trend}				0.02	0.01
Soy food (g/day)					
Low	<38.2	8	50,403	1	1
Middle	38.2–62.7	11	51,056	1.51 (0.60–3.78)	1.68 (0.65–4.35)
High	≥62.8	13	51,393	1.74 (0.71–4.28)	1.74 (0.67–4.52)
p_{trend}				0.22	0.25

[1]Adjusted for age and area.–[2]Adjusted for age, area, HCV, HBsAg, smoking status, alcohol consumption, and intake of coffee and vegetables. Further adjusted for menopausal status in women.

calculated from 6 items in the questionnaire. Testing of the proportional hazards assumption by Schoenfeld and scaled Schoenfeld residuals found no violation of proportionality. We additionally analyzed the association between isoflavone intake and HCC in subjects who were either or both anti-HCV- or HBsAg-positive.

Trends were assessed by assignment of the median value in each category. All p-values were 2-sided, and statistical significance was determined at the $p < 0.05$ level.

Results

During 235,811 person-years of follow-up (average follow-up, 11.8 years) for 19,998 subjects (7,215 men and 12,783 women), a total of 101 cases (69 for men, 32 for women) of HCC were newly diagnosed and included in the analyses.

Subject characteristics at baseline according to tertile of energy-adjusted isoflavone consumption are shown in Table I, with the results for genistein used as a surrogate for isoflavones owing to the high correlation among results for genistein and daidzein. Subjects with high genistein consumption were older, smoked and drank less, consumed less coffee, and consumed more vegetables, notwithstanding sex. The proportion of postmenopausal women increased as genistein intake increased. As expected, soy food and daidzein increased as genistein intake increased. The proportion of subjects positive for anti-HCV,

TABLE III – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR HEPATOCELLULAR CARCINOMA (HCC) ACCORDING TO ISOFLAVONE AND SOY PRODUCT CONSUMPTION AMONG JAPANESE MEN AND WOMEN WHO WERE ANTI-HCV- OR HBSAG-POSITIVE

	No. of cases	Person-years of follow-up	HR (95%CI)[1]	HR (95%CI)[2]
Men (N = 699)				
Genistein				
Low	22	2,481	1	1
Middle	12	2,493	0.58 (0.29–1.20)	0.59 (0.28–1.24)
High	23	2,451	1.06 (0.56–2.00)	1.05 (0.52–2.12)
p_{trend}			0.99	0.96
Daidzein				
Low	22	2,481	1	1
Middle	13	2,483	0.63 (0.31–1.26)	0.63 (0.31–1.31)
High	22	2,460	1.00 (0.52–1.89)	0.97 (0.48–1.98)
p_{trend}			0.88	0.84
Soy food				
Low	22	2,483	1	1
Middle	12	2,482	0.61 (0.30–1.26)	0.61 (0.29–1.28)
High	23	2,459	1.10 (0.58–2.06)	1.10 (0.55–2.20)
p_{trend}			0.86	0.87
Women (N = 890)				
Genistein				
Low	4	3,426	1	1
Middle	11	3,504	3.28 (1.02–10.48)	3.11 (0.92–10.51)
High	10	3,507	3.07 (0.94–10.09)	3.30 (0.92–11.82)
p_{trend}			0.06	0.06
Daidzein				
Low	4	3,427	1	1
Middle	11	3,498	3.27 (1.02–10.47)	3.12 (0.92–10.56)
High	10	3,512	3.08 (0.94–10.13)	3.32 (0.93–11.88)
p_{trend}			0.06	0.06
Soy food				
Low	8	3,393	1	1
Middle	8	3,518	1.11 (0.41–3.05)	0.97 (0.34–2.77)
High	9	3,526	1.18 (0.44–3.12)	1.02 (0.36–2.94)
p_{trend}			0.74	0.98

[1]Adjusted for age and area.–[2]Adjusted for age, area, smoking status, alcohol consumption, and intake of coffee and vegetables. Further adjusted for menopausal status in women.

HBsAg or both among tertiles of genistein consumption were similar.

Table II shows minimally adjusted and multivariable HRs and 95% CIs for HCC by tertile of genistein, daidzein and soy food consumption in men and women. Consumption of genistein, daidzein and soy food showed no association with HCC in men, with respective multivariable HRs for the highest versus lowest tertile of 1.13 (95% CI = 0.60–2.11), 1.09 (95% CI = 0.58–2.05) and 1.10 (95% CI = 0.59–2.03). In women, in contrast, genistein and daidzein were dose-dependently associated with an increased risk of HCC, with multivariable HRs for the highest versus lowest tertile of 3.19 for genistein (95% CI = 1.13–9.00, p_{trend} = 0.03) and 3.90 for daidzein (95% CI = 1.30–11.69, p_{trend} = 0.01). Similarly, soy food consumption also tended to be associated with an increased risk of HCC in women, but without statistical significance (highest versus lowest: multivariable HR = 1.74, 95% CI = 0.67–4.25). Miso soup, natto and tofu consumption also showed no association with HCC in men (data not shown). In women, natto and tofu consumption was positively associated with HCC. Multivariable HRs for the highest *versus* lowest tertile of natto and tofu consumption was 3.71 (95% CI = 1.42–9.71) and 1.67 (95% CI = 0.65–4.28), respectively (data not shown).

These results remained essentially unchanged when analysis was restricted to subjects who were either or both anti-HCV- or HBsAg-positive (Table III); the positive association between genistein and daidzein and HCC in these women remained, albeit with attenuation of the test for linear trend (p_{trend} = 0.06 and 0.06 for genistein and daidzein, respectively). In contrast, soy food was not associated with HCC in women who were either or both HCV- and HBV-positive. No association between isoflavones and soy food and HCC was observed in men. Further, no association with individual soy foods was seen in men (data not shown). In women, the positive association between natto consumption and HCC remained, whereas tofu and miso soup consumption were not associated with HCC risk (data not shown).

Because the effects of isoflavones on HCC might differ between premenopausal and postmenopausal women due to difference in their estrogen levels, we also analyzed the association between soy foods and isoflavones and HCC in postmenopausal women. Results were similar to those for total women in Table II (data not shown). Hazard ratios among premenopausal women could not be calculated, because only one case occurred among them.

Discussion

We found a dose-dependent increase in the risk of HCC with consumption of isoflavones in Japanese women, even after consideration of infection status of hepatitis virus. In contrast, no association between isoflavones and HCC was seen in men. To our knowledge, this is the first study to report a positive association between the consumption of isoflavones and HCC in women.

Previous epidemiological findings for isoflavone and soy food intake and HCC are inconsistent.[11–16] Two prospective[11,12] and one case–control study[13] reported an inverse association between frequency of miso soup intake and HCC mortality. Lei *et al.*[14] reported that genistein consumption was lower at first diagnosis in patients with HCC than in those with cirrhosis. In several case-control studies, in contrast, no association with HCC was seen for frequency of tofu[15] and pulses intake.[16] However, most of these studies did not control for the potentially important confounding effects of infection with either or both HCV and HBV.[11,12,14,15] Additionally, most of these previous studies did not analyze by sex,[11,13–16] notwithstanding that the effects of isoflavones on HCC may differ between men and women.[10]

Although the relation between estrogen and HCC remains obscure,[2,34] previous epidemiological studies have reported the

preventive effects of estrogen against HCC or the progression of liver fibrosis. Yu *et al.* reported that the use of hormone replacement therapy was associated with a lower risk of HCC, and that younger age at menopause and ovariectomy during premenopause were risk factors for HCC.[9] Tanaka *et al.* reported that elevated serum testosterone, together with decreased serum estrogens, may promote the development of HCC in patients with cirrhosis.[35] Additionally, menopause seems to play a role in accelerating the progression of fibrosis.[36] In animal experiments, the degree of fibrosis was increased in males and females with hypoestrogenemia compared with females with normal levels of estrogen.[37] Moreover, variant ERs were more frequently expressed in male HCC patients than female subjects, even in an early stage of chronic liver disease,[38,39] while expression of both ERβ and wild ERα was lower in patients with HCC than in those with chronic liver disease.[40] Taken together, these findings may indicate that a loss of estrogen responsiveness might lead to HCC, and suggest that estrogen and ER status may play a role in hepatic defense.

Several mechanisms may explain the association we found between isoflavone consumption and increased risk of HCC in women. First, because isoflavones compete for estradiol at the receptor complex, they may have an anti-estrogenic effect in women.[17] Indeed, a number of epidemiological studies reporting an association between isoflavone intake and decreased breast cancer risk have suggested that this finding is ascribable to the possibility of anti-estrogenic effects of isoflavone.[10,21,22] Second, serum estradiol concentration shows a significant inverse correlation with soy product intake in women.[41,42] These findings suggest that isoflavones inhibit the preventive effects of estrogen against HCC in women. Moreover, the anti-estrogenic effects of isoflavone in women might impede the preventive effects of estrogen-mediated inhibition of IL-6 on HCC, given that estrogen's inhibitory effect on IL-6 secretion reduced HCC risk in female mice,[8] and that an isoflavone-rich diet increased IL-6 levels in women.[43]

We attempted to specify which kinds of soy products contributed to the increased risk of HCC in women. Our results showed a stronger association for natto (fermented soybean) than other soyfoods. Given that natto is the greatest contributor to isoflavones intake in Japan,[44] this result is plausible. Additionally, the isoflavone aglycones in fermented foods may have greater bioavailability than their glucosides, because genistein and daidzein are absorbed as isoflavone aglycones following hydrolysis of the glycoside by beta-glucosidases present in not only human gut bacteria but also in foods.[45] These findings indicate the need for further study of risk and bioavailability using plasma data.

In this study, most women (97%) who developed HCC during the follow-up period were postmenopausal at baseline. Isoflavone may have not competed with estrogen in postmenopausal women due to their low estrogen levels. However, even when analysis was restricted to postmenopausal women, a positive association between isoflavones and HCC risk remained. This lack of change between premenopausal and postmenopausal women has also been reported for breast cancer.[21,22,46,47] Given the extended period required for carcinogenesis to occur, an anti-estrogenic effect of isoflavones in premenopausal women might remain in postmenopausal women. In contrast, given that isoflavones may have an estrogenic effect in men,[10,21,22] they might be expected to decrease the risk of HCC in men. Here, however, we saw no association between isoflavone consumption and HCC in men. The predominance of androgens in men may obfuscate any estrogenic impact of isoflavones, because testosterone level is positively associated with the risk of HCC in men.[35,48]

The strength of the present study is its prospective design and negligible proportion of loss to follow-up (0.2%). Information on isoflavones and soy food consumption was collected before the subsequent diagnosis of HCC, thereby diminishing the probability of the recall bias that is inherent to case–control studies. Another strength was that virus infection status was determined at baseline for the entire population, allowing us to clarify the association between isoflavones and HCC in a high-risk population.

Several limitations of the study also warrant mention. First, because we estimated consumption from self-reports and at a single point (at baseline), and that validity for isoflavones was moderate, some measurement error in the assessment of isoflavones and soyfoods consumption is inevitable. If present, however, this was probably nondifferential and would have lead to the underestimation of results. Second, it would have been preferable if we had been able to confirm the association between plasma isoflavone level and HCC. Further studies using plasma samples are needed. However, Spearman's correlation coefficients for daidzein and genistein between intakes from the questionnaire and from serum concentrations in a validation study using subsamples in the JPHC Study were 0.31 and 0.33, respectively.[44] Additionally, we previously reported similar results regarding the effects of isoflavone between studies using plasma isoflavone levels and using a FFQ in both breast[21,22] and prostate cancer.[23,24] On the basis of these findings, we expect that results using plasma samples would be similar to our present results. Third, we had no information on the clinical severity of hepatitis or on the treatment of subjects with hepatitis virus infection before and during the study period. If infected subjects had received treatment, the occurrence of HCC may have been decreased. However, this might have lead to the underestimation of HCC occurrence, which would also bias the results toward the null. Finally, any generalization of our results should be done with caution.[49] Our subjects were restricted to those who provided a blood sample and participated in the baseline health checkup survey (28% for men, 45% for women), and subjects already under care for hepatitis infection may have been less willing to provide blood samples.

In conclusion, we found that isoflavones have a relevant role in HCC risk in women. In particular, the unfavorable effect of isoflavones was independent of other major risk factors, namely HBV and HCV infection. It might be therefore necessary for women with hepatitis virus infection to abstain from isoflavone. Because our cases numbers were relatively small, confirmation of these findings in further studies is required.

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Short Communication

Vegetable, fruit and antioxidant nutrient consumption and subsequent risk of hepatocellular carcinoma: a prospective cohort study in Japan

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In a population-based prospective study of 19 998 Japanese individuals, consumption of vegetables, green–yellow and green leafy vegetables was inversely associated with the risk of hepatocellular carcinoma (101 cases), with multivariable hazard ratios for the highest *vs* lowest tertile of 0.61 (95% confidence interval (CI) = 0.36 – 1.03, $P_{trend} = 0.07$), 0.65 (95% CI = 0.39 – 1.08, $P_{trend} = 0.06$) and 0.59 (95% CI = 0.35 – 1.01, $P_{trend} = 0.04$), respectively.
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Keywords: hepatocellular carcinoma; vegetables; fruits; carotenoid; vitamin C; prospective study.

Although the potential roles of fruits and vegetables in cancer prevention have been demonstrated at various cancer sites (Vainio and Weiderpass, 2006), the association with hepatocellular carcinoma (HCC) remains unclear (World Cancer Research Fund/American Institute for Cancer Research, 2007). Fruits and vegetables are a rich source of antioxidants, such as retinol and carotenoids, and vitamin C, and they are thought to exert protective effects against cancer (Stanner *et al*, 2004). In an intervention study, however, not all antioxidant nutrients might be protective against HCC (Bjelakovic *et al*, 2004).

Here, we investigated the association between fruit and vegetable consumption and HCC in a large-scale population-based cohort study in Japan, with due consideration for hepatitis C virus (HCV) and hepatitis B virus (HBV) infection status.

MATERIALS AND METHODS

The Japan Public Health Center-based Prospective Study (JPHC study) Cohort II, initiated during 1993 – 1994, has been described earlier (Kurahashi *et al*, 2009). The study population was defined as all residents aged 40 – 69 years who lived in six PHC areas at the start of the baseline survey. We enrolled 56 635 men and women who provided valid responses to a self-administered questionnaire (82%) and excluded participants with a history of cancer ($n = 1219$). Among them, a total of 20 406 participants (36%) provided a blood sample. These plasma samples were screened for anti-HCV and for HBV antigen (HBsAg).

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[3]Study group members are listed in the Appendix.
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The self-administered food-frequency questionnaire (FFQ) consisted of 52 foods, including beverages. It asked about the usual consumption of six vegetable and three fruit items during the previous year. The vegetables included two pickled vegetables (green leafy vegetables and other vegetables), green leafy vegetables (spinach, Chinese chives, etc), carrot, tomato and 100% vegetable juice, whereas the fruit items included apple, citrus fruits and 100% fruit juice. The questionnaire contained five frequency categories for vegetable and fruit consumption ranging from 'never' to 'almost every day', except for juices. Standard portion sizes were specified for each food item, which were then used to determine the three choice amounts of small (50% smaller), medium (same as the standard) and large (50% larger). Six frequency choices for juice ranged from 'almost never' to '5 or more cups per day'. The consumption of total fruit and total vegetables ($g\,day^{-1}$) was calculated from these responses. We documented the validity of the FFQ in the assessment of vegetable and fruit consumption in subsamples using dietary records. Although validities for vegetables and fruits were relatively low (from 0.22 for vegetables to 0.31 for fruit), correlation coefficients for antioxidant nutrients were considered moderate (from 0.31 for vitamin C to 0.41 for β-carotene).

Among the 20 406 participants who responded to the questionnaire and provided a blood sample, 408 who reported extreme total energy intake (upper 1.0% or lower 1.0%) were excluded, leaving 19 998 participants for analysis, who were followed from the baseline survey until 31 December 2005. Of these, 5% moved out of a study area and 0.2% were lost to follow-up during the study period.

We used Cox regression to compute hazard ratios (HRs) and 95% confidence intervals (CIs) of HCC according to tertiles of consumption of the respective food items or nutrients with adjustment for potential confounders, including HCV or HBV infection status.

RESULTS

During 235 811 person-years of follow-up (11.8 years), a total of 101 new HCC cases were identified. The prevalence of chronic HCV and HBV infection in HCC cases was 70.3 and 12.9%, respectively.

We observed that participants with higher vegetable and fruit consumption tended to be older, smoke less, drink less alcohol, and consume less coffee and more genistein. Body mass index did not substantially differ according to consumption. The proportion of participants positive for anti-HCV, HBsAg or both among tertiles of vegetable and fruit consumption was similar. The prevalence of positive markers for HCV and HBV in this cohort was 5.3 and 2.5%, respectively.

Table 1 presents HRs in relation to vegetable and fruit consumption for HCC cases. Borderline inverse associations were seen between vegetables and green–yellow vegetables and HCC, with multivariable HRs for the highest *vs* lowest tertile of 0.61 (95% CI = 0.36–1.03, $P_{trend} = 0.07$) and 0.65 (95% CI = 0.39–1.08, $P_{trend} = 0.06$), respectively. In particular, green leafy vegetable consumption showed an inverse dose-dependent association with HCC (HR = 0.59, 95% CI = 0.35–1.01 for highest *vs* lowest tertile of consumption, $P_{trend} = 0.04$). Results for vegetables excluding pickled vegetables were similar to those for when they were included. In contrast, fruit consumption including fruit juice appeared to increase the risk of HCC, albeit without statistical significance (HR = 1.45, 95% CI = 0.85–2.48 for highest *vs* lowest tertile of consumption).

Table 2 shows the association between retinol, carotenoids (α-carotene and β-carotene) and vitamin C and HCC risk. A slightly negative association was seen between α- and β-carotene and HCC, with respective multivariable HRs for the highest *vs* lowest tertile of 0.69 (95% CI = 0.42–1.15) and 0.64 (95% CI = 0.38–1.08). Multivariable HR for vitamin C was somewhat increased in the highest category (HR = 1.38, 95% CI = 0.80–2.40).

When the analysis was restricted to participants who were either or both anti-HCV- or HBsAg-positive, these results were substantially unchanged. It is worth noting that our study showed that the preventive effects of α- and β-carotene on HCC strengthened, with respective multivariable HRs for the highest *vs* lowest tertile of 0.60 (95% CI = 0.34–1.08, $P_{trend} = 0.08$) and 0.61 (95% CI = 0.34–1.09, $P_{trend} = 0.08$) (data not shown).

After participants were stratified by smoking status, multivariable HRs for the highest *vs* lowest tertile among never smokers were 0.42 for vegetables (95% CI = 0.19–0.99, $P_{trend} = 0.03$), 0.30 for green–yellow vegetables (95% CI = 0.13–0.70, $P_{trend} < 0.01$) and 0.31 for green leafy vegetables (95% CI = 0.13–0.74, $P_{trend} < 0.01$). Regarding nutrients, β-carotene showed a significant

Table 1 Hazard ratio and 95% confidence intervals for hepatocellular carcinoma according to tertile of intake of vegetables and fruits, JPHC study ($n = 19\,998$)

	Lowest	Middle	Highest	P_{trend}
Total vegetables and fruits				
Median (g day^{-1})	55.3	120.3	200.9	
No. of cases/person-years of follow-up	32/79 057	22/78 938	47/77 816	
Age, area, sex-adjusted HR (95% CI)	1.00	0.71 (0.41–1.23)	1.23 (0.78–1.94)	0.38
Multivariate HR[a] (95% CI)	1.00	0.78 (0.45–1.38)	1.14 (0.70–1.86)	0.56
Vegetables				
Median (g day^{-1})	25.6	51.7	88.5	
No. of cases/person-years of follow-up	37/78 971	31/79 183	33/77 657	
Age, area, sex-adjusted HR (95% CI)	1.00	0.88 (0.55–1.43)	0.81 (0.50–1.29)	0.37
Multivariate HR[b] (95% CI)	1.00	0.79 (0.48–1.31)	0.61 (0.36–1.03)	0.07
Green–yellow vegetables				
Median (g day^{-1})	10.1	23.1	42.3	
No. of cases/person-years of follow-up	44/78 234	24/79 272	33/78 305	
Age, area, sex-adjusted HR (95% CI)	1.00	0.66 (0.40–1.09)	0.81 (0.51–1.28)	0.27
Multivariate HR[b] (95% CI)	1.00	0.55 (0.33–0.94)	0.65 (0.39–1.08)	0.06
Green leafy vegetables				
Median (g day^{-1})	7.1	17.0	32.3	
No. of cases/person-years of follow-up	42/78 473	31/79 018	28/78 320	
Age, area, sex-adjusted HR (95% CI)	1.00	0.82 (0.51–1.30)	0.72 (0.44–1.17)	0.17
Multivariate HR[b] (95% CI)	1.00	0.71 (0.44–1.17)	0.59 (0.35–1.01)	0.04
Fruit				
Median (g day^{-1})	13.4	68.0	120.3	
No. of cases/person-years of follow-up	29/78 795	25/78 872	47/78 144	
Age, area, sex-adjusted HR (95% CI)	1.00	0.91 (0.53–1.56)	1.30 (0.81–2.09)	0.32
Multivariate HR[c] (95% CI)	1.00	1.08 (0.61–1.91)	1.45 (0.85–2.48)	0.19
Fruit excluding 100% fruit juice				
Median (g day^{-1})	11.8	46.8	97.2	
No. of cases/person-years of follow-up	32/78 489	26/78 961	43/78 361	
Age, area, sex-adjusted HR (95% CI)	1.00	0.97 (0.58–1.65)	1.24 (0.77–1.99)	0.40
Multivariate HR[c] (95% CI)	1.00	0.79 (0.45–1.38)	1.08 (0.65–1.82)	0.81

CI = confidence interval; HBsAg = HBV antigen; HCV, hepatitis C virus; HR = hazard ratio. [a]Adjusted for age, area, sex, HCV, HBsAg, smoking status, alcohol consumption, body mass index, history of diabetes mellitus and intake of coffee, genistein. [b]Adjusted for age, area, sex, HCV, HBsAg, smoking status, alcohol consumption, body mass index, history of diabetes mellitus and intake of coffee, genistein and fruit. [c]Adjusted for age, area, sex, HCV, HBsAg, smoking status, alcohol consumption, body mass index, past history of diabetes mellitus and intake of coffee, genistein and vegetable.

Epidemiology

Table 2 Hazard ratio and 95% confidence intervals for hepatocellular carcinoma according to tertile of intake of nutrient, JPHC study ($n = 19\,998$)

	Lowest	Middle	Highest	P_{trend}
Retinol				
Median (mg day^{-1})	114.8	282.7	397.2	
No. of cases/person-years of follow-up	33/78 650	34/78 824	34/78 338	
Age, area, sex-adjusted HR (95% CI)	1.00	1.24 (0.75–2.03)	1.37 (0.84–2.23)	0.20
Multivariate HR[a] (95% CI)	1.00	1.26 (0.76–2.10)	1.07 (0.64–1.79)	0.65
α-carotene				
Median (mg day^{-1})	50.4	146.6	561.2	
No. of cases/person-years of follow-up	40/78 660	28/78 756	33/78 395	
Age, area, sex-adjusted RR (95% CI)	1.00	0.78 (0.48–1.27)	0.81 (0.51–1.29)	0.34
Multivariate HR[a] (95% CI)	1.00	0.73 (0.44–1.22)	0.69 (0.42–1.15)	0.14
β-carotene				
Median (mg day^{-1})	602.2	1355.7	2319.0	
No. of cases/person-years of follow-up	39/78 628	30/79 082	32/78 101	
Age, area, sex-adjusted HR (95% CI)	1.00	0.87 (0.54–1.41)	0.79 (0.49–1.26)	0.31
Multivariate HR[a] (95% CI)	1.00	0.82 (0.50–1.35)	0.64 (0.38–1.08)	0.10
Vitamin C				
Median (mg day^{-1})	36.4	67.8	93.9	
No. of cases/person-years of follow-up	23/78 495	34/78 964	44/78 352	
Age, area, sex-adjusted HR (95% CI)	1.00	1.41 (0.82–2.40)	1.33 (0.79–2.24)	0.39
Multivariate HR[a] (95% CI)	1.00	1.74 (0.996–3.06)	1.38 (0.80–2.40)	0.44

CI = confidence interval; HBsAg = HBV antigen; HCV, hepatitis C virus; HR = hazard ratio. [a]Adjusted for age, area, sex, HCV, HBsAg, smoking status, alcohol consumption, body mass index, history of diabetes mellitus and intake of coffee and genistein.

inverse association with risk among never smokers (highest *vs* lowest: HR = 0.31, 95% CI = 0.13–0.76). In contrast, vitamin C seemed to be positively associated with HCC risk among current smokers, with an increase in multivariable HR for HCC in the second and highest categories (HR = 3.58, 95% CI = 1.21–10.63 and HR = 2.69, 95% CI = 0.89–8.08, respectively) (data not shown).

DISCUSSION

Our study identified inverse associations between the consumption of vegetables, green–yellow and green leafy vegetables and HCC. Concomitantly, an inverse association between α- and β-carotene and HCC risk was shown. These results are plausible, given the abundance of these nutrients in vegetables, particularly green–yellow vegetables.

In an animal experiment, carotenoids were shown to suppress liver carcinogenesis (Murakoshi *et al*, 1992; Moreno *et al*, 2002), whereas in an intervention study in patients with viral hepatitis and cirrhosis, a greater than 50% decrease in HCC incidence was found in the group administered a carotenoid mixture in addition to conventional treatment compared with a group given conventional symptomatic treatment alone (placebo not used) (Nishino, 2007). These findings support our present findings. It is worth noting that our study showed that the preventive effects of α- and β-carotene on HCC were strengthened when participants were limited to those who were either or both HBV and HCV positive. Given that inflammation is accompanied by the excess production of free radicals and that carotenoids have antioxidant potential in the scavenging of free radicals (Krinsky, 1989), carotenoids appear to play an important role in the prevention of hepatitis virus infection-related liver carcinogenesis.

In contrast, vitamin C consumption appeared to be associated with an increased risk of HCC. These relations were strengthened among current smokers in our study (see Results). Although vitamin C has antioxidant potential, it also acts to stimulate the absorption of iron from food (Lynch, 1997), and iron overload is considered a risk factor for HCC (Kowdley, 2004). Dietary vitamin C is positively associated with ferritin, which was used as a measure of body iron stores in the study by Fleming *et al* (1998). Thus, a higher intake of vitamin C might be harmful to hepatic cells, especially among smokers.

Given that the prognosis for HCC is extremely poor, our results would, if confirmed, have important implications for public health. Greater consumption of vegetables that contain α- and β-carotene and restraint in those rich in vitamin C may modify the development of HCC in HBV- and/or HCV-infected participants.

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Appendix

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ORIGINAL PAPER

Metabolic factors and subsequent risk of hepatocellular carcinoma by hepatitis virus infection status: a large-scale population-based cohort study of Japanese men and women (JPHC Study Cohort II)

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Abstract
Objective The association between metabolic factors and hepatocellular carcinoma (HCC) has not been well clarified. We prospectively examined whether metabolic factors predicts the subsequent risk of HCC in the Japan Public Health Center-based Prospective Study Cohort II, in consideration of hepatitis virus infection status.
Methods A total of 17,590 subjects aged 40–69 participating in a questionnaire and health checkup survey during 1993–1994 were followed for incidence of HCC through 2006. A total of 102 cases of HCC were newly documented. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for metabolic factors controlling for potential confounding factors.
Results The presence of metabolic factors in the aggregate was associated with a significantly increased risk of HCC, especially with hepatitis virus infection. HCC was positively associated particularly with high glucose (HR = 1.75, CI = 1.11–2.74) and overweight (HR = 2.22, CI = 1.42–3.48). Results were similar when analyses were limited to subjects with HCV infection.
Conclusions Although metabolic factors in the aggregate may be associated with an increased risk of HCC, the main contributors to this association under HCV infection appear to be overweight and high glucose. Improvement of these factors may be a crucial target in preventing progression to HCC in those with HCV infection.

Keywords Metabolic factor · Hepatocellular carcinoma · Cohort study · Overweight · High glucose

Introduction

Although a link with hepatocellular carcinoma (HCC) has been suggested for some of its individual components, a role for metabolic factors overall as a risk factor for the development of HCC remains unproven [1]. It is known that a significant proportion of cases of HCC develops in cryptogenic cirrhosis, which may actually represent non-alcoholic fatty liver steatohepatitis (NASH) [2]. Non-alcohol fatty liver disease (NAFLD), a category which includes NASH, is closely associated with insulin resistance and several features of metabolic factors [3], in turn, suggesting a link between metabolic factors and HCC. Meanwhile, hepatitis C virus (HCV) is linked with impaired insulin resistance and diabetes, hypocholesterolemia, and steatosis, which together represent a distinct HCV-associated dysmetabolic change [1]. Diabetes is also acknowledged as an independent risk factor for the development of HCC [4], strongly suggesting that HCV

The members of the Japan Public Health Center-based Prospective Study Group are listed in Appendix.

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infection may be a promoter of the development of HCC by way of insulin-mediated pathways.

The primary causes of HCC, which remains one of the most important cancers in Japan [5, 6], are HCV and hepatitis B virus (HBV) infection [7]. Meanwhile, metabolic factors have become recognized as a major public health target worldwide, and associated with the global pandemic of obesity and diabetes [8]. Given the expectation that metabolic factors influence the incidence of HCC, clarification of the association between metabolic factors and HCC is a crucial task. However, epidemiological evidence for this link is limited [9, 10], and most previous epidemiological studies have targeted individual components of metabolic factors, such as diabetes [4, 10–21] and obesity [10, 21–26]. Further, only a few of these studies have taken account of hepatitis virus infection status [10, 13, 15–20].

Here, we conducted a cohort analysis on the association between metabolic factors and risk of HCC using a large-scale population-based study in Japan. Our main purpose was to clarify whether metabolic factors predict the subsequent occurrence of HCC in Japanese, and whether the effect is attributable to specific components or to an aggregate effect, in consideration of hepatitis virus infection status.

Methods

The Japan Public Health Center-based Prospective Study (JPHC Study) Cohort II was initiated in 1993–1994. This cohort consisted of six public health center (PHC) areas across Japan. The study design had been described in detail previously [27]. The study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. The study population was defined as all residents aged 40–69 years at the start of the baseline survey. A part of one PHC area was excluded because its study population was defined differently to the others. Initially, we defined a population-based cohort of 68,975 subjects after exclusion of ineligible subjects ($n = 103$).

Baseline survey

At baseline, a self-administered questionnaire survey on various lifestyle factors was conducted (response rate = 82%). A total of 10 ml of blood was also arranged voluntarily by 39% of the respondents during health checkups provided by the local government. The plasma and buffy layer were divided into four tubes holding 1.0 ml each (three tubes for plasma and one for the buffy layer) and stored at −80°C until analysis.

For this research analysis, we restricted subjects to those who responded to the questionnaire and for whom blood samples and health checkup data on components of metabolic factors were available, i.e., blood pressure, blood glucose, serum HDL-cholesterol, serum triglycerides, height, and weight. We further excluded those with a history of liver cancer and those with missing data on variables to be controlled such as smoking status, weekly ethanol intake, coffee intake, and serum total cholesterol. Finally, a total of 17,590 individuals were included in this analysis.

Measurements

Serum total and HDL-cholesterol, triglycerides, and glucose were measured in 23 laboratories. Precision and accuracy in all the laboratories were found to be satisfactory according to the Osaka Medical Center for Health Science and Promotion [28], a member of the Cholesterol Reference Method Laboratory Network (CRMLN) [29]. Trained technicians measured blood pressure using standard mercury sphygmomanometers. Height was measured in stocking feet and weight in light clothing. Body mass index (BMI) was calculated from the height and weight using the formula weight (kg)/height $(m)^2$.

Subjects were categorized by the number of metabolic factors, according to the definitions of the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) [30] and International Diabetes Federation (IDF) [31]. Waist circumference was not measured in this study. Overweight with a BMI $\geq$ 25.0 kg/m^2 was used as the criterion for central obesity, because this BMI level was reported to correspond well to the Japanese criteria for a high waist circumference of $\geq$85 cm in men and $\geq$90 cm in women, and 100 cm^2 of visceral fat area [32]. The metabolic factors were defined as follows: (1) high blood pressure: blood pressure $\geq$130/85 mmHg and/or medication use; (2) high glucose: blood glucose $\geq$5.55 mmol/l (100 mg/dl) fasting or $\geq$7.77 mmol/l (140 mg/dl) non-fasting, and/or on treatment; (3) low HDL-cholesterol <1.03 mmol/l (40 mg/dl) for men and <1.29 mmol/l (50 mg/dl) for women; (4) high triglycerides: high serum triglycerides $\geq$1.69 mmol/l (150 mg/dl); (5) overweight: BMI $\geq$ 25 kg/m^2. Metabolic aggregate in this study was defined as the presence of three or more of these components (high blood pressure, high glucose, low HDL-cholesterol, high triglycerides, and overweight), similar to the criteria of the AHA/NHLBI; and the presence of two or more of the additional components (high blood pressure, high glucose, low HDL-cholesterol, and high triglycerides) among overweight persons, similar to the criteria of the IDF.

Laboratory assays

Plasma samples were screened for anti-HCV antibody (anti-HCV) using a third-generation immunoassay (Lumipulse II

Ortho HCV, Ortho-Clinical Diagnosis K.K., Tokyo, Japan) [33] and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology Co., Ltd., Tokyo, Japan).

Follow-up and identification of HCC

Subjects were followed from the date of the baseline survey until 31 December 2006. Residence status, including survival, was confirmed through the residential registry. Inspection of the registry is available to anyone under the resident registration law. Information on the cause of death was obtained from the death certificate, provided by the Ministry of Health, Labour, and Welfare with the permission of the Ministry of Internal Affairs and Communications, in which cause of death is defined according to the International Classification of Disease, 10th Version (ICD-10) [34]. Resident and death registration are required by law in Japan and the registries are believed to be complete. Among study subjects, 1,578 died, 961 moved out of the study area, and 47 (0.3%) were lost to follow-up within the follow-up period.

The incidence data on HCC were obtained by active patient notification from major hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Death certificates were used as a supplementary information source. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 4.7%. The site and histology of each HCC case were coded using the International Classification of Diseases for Oncology, Third Edition (ICD-O-3: C22.0) [35]. For this analysis, the earliest date of diagnosis was used in subjects with multiple primary cancers at different times. A total of 102 newly diagnosed cancer cases (67 for men, 35 for women) were identified.

Analysis

The number of person-years in the follow-up period was counted from the date of completion of the baseline questionnaire until the date of HCC diagnosis, date of emigration from the study area, date of death, or end of the study period, whichever occurred first. For subjects who withdrew from or were lost to follow-up, the date of withdrawal and the last confirmed date of presence, respectively, were used as the date of censor.

The relative risk of HCC associated with metabolic factors was described using hazard ratios (HRs) and 95% confidence intervals (CIs). Analyses were conducted among total subjects and among those who were hepatitis virus-positive. The Cox proportional hazards model was employed as a control for potential confounding factors, namely, age at baseline (5-year age categories), area (10 PHC areas), smoking status (never, past, current), weekly ethanol intake (past, never, <weekly, <150 g per week, 150 to <300 g per week, ≥300 g per week), coffee intake (never, 1–2 days/week, 3–4 days/week, and daily (1–2 cups/day, ≥3 cups/day), total cholesterol (mg/dl, continuous), and HCV (anti-HCV-negative, -positive) and HBV infection status (HBsAg-negative, -positive). These variables, obtained from the questionnaire, are either known or suspected from previous studies as risk factors for HCC. Sex, age, and area were treated as strata to allow for a different baseline hazard for each stratum. Testing of the proportional hazards assumption by Schoenfeld and scaled Schoenfeld residuals found no violation of proportionality. In addition, we evaluated whether the effect of overweight and high glucose level influenced each other using a test of interaction by entering into the model multiplicative interaction terms between respective factors. All statistical analyses were performed using Stata 10 (Stata Corporation, College Station, TX) [36].

Results

During 222,800 person-years of follow-up (average follow-up period: 12.7 years) for 17,590 subjects (6,092 men and 11,498 women), a total of 102 cases of newly diagnosed HCC (67 men and 35 women) were identified and included in the analyses.

According to the definition of the respective metabolic factors, 59% of the study subjects had high blood pressure, 21% had high glucose, 23% had low HDL-cholesterol, 24% had high triglycerides, and 31% were overweight. As a consequence, 22% were categorized as having ≥3 metabolic factors, and 16% as having ≥2 factors in addition to being overweight (Table 1).

HRs and CIs of HCC according to the presence of metabolic factors among total subjects are shown in Table 2. The presence of metabolic factors was associated with a significantly increased risk of HCC [HR = 1.68, CI = 1.06–2.66 (≥3 factors); HR = 2.14, CI = 1.27–3.61 (≥2 factors in addition to being overweight)]. HCC was positively associated with components of metabolic factors, namely, high glucose (HR = 1.75, CI = 1.11–2.74) and overweight (HR = 2.22, CI = 1.42–3.48).

When analyzed by sex, a significantly increased risk with the presence of metabolic factors in the aggregate was observed only in men. Increased risk in men was also seen with high glucose and overweight. In women, no clear association with metabolic factors was seen except for a significant increase in risk by overweight (data not shown).

When analyses were limited to subjects with HCV infection (Table 3), results were similar to those among total subjects, although the risk of high glucose was more

Table 1 Baseline characteristics of study subjects ($n = 17,590$)

	Total subjects	HCV-antibody and HBsAg negative subjects	HCV-antibody positive and HBsAg negative subjects	HCV-antibody negative and HBsAg positive subjects	HCV-antibody and HBsAg positive subjects
Number of subjects	17,590	16,213	939	419	19
Total person-years	222,800.6	206,239.1	11,032.9	5,306.3	222.3
Age (mean)	57.0	56.9	59.4	55.6	56.6
Men (%)	34.6	34.0	42.3	42.0	31.6
Smoking status (%)					
Never	71.4	72.2	58.4	69.5	68.4
Past	11.5	11.3	15.1	11.7	5.3
Current	17.1	16.5	26.5	18.9	26.3
Weekly ethanol intake (%)					
Past	2.0	1.8	6.0	1.7	63.2
Never	61.6	62.0	55.4	59.0	5.3
<weekly	6.3	6.3	6.2	6.9	10.5
<150 g per week	15.1	14.9	17.9	14.8	10.5
150 to <300 g per week	8.1	8.1	8.9	7.6	10.5
≥300 g per week	6.9	6.9	5.6	10.0	0.0
Coffee intake (%)					
Almost never	33.2	33.1	35.7	31.0	31.6
1–2 days per week	19.8	19.7	21.1	18.8	10.5
3–4 days per week	10.7	10.6	10.9	14.1	5.3
1–2 cups per day	27.5	27.7	24.6	24.6	42.1
3–4 cups per day	7.1	7.1	6.2	9.1	10.5
≥5 cups per day	1.8	1.8	1.6	2.4	0.0
Serum total cholesterol (mg/dl) (mean)	203.4	204.5	190.0	191.1	193.2
Metabolic factors in the aggregate (%)					
≥3 factors	22.2	22.3	21.5	18.1	15.8
≥2 factors in addition to being overweight	16.1	16.3	13.7	13.1	15.8
Component of metabolic factors (%)					
High blood pressure	59.3	59.0	58.9	61.8	57.9
High glucose	20.7	20.5	23.9	21.0	21.1
Low HDL-cholesterol	23.3	23.2	26.6	19.6	15.8
High triglycerides	23.6	24.1	18.6	15.0	21.1
Overweight	30.8	31.0	25.8	32.9	52.6

attenuated and the risk of overweight was more clearly observed. Likewise, those who were negative for both HCV- and HBV infection revealed a similar tendency to those who were infection-positive, albeit without statistical significance.

Additional analysis was conducted to determine the presence of the effect of BMI and effect modification between overweight and high glucose (Table 4). Increased BMI was associated with HCC in both genders of all subjects and those with HCV infection. The association was more clearly observed in men than in women. The presence of both high glucose and overweight significantly increased the risk of HCC, although no significant effect due to modification between overweight and high glucose level was observed.

Discussion

In this prospective cohort study among a large Japanese population, we found that the presence of metabolic factors in the aggregate predicted the subsequent risk of HCC in men, including those with HCV infection. Our results also confirmed that the main contributors to the effect of