hepatitis C virus or hepatitis B virus [1,2]. Epidemiological data also demonstrate that other environmental factors such as alcohol and tobacco enhance the risk of HCC [3,4].

Although the exact mechanism of hepatocarcinogenesis is still incompletely understood, HCC risk increases with the severity of hepatic inflammation [5,6]. Chronic inflammation developing through the action of various inflammatory mediators is known as a cofactor in carcinogenesis [7]. Among inflammatory mediators, proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α play essential roles and have been implicated in inflammation-associated tumors [8].

IL-1B gene polymorphisms in the promoter region at positions −511C/T and −31T/C, which are in tight linkage disequilibrium [9], have been associated with susceptibility to HCC [10–13] although the direction of this association has been conflicting. A *TNF-A* polymorphism in the promoter region at −308G/A, which has been related to various diseases [14,15] but is known to be rare in the Japanese [16], has also been linked to HCC risk [17]. Another *TNF-A* polymorphism in the promoter region at −1031T/C, which has been related to several diseases [18,19] and is observed in a relatively large proportion of the Japanese [16], has not been examined in causation of HCC.

We conducted a case-control study to investigate whether the above gene polymorphisms of *IL-1B* and *TNF-A* affect HCC risk with any interaction with alcohol and tobacco, both of which have been implicated as not only risk factors of HCC but also correlates with the production of inflammatory cytokines [20,21]. Two different control groups (hospital controls and patients with chronic liver disease [CLD] without HCC) were employed in this study; the former represents a conventional control group, and the latter was selected based on the clinically established finding that the majority of HCC patients in Japan have preexisting CLD [22].

2. Methods

2.1. Subjects

The details of this study have been described elsewhere [23]. Briefly, all study subjects were restricted to residents of Saga Prefecture, Japan, who were aged 40–79 years at the time of identification. We recruited 209 incident HCC cases (participation rate [PR] = 92%) who were admitted or outpatients of two main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital) between April 2001 and March 2004; 198 cases (95%) had preexisting cirrhosis ($n = 167$) or chronic hepatitis ($n = 31$).

Hospital controls ($n = 275$, PR = 73%) were first-time visitors at the general outpatient clinic of Saga Medical School Hospital between May 2001 and April 2003, who had no evidence of HCC. We selected these controls based on the following order of priority: (i) men aged 50–79 years; (ii) women aged 60–79 years; (iii) men aged 40–49 years; and (iv) women aged 40–59 years. This order was determined by the sex and age distribution of mortality from liver cancer in Saga Prefecture in 1998. The controls had various, mostly minor, diseases ($n = 190$), undiagnosed symptoms ($n = 49$), and no definite abnormality ($n = 36$). Patients with CLD without HCC ($n = 381$, 298 patients with chronic hepatitis and 83 cirrhotic patients, PR = 96%) were out- or in-patients of the two hospitals between September 2001 and March 2004; patients with special types of CLD (primary and secondary biliary cirrhosis, autoimmune hepatitis, and liver disease due to parasitosis, congestive heart failure, or metabolic disorders) were excluded.

The study protocol was approved by the Ethics Committees of the two hospitals, and written informed consent to the use of their blood and clinical information for this study was obtained from all subjects.

2.2. Interview

Research nurses interviewed study subjects on drinking and smoking habits using a uniform questionnaire. We defined "never drinkers" as those who had never drunk or had drunk less than once per week and/or for less than one year, "former drinkers" as those who had quit alcohol one or more years before, and "current drinkers" as those who currently drank or had quit alcohol less than one year before. A "history of heavy drinking" was regarded as present if subjects had imbibed 69 g or more of ethanol per day for 10 or more years. "Never smokers" were defined as individuals who had never smoked or had smoked for less than one year, "former smokers" as those who had stopped smoking one or more years before, and "current smokers" as those who currently smoked or had stopped smoking less than one year before.

2.3. Serologic tests and genotyping

Venous blood was drawn, and plasma samples were tested for hepatitis B surface antigen (HBsAg) by a chemiluminescent immunoassay (CLIA; Dainabot, Tokyo, Japan) and for antibody to HCV (HCVAb) by a 2nd generation enzyme immunoassay (Abott HCV EIA II; Dainabot, Tokyo).

DNA was extracted from buffy coat preparations by using a commercial kit (QIAmp DNA Blood Mini kit; QIAGEN Inc, Tokyo). Genotyping of *IL-1B* −31T/C and *TNF-A* −1031C/T polymorphisms was carried out by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) according to Hamajima et al. [9,24]. *TNF-A* −308G/A polymorphism was analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) according to Ho *et al* [17]. We confirmed the above results for several specimens with different genotyping patterns by direct sequencing. In addition, about 10% random samples were rechecked by the same method (PCR-CTPP or PCR-RFLP).

2.4. Statistical analyses

χ^2 tests were used for unadjusted comparisons based on frequency. Unconditional logistic regression analyses were conducted to estimate odds ratios (ORs) and their 95 percent confidence intervals (CIs) of HCC for *IL-1B* and *TNF-A* genotypes and drinking and smoking habits with adjustment for sex, age category (40–49, 50–59, 60–69, and 70–79 years), heavy drinking history (never and ever), smoking status (never, former, and current smokers), and HBsAg and HCVAb status. Tests of linear trend for *IL-1B* and *TNF-A* genotypes were performed by assigning an ordinal variable to the genotype in the logistic model. Interactions between each genotype and alcohol/tobacco on HCC risk were evaluated by the likelihood ratio test. All statistical analyses were performed with

Table 1
Selected characteristics of study subjects

Factor	HCC cases no.(%)	Hospital controls no. (%)	CLD patients no.(%)	P[a]	P[b]
Sex				0.64	<0.01
Male	141 (67.5)	180 (65.5)	205 (53.8)		
Female	68 (32.5)	95 (34.5)	176 (46.2)		
Age (year)				<0.01	<0.01
40–49	6 (2.9)	42 (15.3)	73 (19.2)		
50–59	28 (13.4)	85 (30.9)	93 (24.4)		
60–69	76 (36.4)	86 (31.3)	136 (35.7)		
70–79	99 (47.4)	62 (22.6)	79 (9.2)		
HBsAg				<0.01	0.97
Negative	190 (90.9)	269 (97.8)	346 (90.8)		
Positive	19 (9.1)	6 (2.2)	35 (9.2)		
HCVAb				<0.01	0.95
Negative	30 (14.4)	254 (92.4)	54 (14.2)		
Positive	179 (85.7)	21 (7.6)	327 (85.8)		
Heavy drinking history, male				<0.01	<0.01
No	95 (67.4)	158 (87.8)	170 (82.9)		
Yes	46 (32.6)	22 (12.2)	22 (17.1)		
Heavy drinking history, female				0.17	0.37
No	65 (95.6)	94 (99.0)	172 (97.7)		
Yes	3 (4.4)	1 (1.1)	4 (2.3)		
Smoking status, male				0.03	0.07
Never smoker	24 (17.0)	50 (27.8)	54 (26.3)		
Former smoker	51 (36.2)	67 (37.2)	76 (37.1)		
Current smoker	66 (46.8)	63 (35.0)	75 (36.7)		
Smoking status, female				0.70	0.66
Never smoker	61 (89.7)	88 (92.6)	150 (85.2)		
Former smoker	4 (5.9)	3 (3.2)	15 (8.5)		
Current smoker	3 (4.4)	4 (4.2)	11 (6.3)		

[a] Comparisons were made between HCC cases and hospital controls.
[b] Comparisons were made between HCC cases and CLD patients.

Table 2
Adjusted ORs of HCC for IL-1B −31 and TNF-A −1031 and −308 genotypes

	HCC cases no. (%)	Hospital controls no. (%)	CLD patient no. (%)	OR[a,b] (95% CI)	OR[a,c](95% CI)
IL-1B −31					
C/C	41 (19.6)	66 (24.0)	95 (25.2)	1.00 (reference)	1.00 (reference)
C/T	101 (48.3)	146 (53.1)	176 (45.7)	0.84 (0.35–2.03)	1.46 (0.90–2.37)
T/T	67 (32.1)	63 (22.9)	110 (29.1)	1.32 (0.48–3.65)	1.46 (0.86–2.47)
P for trend				0.53	0.14
TNF-A −1031					
T/T	151 (72.3)	196 (71.3)	264 (69.3)	1.00 (references	1.00 (reference)
T/C	49 (23.4)	69 (25.1)	109 (28.6)	0.35 (0.16–0.78)	0.75 (0.48–1.15)
C/C	9 (4.3)	10 (3.6)	8 (2.1)	2.35 (0.40–13.75)	1.51 (0.49–4.66)
P for trend				0.15	0.53
TNF-A −308					
G/G	205 (98.1)	270 (98.2)	371 (97.4)	1.00 (reference)	1.00 (reference)
G/A	4 (1.9)	5 (1.8)	10 (2.6)	9.13 (0.91–91.63)	0.86 (0.24–3.10)

[a] Adjusted for sex, age category, HBsAg, HCVAb, heavy drinking history, and smoking status.
[b] Comparisons were made between HCC cases and hospital controls.
[c] Comparisons were made between HCC cases and CLD patient.

the SAS/PC statistical package (SAS Institute Inc., Cary, NC).

3. Results

Basic characteristics of study subjects are shown in Table 1. As compared with at least either control group, HCC cases presented significantly higher proportions of males (against CLD patients), older subjects (against both control groups), HBsAg positives (against hospital controls), HCVAb positives (against hospital controls), males with a heavy drinking history (against both control groups), and male current smokers (against hospital controls).

Table 2 shows the distribution of *IL-1B* and *TNF-A* genotypes among study subjects and associated ORs of HCC against either control group. When HCC cases were

Table 3
Adjusted ORs of HCC for *IL-1B* −31 genotype according to drinking and smoking status

	HCC cases no. (%)	Hospital controls no. (%)	CLD patients no. (%)	OR[a,b] (95% CI)	OR[a,c] (95% CI)
Never drinker					
C/C	12 (15.4)	38 (24.4)	50 (25.5)	1.00 (reference)	1.00 (reference)
C/T	38 (48.7)	80 (51.3)	86 (43.9)	1.50 (0.30–7.45)	1.70 (0.76–3.77)
T/T	28 (35.9)	38 (24.4)	60 (30.6)	1.80 (0.31–10.58)	2.46 (1.05–5.76)
P for trend				0.51	0.03
Current/former drinker					
C/C	29 (22.1)	28 (23.5)	45 (24.3)	1.00 (reference)	1.00 (reference)
C/T	63 (48.1)	66 (55.5)	90 (48.7)	0.63 (0.23–1.77)	1.28 (0.69–2.38)
T/T	39 (29.8)	25 (21.0)	50 (27.0)	1.10 (0.33–3.74)	1.08 (0.54–2.17)
P for trend				0.89	0.79
Never/former smoker					
C/C	26 (18.6)	47 (22.6)	68 (23.1)	1.00 (reference)	1.00 (reference)
C/T	68 (48.6)	111 (53.4)	130 (48.6)	0.76 (0.26–2.20)	1.50 (0.85–2.66)
T/T	46 (32.9)	50 (24.0)	97 (32.9)	1.02 (0.31–3.39)	1.33 (0.72–2.45)
P for trend				0.91	0.44
Current smoker					
C/C	15 (21.7)	19 (28.4)	27 (31.4)	1.00 (reference)	1.00 (reference)
C/T	33 (47.8)	35 (52.2)	46 (53.5)	1.27 (0.16–10.31)	1.53 (0.60–3.90)
T/T	21 (30.4)	13 (19.4)	13 (15.1)	1.76 (0.15–20.50)	2.54 (0.81–7.95)
P for trend				0.65	0.11

[a] Adjusted for sex, age category, HBsAg, HCVAb, and either smoking status or heavy drinking history.
[b] Comparisons were made between HCC cases and hospital controls.
[c] Comparisons were made between HCC cases and CLD patients.

compared with hospital controls, ORs were very unstable after adjustment for HBsAg and HCVAb in addition to other covariates, as evidenced by wide CIs. This was because only 2% and 8% of hospital controls tested positive for HBsAg and HCVAb, respectively. Multiple logistic regression analyses did not reveal any significant trends in HCC risk associated with the *IL-1B* −31 or *TNF-A* −1031 genotype. For the *TNF-A* −308 genotype, the minor A allele was too few to make meaningful analyses.

Table 3 presents the HCC risk for the *IL-1B* −31 genotype according to drinking and smoking status. Based on comparison of HCC cases with CLD patients, we detected a significant trend in HCC risk associated with the genotype among never drinkers (P = 0.03); the adjusted ORs (and 95% CIs) for the C/T and T/T genotypes compared with the C/C genotype were 1.70 (0.76–3.77) and 2.46 (1.05–5.76), respectively. Such an association was not observed in former and current drinkers combined. A similar upward tendency was observed among current smokers (P for trend = 0.11); the corresponding ORs (and 95% CIs) were 1.53 (0.60–3.90) and 2.54 (0.81–7.95), respectively. Similar but statistically insignificant results were obtained by the comparison between HCC cases and hospital controls. Regarding the *TNF-A* −1031 and −308 genotypes, we did not find any appreciable differences by drinking or smoking status (data not shown).

Finally, we evaluated if the HCC risk due to heavy alcohol intake and current smoking, both of which were risk factors for HCC in this study [23,25], differed by the *IL-1B* −31 genotype based on comparison of HCC cases with CLD patients (Fig. 1). The HCC risk associated with heavy alcohol intake decreased with increasing T allele; the adjusted ORs (and 95% CIs) for heavy drinking history among C/C, C/T, and T/T carriers were 5.70 (1.72–18.92), 3.13 (1.35–7.24), and 0.80 (0.28–2.33), respectively (P for interaction = 0.10). Conversely, the risk associated with current smoking increased with increasing T allele; the corresponding ORs (and 95% CIs) were 1.62 (0.53–4.95), 2.21 (1.07–4.58), and 5.66 (1.91–16.73), respectively (P for interaction = 0.55).

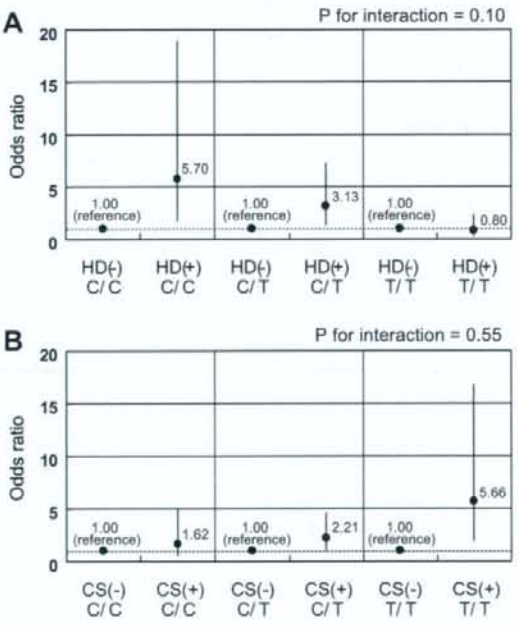

Fig. 1. Adjusted ORs (and 95% CIs) of HCC for either heavy drinking history (HD, Fig. 1A) or current smoking (CS, Fig. 1B) according to *IL-1B* −31 genotype. Comparisons were made between HCC cases and CLD patients, and ORs were adjusted for sex, age category, HBsAg, HCVAb, and either smoking status or heavy drinking history. P values for interaction were calculated by the likelihood ratio test in unconditional logistic regression analysis.

4. Discussion

The present study assessed the association between *IL-1B* and *TNF-A* gene polymorphisms and HCC. IL-1β and TNF-α are proinflammatory cytokines with multiple biological effects and play major roles in inflammation-linked tumor development. Both can induce inflammation, which leads to tissue damage resulting in increased cellular turnover. Nitric oxide and reactive oxygen species from inflammatory cells may induce DNA damage, which increases the possibility of the emergence of cells possessing a high risk of malignant transformation. In addition, these proinflammatory cytokines can activate NF-κB and AP-1 transcription factors, which not only induce inflammatory mediators, cytokines, and growth factors but also regulate cell proliferation, antiapoptosis, and immune response in target tumors and pretumorous cells [8]. Therefore, we hypothesized that gene polymorphisms of *IL-1B* and *TNF-A* might be associated with the HCC risk. Several epidemiological studies have examined this association although the results have been controversial [10–13,17]. In addition, no studies but one [12] evaluated potential interactions between the genes and environmental factors such as alcohol and tobacco.

In this study, from the comparison between HCC cases and CLD patients, we noticed that the HCC risk associated with the *IL-1B* −31T/C polymorphism increased with increasing T allele among never drinkers and current smokers although this polymorphism did not significantly affect the overall risk. Similarly, the risk associated with heavy alco-

hol intake and current smoking was different according to the polymorphism. The *IL-1B* −31T/C polymorphism is included in a TATA-box and has been shown to affect DNA–protein interactions in vitro; the −31T allele, which preserves the TATA-box, was reported to have a five-fold elevated binding activity with the transcription initiation factor [26]. Accordingly, this allele may enhance IL-1β production in the liver and may cause higher susceptibility to HCC.

Alcohol intake and cigarette smoking are also known to influence the production of inflammatory cytokines. There is evidence showing that moderate alcohol intake in humans reduces the production of IL-1β and TNF-α through inhibition of NF-κB [20] and that in vitro exposure of cultured cells to ethanol in high concentrations reduced the IL-1β and TNF-α induced cytokine generation by inhibiting translocation of NF-κB subunits to the nucleus [27]. Smoking has been shown to increase the production of IL-1β and TNF-α by macrophages [21,28]. These properties might contribute to the potential risk modification for the *IL-1B* −31T/C genotype by alcohol and tobacco although the precise mechanisms remain to be elucidated.

The association of the *IL-1B* −31 genotype with HCC was not statistically significant based on comparison between HCC cases and hospital controls although the point estimates of ORs against hospital controls demonstrated similar tendencies with those against CLD patients. One possible reason may be that the ORs against hospital controls became very unstable after adjustment for hepatitis virus markers that were positive in less than 10% of hospital controls.

The *TNF-A* −1031T/C and −308G/A polymorphisms were not associated with HCC in this study. *TNF-A* is known to have five biallelic single-nucleotide polymorphisms in the promoter region at −1031T/C, −863C/A, −857C/T, −308G/A, and −238G/A [16]. In the Japanese, the −308A and −238A alleles are rare [16], and −1031T/C is tightly linked with −863C/A [29]. The −308A allele has been associated with an eight-fold higher transcriptional activation in vitro [30] and higher TNF-α production in human whole blood cell culture stimulated by lipopolysaccharide [31]. A recent study reported a positive association between this polymorphism and HCC [17], but we could not detect such an association. As another candidate, we examined the −1031T/C polymorphism for which a possible difference of *TNF-A* expression [29,32] and potential relationships with several diseases among the Japanese [18,19] were reported, yet no significant relation was observed.

In conclusion, we found that the impact of *IL-1B* −31T/C polymorphism on HCC was different by drinking and smoking status among CLD patients, and this polymorphism appeared to modify the HCC risk in relation to alcohol and tobacco. Further well-designed large studies are required to confirm these results.

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Original Article

Does a late evening meal reduce the risk of hepatocellular carcinoma among patients with chronic hepatitis C?

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Aim: Some studies have suggested that nutritional support might protect against the recurrence of hepatocellular carcinoma (HCC) among postoperative HCC patients. However, no epidemiological studies have evaluated the effect of nutritional support on HCC incidence. This study aimed to investigate the association between a late evening meal and HCC.

Methods: We conducted a hospital-based, case-control study comparing 73 cases with HCC to 253 matched controls among patients with chronic hepatitis C. A questionnaire elicited information on the consumption of a late evening meal, which was defined as a snack or meal within 2 h before bedtime. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by the conditional logistic regression model.

Results: After adjustment for potential confounders, patients who consumed a late evening meal had a lower OR as compared to those who did not consume one (OR, 0.08; 95% CI, 0.01–0.48). In terms of frequency of intake, a clear inverse exposure–response relationship was observed (trend $P = 0.009$). In addition, a negative association between a late evening meal and HCC was more pronounced among patients with an α-fetoprotein level of less than 20 ng/mL and those with a body mass index of less than 25 kg/m^2.

Conclusion: A late evening meal might protect against HCC, particularly among patients with a normal α-fetoprotein level and who are not obese, although these relations might be accounted for other factors, including total energy intake. Further studies with larger study sizes are needed to corroborate these findings.

Key words: case-control study, hepatitis C virus, hepatocellular carcinoma, late evening meal, risk factor

INTRODUCTION

PROTEIN–ENERGY MALNUTRITION is often observed in patients with advanced liver cirrhosis because of nutritional and metabolic abnormalities.[1–3] Several previous papers suggested that protein–energy malnutrition is significantly associated with the development of life-threatening complications and increased mortality.[4–7] In particular, nocturnal starvation in those with liver cirrhosis seems to be an important problem because a severe catabolic state is present overnight.[8] One study showed that nocturnal starvation might be a potential risk factor for the aggravation of liver disease.[9]

To improve nocturnal starvation, current guidelines recommend late evening snacks for patients with cirrhosis,[1,10] and therefore, the administration of branched chain amino acids (BCAA) or divided meal, partly consumed as a late evening snack, is now often prescribed. Previous studies have consistently demonstrated that BCAA administration corrects malnutrition in patients with cirrhosis.[11,12] Administration before bedtime seems to be most effective in terms of nutritional metabolism.[13–15] Recent studies have also suggested that BCAA might decrease mortality among patients with liver cirrhosis.[16] Before BCAA prescription for patients with cirrhosis became popular, carbohydrate-rich snacks were considered as a late evening snack. Carbohydrate-rich snacks also improve nitrogen balance and abnormal fuel metabolism in patients with cirrhosis.[8,17–19]

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A previous study indicated that a late evening meal, including carbohydrate-rich snacks, had the same effect as BCAA administration,[6] although a recent randomized, controlled trial suggested that the impact of BCAA administration on the improvement of nutritional parameters was superior to that of ordinary food containing matched daily energy and protein intake.[20]

However, few studies have investigated the long-term effect of nutritional support on hepatocellular carcinoma (HCC) development. To the best of our knowledge, only two studies have evaluated the effect of nutritional intervention on the risk of HCC recurrence among postoperative HCC patients.[21,22] In these studies, the intervention group had a significantly lower recurrence rate when compared to the control group. These reports suggested that nutritional support might act to prevent HCC occurrence. Thus we conducted a case-control study to examine the hypothesis that nutritional support might reduce the risk of HCC incidence. The present study took special notice of a late evening meal as a nutritional factor since this has been considered to be one of the most effective approaches for improvement of nocturnal starvation. In Japan, 80% of HCC cases are caused by hepatitis C virus (HCV) infection,[23] so the source population was restricted to patients with chronic type C liver disease.

METHODS

Selection of cases and controls

THE METHOD OF the present study has been described elsewhere.[24,25] We identified all consecutive patients with chronic hepatitis C who visited the Department of Hepatology of Osaka City University Hospital (OCUH; Osaka, Japan) for clinical follow up between 1 November 2001 and 31 January 2002 (the recruitment period). The following patients were excluded: patients with other types of liver disease (e.g. co-infection with hepatitis B virus, primary biliary cirrhosis, autoimmune hepatitis, and idiopathic portal hypertension), referred patients who had already been diagnosed with HCC at other hospitals, and patients in poor health (e.g. liver failure and terminal stage of HCC). This resulted in 1159 patients who were regarded as a source population.

From the source population, 86 patients were first diagnosed with HCC between 1 November 1998 and 31 March 2002. The diagnosis of HCC was based either on a histopathological examination or a positive result in at least one imaging study (computed tomography, magnetic resonance imaging, angiography) combined with an elevated serum á-fetoprotein level. For each HCC case, we selected 1–5 control patients, matching for age (±2 years), sex, and the date of the first OCUH visit (±2 years). Eventually, 86 cases and 333 controls were identified as candidates.

The study protocol was approved by the ethics committee at the Osaka City University Graduate School of Medicine.

Information collection

From 1 June 2002 to 31 December 2002 (the study period), the physician-in-charge explained this study to the candidate cases and controls each time they underwent regular medical examinations. After obtaining informed consent verbally, the physician-in-charge gave the patients a self-administered, mail-back questionnaire. We mailed reminders to non-respondents twice at 1-month intervals. The questionnaire included items on demographic factors; past medical history; age of first identification of liver disease (e.g. abnormality of liver enzyme level or positive results for HCV infection); family history of liver diseases; smoking; alcohol drinking; dietary habits, including a late evening meal; occupation; physical exercise; and reproductive history. A late evening meal was defined as a snack or meal within 2 h before bedtime. The habit of eating a late evening meal after first identification of liver disease was investigated retrospectively by reporting a dichotomous answer (yes or no). Patients who answered "yes" also reported the average weekly frequency of eating a late evening meal and the major food items they consumed.

We also collected the findings of abdominal ultrasonography and laboratory data at the first OCUH visit from medical records. At OCUH, the findings from the abdominal ultrasonography had been scored to show disease severity on a semiquantitative scale called the "US score." This score is the sum of the five leveled scores (0, 0.5, 1.0, 1.5, and 2.0) for five variables (liver deformity, nature of the liver edge, nature of the liver surface, coarsening of intrahepatic echo signals, and size of the spleen). This was evaluated in patients with chronic type C liver disease and proved to be highly correlated with the degree of liver fibrosis according to the new European classification or Child–Turcotte criteria.[26] While US scores ≥3.5 indicated chronic liver disorders and US scores >5.0 indicated liver cirrhosis, the sensitivity and specificity of this approach to classifying the presence or absence of liver cirrhosis were estimated to be 83–97% and 91–96%, respectively.[27,28] Laboratory data included white blood cell, red blood cell, platelet

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count, total bilirubin, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, α-fetoprotein, virus titer of HCV-RNA, and fasting blood sugar. Information about interferon therapy was obtained from medical records.

Data analyses

The frequency of intake of a late evening meal was recategorized into three levels according to the distribution of controls, with category boundaries that were drawn to make the size of groups as equal as possible. The χ^2-test and Wilcoxon rank sum test were used to compare selected characteristics between cases and controls. To consider the presence of confounding, the distribution of potential confounders was compared between patients who consumed a late evening meal and those who did not only among the control patients using χ^2-test or Wilcoxon rank sum test. The conditional logistic regression model was used to calculate the odds ratios (OR) and 95% confidence intervals (CI) for HCC risk. Variables that showed *P*-values less than 0.1 or seemed to correlate with the late evening meal were considered to be potential confounders for adjustment.

We performed an additional analysis to consider the effect of possible confounding variables, such as markers of progression of liver disease, potential malnutrition, obesity, and treatment with interferon. In the additional stratified analyses, patients were divided into two groups according to the following cut-off point: median level of US score, presence or absence of suspected liver cirrhosis (ratio of aspartate to alanine aminotransferase >1.0,[29–31] platelet count $<10 \times 10^4/\mu l$[29]), normal level of α-fetoprotein, median level of serum albumin, and presence or absence of obesity.[32] In the stratified analyses, the unconditional logistic regression model was used to calculate OR and 95% CI of a late evening meal for HCC. Each model included three matching factors (i.e. age, sex, and duration from first OCUH visit) and the potential confounders other than stratified factors. The homogeneity of OR across stratified categories was tested as the *P*-value of the interaction term between a late evening meal and each stratified variable.

All statistical analyses were performed using SAS version 8.2 (SAS Institute, Cary, NY, USA).

RESULTS

AMONG THE 419 enrolled patients, 51 were excluded. Ten patients were subsequently found to be ineligible (e.g. co-infection with HBV and complete recovery from HCV infection) and 41 patients did not visit OCUH during the study period. There were 23 non-respondents (6%) for the following reasons: death (4 patients: 3 cases and 1 control), poor health (6 patients: 3 cases and 3 controls), and refusal to participate (13 patients: 1 case and 12 controls). Eventually, 326 patients (73 cases and 253 controls, 73 matched sets) maintained the initial matched combination and comprised the patients for the analysis.

Table 1 shows the selected characteristics of the cases and controls. Cases and controls were well matched for age, sex, and duration from the first OCUH visit until the beginning of the study. A significant difference between cases and controls was observed in the duration from first identification of liver disease until the beginning of the study period (17 *vs* 13 years). Cases had more family history of liver diseases and received less interferon therapy with marginal significance. Laboratory data and US scores at the first OCUH visit indicated that cases had more severe disease condition than controls during the 7 years before the beginning of the study period.

Table 2 provides the distribution of selected potential confounders between patients who had consumed a late evening meal and those who did not among the control patients. No measurable differences were found in the distribution of potential confounders, including liver disease progression, body mass index, and interferon therapy, across the groups who did or did not consume a late evening meal.

Table 3 shows the OR for HCC according to late evening meal, adjusted for duration from first identification of liver disease, disease severity at the first OCUH visit (US score, platelet count, aspartate aminotransferase, α-fetoprotein, and fasting blood sugar), and interferon therapy. The group who consumed a late evening meal had a reduced risk of HCC as compared to those who did not consume one (OR, 0.08; 95% CI, 0.01–0.48). In addition, higher frequency intake of a late evening meal was associated with lower OR with a significant dose–response relationship (trend $P = 0.009$). Thus a late evening meal was associated with a lower risk of HCC.

The inverse associations of a late evening meal with the development of HCC did not differ between groups with or without possible liver cirrhosis (Table 4). When the study patients were divided according to the absence or presence of possible liver cirrhosis (e.g. a platelet count of less than $10 \times 10^4/\mu l$ or ratio of aspartate to alanine aminotransferase of more than 1.0), no measurable difference was observed in the inverse association

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Table 1 Comparison of selected characteristics between cases and controls†

Characteristics	Case ($n = 73$)	Control ($n = 253$)	*P*-value‡
Age (years)	69 (65–73)	69 (65–72)	0.389
Sex (%)			
Male	47	52	0.434
Duration until beginning of the study (years)			
From first identification of liver disease	17 (12–26)	13 (10–21)	0.011
From first OCUH visit	7 (4–9)	7 (4–9)	0.289
Family history of liver diseases (%)			
Present	38	27	0.069
Interferon therapy (%)			
Present	25	36	0.072
Body mass index (kg/m^2)	22 (21–26)	23 (21–25)	0.986
Platelet count ($\times 10^4/\mu$L)	11 (8–15)	16 (12–20)	0.000
Aspartate aminotransferase (IU/L)	86 (59–112)	67 (43–101)	0.003
Albumin (g/dL)	3.8 (3.6–4.1)	4.1 (3.9–4.3)	0.000
Alpha-fetoprotein (ng/mL)	15 (7–36)	5 (4–11)	0.000
Fasting blood sugar (mg/dL)	100 (94–118)	98 (92–108)	0.066
US score	4.0 (3.0–5.5)	3.0 (2.0–3.5)	0.000

†Data are expressed as median (inter-quartile range) unless otherwise indicated. ‡χ^2-test and Wilcoxon rank sum test were used where appropriate. OCUH, Osaka City University Hospital; US score, ultrasonography score.

Table 2 Comparison of selected characteristics between patients who consumed a late evening meal and those who did not among control patients†

Characteristics	Patients who consumed a late evening meal ($n = 46$)	Patients who did not consume a late evening meal ($n = 207$)	*P*-value‡
Age (years)	68 (64–74)	69 (65–72)	0.960
Sex (%)			
Male	52	52	0.953
Duration until beginning of the study (years)			
From first identification of liver disease	16 (11–26)	13 (10–21)	0.152
From first OCUH visit	7 (4–9)	7 (4–9)	0.974
Family history of liver diseases (%)			
Present	28	27	0.868
Interferon therapy (%)			
Present	37	36	0.877
Body mass index (kg/m^2)	23 (21–25)	23 (21–25)	0.842
Platelet count ($\times 10^4/\mu$L)	17 (11–21)	16 (12–19)	0.677
Aspartate aminotransferase (IU/L)	66 (41–96)	67 (47–101)	0.482
Albumin (g/dL)	4.1 (3.8–4.3)	4.1 (3.9–4.3)	0.352
α-Fetoprotein (ng/mL)	5 (4–10)	6 (4–11)	0.452
Fasting blood sugar (mg/dL)	99 (93–110)	98 (92–107)	0.330
US score	3.0 (2.0–4.0)	3.0 (2.5–3.5)	0.471

†Data are expressed as median (interquartile range) unless otherwise indicated. ‡χ^2-test and Wilcoxon rank sum test were used where appropriate. OCUH, Osaka City University Hospital; US score, ultrasonography score.

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Table 3 Odds ratio (OR)† for hepatocellular carcinoma according to frequency of intake of a late evening meal

Characteristics	Level	Case ($n = 73$) n (%)	Control ($n = 253$) n (%)	Univariate			Multivariate‡		
				OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
Late evening meal	Never	66 (90)	207 (82)	1			1		
	Intake	7 (10)	46 (18)	0.47	(0.20–1.07)	0.071	0.08	(0.01–0.48)	0.005
Frequency	Never	66 (90)	207 (82)	1			1		
	<4 times/week	6 (8)	26 (10)	0.70	(0.28–1.75)	0.440	0.12	(0.02–1.02)	0.052
	≥4 times/week	1 (1)	20 (8)	0.16	(0.02–1.19)	0.073	0.06	(0.01–0.57)	0.015
					(Trend $P = 0.041$)			(Trend $P = 0.009$)	

†Calculated by the conditional logistic regression model. ‡Model includes: duration from first identification of liver disease, body mass index at first identification of liver disease, severity of liver disease at first Osaka City University Hospital visit (ultrasonography score, platelet count, aspartate aminotransferase, albumin, α-fetoprotein, fasting blood sugar), family history of liver disease, and interferon therapy. CI, confidence intervals.

of a late evening meal with HCC across the groups, although valid estimates could not be calculated in the assessment of the ratio of aspartate to alanine aminotransferase of more than 1.0. As for the α-fetoprotein level, the relationship between a late evening meal and HCC was demonstrated with smaller OR among patients with a normal α-fetoprotein level. Regarding albumin level or obesity, inverse associations of a late evening meal with HCC were more pronounced in patients with an albumin level less than 4 g/dL and those with a body mass index less than 25 kg/m^2. Furthermore, the interaction between body mass index and a late evening meal for HCC was statistically significant ($P = 0.022$ for the homogeneity of OR). A late evening meal indicated a smaller OR for HCC risk irrespective of the absence or presence of a history of interferon therapy, although the relationship reached statistical significance only in patients without a history of interferon therapy.

The results from stratified analyses suggested that the interaction between a late evening meal and α-fetoprotein level, albumin level, or body mass index existed. In that case, it may be more appropriate that the interaction terms were included in the overall multivariate analyzes. Thus we conducted additional multivariate analyses in which each interaction term was added as an adjustment. When the interaction term between a late evening meal and α-fetoprotein was included in the multivariate analysis, OR of a late evening meal was almost similar with the results in Table 3 (OR, 0.02; 95% CI = 0.00–1.21; $P = 0.062$). Considering the interaction with albumin level, OR were nearly the same as the results in Table 3 (OR, 0.09; 95% CI = 0.01–0.62; $P = 0.014$). When we included the interaction term between a late evening meal and body mass index, the model did not converge because there was only one case who consumed a late evening meal and had a body mass index of less than 25 kg/m^2. Thus we could not simultaneously consider these three interactions. In order to consider these interactions, further large-scale studies are needed.

DISCUSSION

THE PRESENT RESULTS support the hypothesis that a late evening meal may decrease the risk of HCC. This finding is consistent with those of previous studies in which nutritional intervention was associated with a lowered recurrence rate of HCC among postoperative HCC patients.[18,19] In addition, a past experimental study indicated that higher administration of a nutritional

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Table 4 Adjusted odds ratio (OR) of late evening meal intake for hepatocellular carcinoma stratified according to selected potential confounders

Stratified category	Proportion of late evening meal intake		OR†‡	(95% CI)	*P*-value	Homogeneity of OR across stratified categories§
	Case n/N (%)	Control n/N (%)				
US score (Severity of liver disease)						
<3.5¶	0/20 (0)	27/162 (17)	-	-	-	0.951
3.5+	6/51 (12)	19/90 (21)	0.39	(0.11–1.37)	0.142	
Ratio of aspartate to alanine aminotransferase						
<1.0	6/57 (11)	33/194 (17)	0.50	(0.17–1.47)	0.209	0.947
1.0+¶	1/16 (6)	13/59 (22)	-	-	-	
Platelet count ($\times 10^4/\mu L$)						
10	4/42 (10)	36/225 (16)	0.36	(0.08–1.66)	0.191	0.563
<10	3/29 (10)	10/28 (36)	0.26	(0.04–1.53)	0.136	
α-Fetoprotein (ng/mL)						
20.0+	5/25 (20)	6/37 (16)	0.75	(0.10–5.62)	0.779	0.067
<20.0	2/44 (5)	37/194 (19)	0.02	(0.001–0.36)	0.007	
Albumin level (g/dL)						
4.0+	3/30 (10)	25/167 (15)	0.72	(0.16–3.23)	0.665	0.148
<4.0	4/43 (9)	21/86 (24)	0.13	(0.02–0.68)	0.016	
Body mass index (kg/m^2)						
25.0+	5/18 (28)	10/54 (19)	1.59	(0.22–11.2)	0.644	0.022
<25.0	1/53 (2)	36/198 (18)	0.05	(0.01–0.44)	0.008	
History of interferon therapy						
Absent	6/55 (11)	29/162 (18)	0.27	(0.08–0.99)	0.048	0.672
Present	1/18 (6)	17/91 (19)	0.15	(0.01–2.76)	0.203	

†Calculated by unconditional logistic regression model. ‡Model includes three matching factors (age, sex, and duration from first OCUH visit) and the following potential confounders other than stratified factor: body mass index at first identification of liver disease, severity of liver disease at first OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, α-fetoprotein, fasting blood sugar), family history of liver disease, duration from first identification of liver disease, and interferon therapy. §Homogeneity of OR across stratified categories was tested as the *P*-value of the interaction term between a late evening meal and each stratified variable. ¶Model did not converge because there were no cases or too limited cases who consumed a late evening meal. CI, confidence intervals; OCUH, Osaka City University Hospital visit US score; ultrasonography score.

factor prevented human HCC cells from increasing.[33] Thus it seems reasonable to infer that a late evening meal has a protective effect against HCC.

It is important to clarify the optimal timing of nutritional support. Some studies have indicated that starting nutritional support in the early stage of cirrhosis may be useful in improving nutritional parameters.[34,35] In the present stratified analyses, the protective impact of a late evening meal was observed irrespective of the presence or absence of possible liver cirrhosis. The inverse effect of a late evening meal for HCC development was more pronounced among patients with an α-fetoprotein level of less than 20 ng/mL. About this association, we considered the following: (i) patients with a higher α-fetoprotein level might have a higher risk for HCC development. This background caused difficulty in the detection of the negative association with a late evening meal among these patients, but ease in determining the relationship among those with a normal α-fetoprotein level; (ii) potentially undetectable HCC cells might be developed among patients with a higher α-fetoprotein level. A late evening meal might no longer operate on the prevention of HCC among these patients. Contrary to this, the impact of late evening meal might be more easily demonstrated among those with a normal α-fetoprotein level. Further studies with larger study sizes are needed to corroborate these findings in order to consider the underlying mechanisms.

As for the interaction between a late evening meal and body mass index, the inverse associations of a late evening meal with HCC were further pronounced in

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patients with a body mass index less than 25 kg/m^2. It was recently indicated that obesity might be a risk factor of HCC development. Thus it brought about difficulties in the detection of the negative association with a late evening meal among the obesity group, but ease in demonstrating the decreasing OR of a late evening meal for HCC among patients with a body mass index less than 25 kg/m^2. A recent randomized, controlled trial among patients with decompensated liver cirrhosis demonstrated that the impact of BCAA in reducing the risk of liver cancer is superior to that of the ordinary food group among patients with a body mass index of more than 25 kg/m^2, although there was no difference in the risk of HCC between BCAA and ordinary food among those with a body mass index below 25 kg/m^2.[36] Taken together, these findings seem to indicate that a late evening meal has a preventive effect against HCC to the same extent as BCAA administration among patients who are not obese, while the effect of a late evening meal for HCC prevention is less than that of BCAA among those who are obese. It is therefore likely that BCAA and a late evening meal exert their effects by different mechanisms among patients who are obese.

The effect of a late evening meal was found to be statistically significant only in patients without a history of interferon therapy. However, point estimates of the effect of a late evening meal were similar in the absence or presence of a history of interferon. Thus decreased statistical power in the category of the presence of interferon therapy (i.e. only a small number of patients had experienced interferon therapy) might be responsible for the lack of statistical significance.

Regarding the mechanism of a late evening meal in HCC prevention, several previous studies indicated that malnutrition, including nocturnal starvation, is related to a poorer prognosis of liver cirrhosis[4–9] and that a late evening meal or BCAA supplement before bedtime improves protein–energy nutrition, imbalance of amino acids, or glucose tolerance.[13–15,17–19] In addition, some reports have indicated that a nibbling pattern of food intake, including a good breakfast and a late evening meal, would be preferable in order to have shorter episodes of catabolism during the day.[37–39] Some intervention studies have suggested that nutritional supplementation with oral BCAA is useful in preventing progressive hepatic failure and improving surrogate markers and perceived health status.[40–42] Thus it seems quite probable that a late evening meal acts to counteract malnutrition or nocturnal starvation, suppress the aggravation of liver disease, and as a result, prevent the development of HCC.

The strength of the present study is that the source population was restricted to patients with chronic type C liver disease, which enabled us to make a straightforward interpretation regarding any risk factors for HCV-associated HCC. In addition, we could analyze the data allowing for differences of background factors between the compared groups (e.g. severity of liver disease and the duration from first identification of liver disease).

However, due to the case-control study design within a very special population, that is, patients with chronic hepatitis C, the following three limitations may be present. First, selection bias might be introduced since the source population consisted of patients who had survived to the recruitment period. Patients who developed HCC but died before the recruitment period were not included in the case series, although cases were defined as those patients who had been first diagnosed with HCC in the recent past, that is, within 3 years. However, previous studies have reported that the mortality rate was significantly lower among a nutritional intervention group than among a placebo group.[40,43] It is therefore likely that patients without nutritional support have a higher risk of death. If, hypothetically speaking, cases excluded because of death had been included in this study, the prevalence of never consuming a late evening meal would increase in the hypothetical case series and the OR would decrease. Thus this selection bias may operate to bias the association toward the null, but not lead to exaggerated results.

A second limitation is an information bias resulting from imperfect memory of distant past history of consuming late evening meals. However, the hypothesis that a late evening meal is related to HCC or chronic liver disease was not generally recognized. Thus all patients would receive similar recall stimuli about past late evening meals. The misclassification due to such information bias, if any, is probably non-differential and would not affect the plausibility of the results.

Reverse causation is a third limitation for the observed association, although most retrospective studies suffer from this limitation. The habit of a late evening meal may change over time. However, this results of this study were interpreted from the information of a late evening meal at only one point without considering the potential changes in a late evening meal associated with liver dysfunction. Since more than 30 years may elapse between HCV infection and developing HCC, a late evening meal in the recent past may be affected by already manifested liver dysfunction. A long induction period in HCC can bring about the apparent causative associations, and exposure might be

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of importance only during an age-specific window or a specific time interval before diagnosis.

It is possible that other lifestyle characteristics can account for the protective effect of a late evening meal. However, we estimated the effect of late evening meal after correcting for the known HCC risk factors (liver disease severity, diabetes mellitus, family history, and interferon therapy) and for other putative confounders (duration of liver disease, and body mass index). In addition, similar results were obtained even when alcohol drinking and smoking were included in the analysis as additional potential confounders (data not shown). However, other uncontrolled factors might have affected the validity of our results. Previous studies indicated that riboflavin or vitamin B12 might reduce the risk of HCC.[44,45] One report indicated that some nutrients were positively associated with liver cirrhosis.[46] In addition, current guidelines define a late evening meal as a type of divided meal, and thus recommend fixing the total energy intake.[1,10] Due to the retrospective epidemiological analysis, a late evening meal in the present study could not be well characterized in terms of total energy intake as well as specific nutrients. Thus a late evening meal could be correlated with energy intake or specific nutrients.

In summary, this study showed a negative association between a late evening meal and HCC occurrence among patients with chronic hepatitis C. Further studies with larger study sizes are needed to corroborate these findings.

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