three with both, and nine with other origins) or cirrhosis ($n = 83$; 56 with hepatitis C alone, 10 with hepatitis B alone, two with both, and 15 with other origins) and had no evidence of HCC by radiological findings.

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

Interviews. Research nurses interviewed the study subjects using a structured questionnaire on demographic and lifestyle factors. The questionnaire elicited information on whether they had a 'heavy drinking history', which was defined as having drunk 69 g or more of ethanol per day for 10 or more years. A closed-end question queried about current smoking habit (never, former, or current smokers), with subsequent inquiries to former and current smokers about the number of cigarettes smoked per day and the duration of smoking in years, as well as the time of quitting smoking for former smokers. We defined 'never smokers' 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. The cumulative amount of smoking was calculated as pack-years (packs [1 pack = 20 cigarettes]/day × years of smoking) during lifetime or different time periods of life (e.g. last 10 years).

Hepatitis virus markers. Venous blood was collected from each subject, and plasma HBsAg and anti-HCV were assayed using a chemiluminescent immunoassay (CLIA; Dinabot, Tokyo) and a second-generation enzyme immunoassay (Abbott HCV EIA II; Dinabot, Tokyo), respectively, at an external laboratory (SRL, Tokyo).

Statistical analysis. χ^2 tests (for numbers and proportions) and Mann–Whitney tests (for continuous variables) were used for univariate analyses. The odds ratios (OR) and 95% confidence intervals (CI) of HCC for smoking habits were estimated by using unconditional logistic regression analysis, with adjustment for sex, age category (40–49, 50–59, 60–69, and 70–79), heavy drinking history (never and ever), and HBsAg and anti-HCV status. To assess linear trends in HCC risk associated with pack-years, a continuous variable of pack-years as well as covariates was included in the logistic model. Since female smokers were very few, we made analyses for men and women combined with adjustment for sex. All reported P-values are two-sided, and P-values less than 0.05 were considered statistically significant. All statistical analyses were carried out with the STATA statistical package (StataCorp, College Station, TX, USA).

Results

Table 1 shows basic characteristics of study subjects. As compared with at least either control group, HCC cases presented higher proportions of males ($P < 0.01$ against CLD patients), older subjects ($P < 0.01$ against both control groups), HBsAg positives ($P < 0.01$ against hospital controls), anti-HCV positives ($P < 0.01$ against hospital controls), males with a heavy drinking history ($P < 0.01$ against both control groups), and male current smokers ($P = 0.03$ against hospital controls, $P = 0.07$ against CLD patients). The median years since smoking cessation among former smokers ranged from 10 to 22 and did not significantly differ between HCC cases and either control group in either sex.

The relationship between smoking histories and HCC is shown in Table 2. After adjustment for sex, age, heavy drinking history, HBsAg and anti-HCV, the HCC risk was elevated for current versus never smokers (OR 1.8, 95% CI 0.6–5.1 against hospital controls; OR 2.5, 95% CI 1.4–4.6 against CLD patients) but not for former versus never smokers (OR 0.8 and 1.0, respectively). In terms of pack-years during lifetime, the dose–response relationship with HCC risk was not evident against either control group (P trend = 0.43), although some risk excess was observed for light to moderate consumption categories.

Since the comparison between HCC cases and CLD patients demonstrated a significantly increased risk for current smoking but not for pack-years during lifetime, we speculated that more recent cigarette use might be associated with higher HCC risk. To examine this possibility quantitatively, we calculated pack-years during different time periods (last 40, 20, 10 and 5 years) and associated OR (Table 3). Although no significant association was detected against hospital controls, significant dose–response relationships with pack-years during the last 10 or 5 years were observed against CLD patients. For example, the adjusted OR (and 95% CI) for 1–4 and 5+ pack-years during the

Table 1. Basic characteristics of study subjects

Factor	HCC cases ($n = 209$)	Hospital controls ($n = 256$)	CLD patients ($n = 381$)	$P^{\dagger,\ddagger}$	$P^{\S,\ddagger}$
Male : female (number)	141:68	167:89	205:176	0.61	<0.01
Age (years, median)	69	61	61	<0.01	<0.01
HBsAg-positive (%)	9.1	2.3	9.2	<0.01	0.97
Anti-HCV-positive (%)	85.6	7.8	85.8	<0.01	0.95
Heavy drinking history, male (%)	32.6	12.6	17.1	<0.01	<0.01
Heavy drinking history, female (%)	4.4	1.1	2.3	0.20	0.37
Smoking habit, male (%)					
Never smoker	17.0	28.1	26.3	0.03	0.07
Former smoker	36.2	37.7	37.1		
Current smoker	46.8	34.1	36.6		
Smoking habit, female (%)					
Never smoker	89.7	94.4	85.2	0.47	0.66
Former smoker	5.9	2.2	8.5		
Current smoker	4.4	3.4	6.3		
Years since smoking cessation (median)					
Male former smoker	18.0	15.0	15.5	0.09	0.22
Female former smoker	14.0	21.5	10.0	1.00	0.58

†P-value for the difference between hepatocellular carcinoma (HCC) cases and hospital controls.‡χ^2 tests (for numbers and proportions) or Mann–Whitney tests (for continuous variables).§P for the difference between HCC cases and chronic liver disease (CLD) patients. Anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen.

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Table 2. Adjusted odds ratios (OR) (and 95% confidence intervals [CI]) of hepatocellular carcinoma (HCC) according to smoking habits

Smoking habits	HCC cases versus hospital controls		HCC cases versus CLD patients	
	Number of cases/controls	OR† (95% CI)	Number of cases/controls	OR† (95% CI)
Never smoker	85/131	1.0 (reference)	85/204	1.0 (reference)
Former smoker	55/65	0.8 (0.3–2.3)	55/91	1.0 (0.6–1.7)
Current smoker	69/60	1.8 (0.6–5.1)	69/86	2.5 (1.4–4.6)
1–19 cigarettes/day	30/20	2.5 (0.7–9.2)	30/37	2.3 (1.1–4.7)
20+ cigarettes/day	39/40	1.4 (0.4–4.6)	39/49	2.7 (1.4–5.6)
Pack-years during lifetime				
0	85/131	1.0 (reference)	85/204	1.0 (reference)
1–19	32/31	3.0 (0.9–10.3)	32/63	1.3 (0.7–2.5)
20–39	48/58	0.9 (0.3–2.7)	48/62	2.0 (1.1–3.8)
40+	44/36	0.8 (0.2–2.5)	44/52	1.1 (0.6–2.2)
		P trend = 0.43		*P* trend = 0.43

†Adjusted for sex, age, heavy drinking history, hepatitis B surface antigen, and antibody to hepatitis C virus. CLD, chronic liver disease.

Table 3. Adjusted odds ratios (OR) (and 95% confidence intervals [CI]) of hepatocellular carcinoma (HCC) according to pack-years during different time periods

Pack-years	HCC cases versus hospital controls		HCC cases versus CLD patients	
	Number of cases/controls	OR† (95% CI)	Number of cases/controls	OR† (95% CI)
During last 40 years				
0	90/136	1.0 (reference)	90/207	1.0 (reference)
1–39	81/95	1.1 (0.4–2.9)	81/135	1.4 (0.8–2.2)
40+	38/25	1.4 (0.4–4.8)	38/39	1.6 (0.8–3.1)
		P trend = 0.58		*P* trend = 0.18
During last 20 years				
0	110/151	1.0 (reference)	110/239	1.0 (reference)
1–19	56/61	0.6 (0.2–1.6)	56/82	1.4 (0.8–2.3)
20+	43/44	1.0 (0.3–2.8)	43/60	2.0 (1.1–3.6)
		P trend = 0.99		*P* trend = 0.06
During last 10 years				
0	129/176	1.0 (reference)	129/264	1.0 (reference)
1–9	40/39	1.4 (0.5–3.7)	40/64	1.4 (0.8–2.3)
10+	40/41	1.4 (0.5–3.9)	40/53	2.3 (1.3–4.3)
		P trend = 0.49		*P* trend = 0.01
During last 5 years				
0	135/187	1.0 (reference)	135/285	1.0 (reference)
1–4	34/28	2.2 (0.8–6.4)	34/47	1.9 (1.1–3.6)
5+	40/41	1.6 (0.5–4.4)	40/49	2.8 (1.5–5.2)
		P trend = 0.37		*P* trend = 0.003

†Adjusted for sex, age, heavy drinking history, hepatitis B surface antigen, and antibody to hepatitis C virus. CLD, chronic liver disease.

last 5 years compared with no use were estimated at 1.9 (1.1–3.6) and 2.8 (1.5–5.2), respectively, with a *P* trend of 0.003.

Discussion

Our findings from the comparison between HCC cases and CLD patients lend further support to the positive association between cigarette smoking and HCC risk. Several epidemiologic studies on patients with CLD[11–14] demonstrated a clearer association between smoking and HCC, as seen in this study. On the other hand, our comparison between HCC cases and hospital controls did not show any significant association with cigarette smoking, although some risk elevation was noted for current smokers and more recent cigarette use. This finding also accords with the results from most Japanese case–control studies using hospital or community controls.[5] The above discrepancy was partly because only 2% and 8% of our hospital controls tested positive for HBsAg and anti-HCV, respectively, and adjustment for both markers made the relevant OR very unstable.

The dose–response relationship between cigarette smoking and HCC has been unclear in most epidemiologic studies,[15–19] although a part of cohort studies showed a clearer relation.[11,12,20–22] Based on our comparison of HCC cases with CLD patients, no dose–response relation was evident for pack-years during lifetime, yet more recent cigarette consumption such as pack-years during the last 5 years was significantly associated with HCC risk in a dose-dependent manner. Similarly, Tanaka *et al.* reported that current, but not former, heavy smoking (Brinkman index ≥800) was an independent risk factor for HCC (RR = 4.9) in a case–control study using hospitalized patients.[23] This suggests the possibility that a change in recent smoking habit may have a large effect on smoking-HCC relations, thereby distorting dose–response relationships with pack-years during lifetime or cigarette consumption measured in the remote past.

Based on the results from large cohort studies, Hirayama,[20] and Tsukuma *et al.*[11] suggested that cigarette smoking may be involved in end-stage development of liver cancer, such

as cirrhosis to HCC. Our results supported their hypothesis, since most smokers among HCC cases had lately suffered from advanced CLD such as cirrhosis. For information, the comparison between CLD patients and hospital controls without CLD in this study did not show increased risk for the development of CLD among smokers (data not shown). In light of these findings, cigarette smoking may facilitate tumor promotion or progression, rather than initiation, in multistage hepatocarcinogenesis. Experimental data suggest that rodents exposed to tobacco constituents demonstrate a higher incidence of liver tumor than control animals,[24,25] and that tobacco smoke enhances chemically induced rat hepatocarcinogenesis.[26]

In Japan, only a few epidemiologic studies have considered serologic markers for both hepatitis B and C viruses as potential confounders for the smoking-HCC relation,[11,12,23] although several studies in foreign countries took this consideration.[27–35] Among these, three cohort studies,[11,12,34] and three case–control studies,[23,28,33] reported an overall significant risk increase for smoking although some insignificant risk increase was observed in other studies.[27,29,30] Except for studies on hepatitis virus carriers or CLD patients,[11,12] statistical adjustment for both viral markers generally renders relevant risk estimates imprecise as a result of a relatively low seropositivity among study populations or control groups. Such was the case in our comparison of HCC cases with hospital controls, and much more hospital controls would be required to overcome this problem. However, studying defined high risk populations such as hepatitis virus carriers or CLD patients, rather than making a strenuous effort to recruit a large number of hospital controls, would provide more practical information if one considers that the majority of HCC patients develop from such high risk individuals.

Several studies reported that the positive association with smoking was restricted to or stronger in subjects seronegative for HBsAg and/or anti-HCV,[6,7,27,33,34] as compared with seropositive subjects although other studies demonstrated almost opposite findings.[8,35,36] In the present study, only 17 HCC cases (8.1%) tested negative for both HBsAg and anti-HCV, and thus it was difficult to examine the above virus–smoking interaction. However, our results revealed that cigarette smoking was associated with an increased risk of HCC among CLD patients (predominantly of hepatitis C origin), who can be regarded as a target population for possible smoking intervention. CLD patients may benefit from their earliest smoking cessation, which has not yet been commonly recommended by clinicians or the general public in Japan.

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HEPATOLOGY (In press)

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

Satoko Ohfuji[a,*], Wakaba Fukushima[a], Takashi Tanaka[b], Daiki Habu[c], Tadashi Takeda[c], Akihiro Tamori[c], Hiroki Sakaguchi[c], Shuichi Seki[c], Norifumi Kawada[c], Shuhei Nishiguchi[d], Susumu Shiomi[e], and Yoshio Hirota[a]

[a]Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan; [b]Department of Internal Medicine, Hoai Hospital, Osaka, Japan; [c]Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Japan; [d]Departement of Hepatobiliary and Pancreatic Disease, Hyogo Medical College, Hyogo, Japan; [e]Department of Nuclear Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

Abstract.
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 incident cases with HCC to 253 matched controls among patients with chronic hepatitis C. A questionnaire survey elicited information on the consumption of a late evening meal, which was defined as a snack or meal within 2 hours 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, subjects who consumed a late evening meal had a lowered OR as compared to those who never consumed 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 alpha-fetoprotein level of less than 20.0 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 normal alpha-fetoprotein level and without obesity, although these relations might be accounted for other factors including total energy intake. Further studies with larger study size 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 was 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 aggravation of liver disease.[9]

To improve nocturnal starvation, current guidelines recommends the use of 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 consistently demonstrated that BCAA administration correct malnutrition in patients with cirrhosis. [11-12] Administration before bedtime seems to be most effective in terms of nutritional metabolism.[13-15] Recent studies also suggested that BCAA might decrease mortality among patients with liver cirrhosis.[16] Before BCAA prescription for patients with cirrhosis become popular, carbohydrate-rich snacks were considered as a late evening snack. Carbohydrate-rich snacks also improves nitrogen balance and abnormal fuel metabolism in patients with cirrhosis.[8, 17-19] A previous study indicated that a late evening meal including carbohydrate-rich snacks had the same effect as BCAA administration,[8] 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 investigated the long term effect of nutritional supports on HCC development. To the best of our knowledge, only two studies have evaluated the effect of nutritional intervention on the risk of hepatocellular carcinoma (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 cases of HCC are caused by hepatitis C virus (HCV) infection,[23] so the source population was restricted to patients with chronic type C liver disease.

2. Methods

1.1. Selection of Cases and Controls

The method of the present study was 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) 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, auto-immune hepatitis, idiopathic portal hypertension); referral patients who had already been diagnosed with HCC at other hospitals; and patients in poor health (e.g., liver failure, terminal stage of HCC). This resulted in 1,159 patients, who were regarded as a source population.

From the source population, 86 patients were identified who were first diagnosed with HCC between 1 November 1998 and 31 March 2002. The diagnosis of HCC was based either on histopathologic examination or on a positive result in at least one imaging study (CT, MRI, angiography) combined with an elevated serum alpha-fetoprotein level. For each case with HCC, we selected 1 to 5 control patients, matching for age (± 2 years), gender, 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.

2.2. 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 intervals of one month. 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 hours 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). Subjects 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, findings from 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 intra-hepatic 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 we assessed "US score" ≥ 3.5 as indicating chronic liver disorder and "US score" >5.0 as indicating 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 count, total bilirubin, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, alpha-fetoprotein, virus titer of HCV-RNA, and fasting blood sugar. Information about interferon therapy was obtained from medical records.

2.3. Data Analyses

The frequency of intake of a late evening meal was re-categorized 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 chi-square 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 late evening meal and those who did not only among control subjects, using

chi-square test or Wilcoxon rank sum test. The conditional logistic regression model was used to calculate 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, subjects 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<$10\times10^4/\mu l$ [29]), normal level of alpha-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 ORs and 95% CIs of late evening meal for HCC. Each models included three matching factors (i.e., age, sex, and duration from first OCUH visit) and the potential confounders other than stratified factors. Homogeneity of ORs across stratified categories was tested as p value of the interaction term between late evening meal and each stratified variable.

All statistical analyses were performed using SAS version 8.2 (SAS Institute Inc.).

3. Results

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

Table 1 shows selected characteristics of cases and controls. Cases and controls were well-matched for age, sex, and duration from 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 score" 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 late evening meal and those who never consumed only among control subjects. 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 late evening meal.

Table 3 shows the ORs for HCC according to late evening meal, adjusted for duration from first identification of liver disease, disease severity at first OCUH visit (US score, platelet count, aspartate aminotransferase, alpha-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 never consumed one (OR, 0.08; 95% CI, 0.01-0.48). In addition, higher frequency intake of a late evening meal was associated with lower ORs, with a significant dose-response relationship (Trend P=0.009). Thus, a late evening meal was associated with lowered risk of HCC.

The inverse associations of late evening meal with the development of HCC did not differ between group with or without possible liver cirrhosis (Table 4). When study subjects were divided according to the absence or presence of possible liver cirrhosis (e.g., a platelet count of less than $10\times10^4/\mu l$ or ratio of aspartate to alanine aminotransferase of more than 1.0), no measurable difference was observed in inverse association of late evening meal with HCC across the groups, although valid estimates could not be calculated in the assessment of ratio of aspartate to alanine aminotransferase of more than 1.0. As for alpha-fetoprotein level, the relationship between late evening meal and HCC demonstrated with smaller OR among patients with normal alpha-fetoprotein level. Regarding albumin level or obesity, inverse associations of late evening meal with HCC were more pronounced in patients with an albumin level less than 4.0 g/dl and those with a body mass index less than 25.0 kg/m^2. Furthermore, the interaction between body mass index and late evening meal for HCC was statistically significant (p=0.022 for homogeneity of OR). A late evening meal indicated a smaller OR for HCC risk irrespective of the absence or presence of 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 late evening meal and alpha-fetoprotein level, albumin level or body mass index were existed. In that case, it may be more appropriate that the interaction terms were included in overall multivariate analyses. Thus, we conducted additional multivariate analyses in which each interaction term was added as adjustment. When the interaction term between late evening meal and alpha-fetoprotein was included in the multivariate analysis, OR of late evening meal was almost similar with the results in table 3 (OR, 0.02; 95% CI=0.00-1.21; p=0.062). Considered the interaction with albumin level, OR was 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 late evening meal and body mass index, the model did not converge because there was only 1 case who ate a late evening meal in the category of body mass index of less than 25.0 kg/m^2. Thus we could not simultaneously consider these three interactions. In order to consider these interactions, further large scale studies are needed.

4. 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, past experimental study indicated that higher administration of a nutritional 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. On the other hand, the inverse effect of a late evening meal for HCC development was more pronounced among patients with an alpha-fetoprotein level of less than 20.0 ng/ml. It was possible to consider following speculations about this association: 1) patients with higher alpha-fetoprotein level might have a higher risk for the development of HCC. Thus, this background caused a difficulty in detection of the negative association with a late evening meal among these patients but to find more easily the relationship among those with normal alpha-fetoprotein level. 2) Potential undetectable HCC cells might be developed among patients with higher alpha-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 normal alpha-fetoprotein level. Further studies with larger study size are needed to corroborate these findings and to consider the underlying mechanisms.

As for the interaction between late evening meal and body mass index, the inverse associations of late evening meal with HCC were further pronounced in patients with a body mass index less than 25.0 kg/m^2. It was recently indicated that obesity might be a risk factor of HCC development. Thus, it brought about a difficulty in detection of the negative association with late evening meal among obesity group but to demonstrate more easily the decreasing ORs of late evening meal for HCC among patients with a body mass index less than 25.0 kg/m^2. On the other hand, 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 ordinary food group among patients with a body mass index more than 25.0 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 25.0 kg/m^2.[36] Taken together, these findings seem to indicate that late evening meal has a preventive effect against HCC to the same extent as BCAA administration among patients without obesity, while the effect of late evening meal for HCC prevention is less than that of BCAA among those with obesity. It is therefore likely that BCAA and a late evening meal exert their effects by different mechanisms among patients with obesity.

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

As to the mechanism of late evening meal in HCC prevention, several previous studies indicated that malnutrition including nocturnal starvation was related to poorer prognosis of liver cirrhosis[4-9] and that a late evening meal or BCAA supplement before bedtime improved 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 to prevent progressive hepatic failure and to improve 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.

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, the duration from first identification of liver disease, etc.).

However, due to the case-control study design within a very special population, i.e., 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, i.e., 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 OR would decrease. Thus, this selection bias may operate to bias the association toward the null but not to lead to exaggerated results.

A second limitation is an information bias resulting from imperfect memory of distant past history of eating 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 subjects would receive similar recall stimuli about past late evening meals. The misclassification due to such information bias, if any, is probably nondifferential and would not affect the plausibility of the results.

Reverse causation is a third limitation for observed association, although most retrospective studies suffer from this limitation. The habit of a late evening meal may change over time. However, this study interpreted just from information of a late evening meal at one point without considering the potential changes in a late evening meal associated 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 liver dysfunction already manifested. A long induction period in HCC can bring about the apparent causative associations, and exposure might be 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, interferon therapy) and for other putative confounders (duration of liver disease, BMI). 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 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, late evening meal in 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 size are needed to corroborate these findings.

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Table 1
Comparison of Selected Characteristics between Cases and Controls†

Characteristics	Case (N=73)	Control (N=253)	P value‡
Age (yrs)	69 (65-73)	69 (65-72)	0.389
Gender (%)			
Male	47	52	0.434
Duration until beginning of the study (yrs)			
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.

‡ Chi-squre test, Wilcoxon rank sum test were used where appropriate.

Table 2
Comparison of Selected Characteristics between Patients who Have Consumed Late Evening Meal and Those who Never Consumed among Control Subjects†

Characteristics	Patients who Have Consumed Late Evening Meal (N=46)	Patients who Never Consumed (N=207)	P value‡
Age (yrs)	68 (64-74)	69 (65-72)	0.960
Gender (%)			
Male	52	52	0.953
Duration until beginning of the study (yrs)			
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
Alpha-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 (inter-quartile range) unless otherwise indicated.

‡ Chi-squre test, Wilcoxon rank sum test were used where appropriate.

Table 3

Odds Ratio† for Hepatocellular Carcinoma According to Frequency of Intake of a Late Evening Meal: Japan

Characteristics	Level	Case (N=73)	Control (N=253)	Univariate			Multivariate‡		
		n (%)	n (%)	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 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 OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, alpha-fetoprotein, fasting blood sugar), family history of liver disease and interferon therapy.

Table 4

Adjusted Odds Ratio 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 ORs across stratified categories¶
	Case	Control				
	n / N (%)	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	
Alpha-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, alpha-fetoprotein, fasting blood sugar), family history of liver disease, duration from first identification of liver disease and interferon therapy.

§ the model did not converge because there were no case or too limited cases who ate a late evening meal.

¶ Homogeneity of ORs across stratified categories was tested as P value of the interaction term between late evening meal and each stratified variable.