Research Article

Cancer Epidemiology, Biomarkers & Prevention

Plasma Levels of Adiponectin and Primary Liver Cancer Risk in Middle-Aged Japanese Adults with Hepatitis Virus Infection: A Nested Case–Control Study

Takehiro Michikawa[1,2], Manami Inoue[2,3], Norie Sawada[2], Shizuka Sasazuki[2], Yasuhito Tanaka[4], Motoki Iwasaki[2], Taichi Shimazu[2], Taiki Yamaji[2], Masashi Mizokami[5], and Shoichiro Tsugane[2], for the Japan Public Health Center–based Prospective Study Group

Abstract

Background: Excess body weight is an independent risk factor for primary liver cancer, and the role of adiponectin in the pathogenesis of obesity-related malignancies is a focus of research interest. Few prospective studies have examined the association between circulating adiponectin and liver cancer risk, so we investigated this association in a nested case–control study of a population-based prospective cohort in Japan.

Methods: From 18,628 target participants of ages 40 to 69 years who returned the baseline questionnaire and provided blood samples, we selected those with either hepatitis B or C virus infection at baseline ($n = 1{,}544$). Among these, 90 were newly diagnosed with primary liver cancer from 1993 through 2006, and matched to 177 controls. The ORs of liver cancer development based on plasma levels of adiponectin were estimated with a conditional logistic regression model.

Results: Median values of total and high-molecular-weight (HMW) adiponectin tended to be higher in the patients with liver cancer, and plasma levels of adiponectin were positively associated with liver cancer risk. Body mass index– and diabetes-adjusted ORs for the highest tertile of total and HMW adiponectin levels versus the lowest were 3.30 [95% confidence interval (CI), 1.45–7.53; $P_{trend} < 0.01$] and 3.41 (95% CI, 1.50–7.73; $P_{trend} < 0.01$), respectively. There was no effect modification by body mass index and diabetes.

Conclusions: Higher plasma adiponectin levels were associated with an increased risk of primary liver cancer in middle-aged Japanese adults with hepatitis virus infection.

Impact: Circulating adiponectin levels may be a risk marker for primary liver cancer. *Cancer Epidemiol Biomarkers Prev; 22(12); 2250–7. ©2013 AACR.*

Introduction

Accumulating epidemiologic evidence suggests that excess body weight is an independent risk factor for primary liver cancer (1, 2). Even in Japan, where the prevalence of overweight and obesity is lower than that in Western countries (3) and the main cause of liver cancer is chronic hepatitis C virus (HCV) infection (4), overweight or obesity seem to increase the risk of liver cancer (5). We have also reported that high body mass index (BMI) is associated with an increased risk of primary liver cancer, irrespective of hepatitis virus infection (6). The causal association between excess body weight and liver cancer development is probably connected with insulin resistance (7, 8). However, the underlying mechanism by which excess body weight promotes liver cancer development is still not fully understood.

The role of adiponectin in the pathogenesis of obesity-related malignancies is a focus of research interest. Adiponectin is a physiologically active polypeptide secreted by adipose tissues and its circulating levels are inversely associated with obesity (9). Earlier experimental studies suggested that adiponectin played a protective role in carcinogenesis via insulin sensitization, antiproliferation, anti-inflammation, and angiogenesis regulation (10), and data supported epidemiologic evidence that adiponectin levels were inversely associated with the risk of obesity-related malignancies, such as breast, colorectal, endometrium, and prostate cancers (10). These results also suggested that elevated levels of adiponectin would be associated with a reduced risk of primary liver cancer linked with obesity, and that hyperadiponectinemia

Authors' Affiliations: [1]Environmental Epidemiology Section, Center for Environmental Health Sciences, National Institute for Environmental Studies, Tsukuba, Ibaraki; [2]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center; [3]Graduate School of Medicine, The University of Tokyo, Tokyo; [4]Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya; and [5]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba, Japan

Corresponding Authors: Manami Inoue, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Phone: 81-3-5841-3688; Fax: 81-3-5841-3637; E-mail: mnminoue@m.u-tokyo.ac.jp; and Shoichiro Tsugane, stsugane@ncc.go.jp

doi: 10.1158/1055-9965.EPI-13-0363

might suppress liver tumorigenesis (11). Indeed, experimental studies indicated that adiponectin treatment increased apoptosis of hepatocelluar carcinoma, the most common form of primary liver cancer, and inhibited its proliferation (12, 13). However, it has been pointed out that hyperadiponectinemia reflects the progression of liver disease leading to the development of liver cancer, as the liver is the main organ of adiponectin metabolism (9, 11). A recent hospital-based cohort study showed that high serum levels of adiponectin were positively associated with the development of hepatocelluar carcinoma in patients with chronic hepatitis C (14). Because there have been few prospective studies (14–16), further examination of the potential association between adiponectin and liver cancer risk is warranted.

The aim of the present study was to investigate the association between plasma adiponectin levels and primary liver cancer risk in middle-aged adults with hepatitis virus infection. We used a nested case–control design based on data from a large-scale population-based prospective cohort study in Japan.

Materials and Methods

Study population

The Japan Public Health Center–based Prospective Study (JPHC Study) is an ongoing cohort study of cancer, cardiovascular disease, and other lifestyle-related diseases. The first cohort (cohort I) started in 1990 and the second cohort (cohort II) in 1993. In cohort I, the study population included all registered Japanese residents of ages 40 to 59 years of five public health center areas, and in cohort II the study population included all residents of ages 40 to 69 years of six other areas; the study design is described elsewhere (17). We investigated the hepatitis B surface antigen (HBsAg) and anti-HCV antibody (anti-HCV) measurements from the cohort II data. Our study was approved by the Institutional Review Board of the National Cancer Center (Tokyo, Japan).

In cohort II (1993–1994), 56,542 participants (response rate: 82%) answered a baseline questionnaire in sociodemographic characteristics, medical history, smoking and drinking habits, diet, and so on. Of the questionnaire respondents, 37% voluntarily provided 10 mL of blood in health checkups during the baseline survey (1993–1995). The blood samples were divided into plasma and buffy layers and preserved at −80°C until analysis. Study participants were informed of the objectives and methods of the study in writing, and those who responded to the questionnaire and donated blood were regarded as having given informed consent to participate. Of these, we selected only those who had no history of cancer at baseline and had provided data on basic characteristics, leaving us with a total of 18,628 participants (6,401 men and 12,227 women).

Follow-up

Participants were followed up from the date of blood collection until December 31, 2006. Residence status and survival were confirmed annually through residential registers in the respective public health center areas. During the follow-up period, 0.3% ($n = 49$) of participants were lost to follow-up.

Incident cases of primary liver cancer were identified by active patient notification from major hospitals in the study area and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. Cases were coded using the International Classification of Diseases for Oncology, Third Edition (code: C22.0; ref. 18). In our cancer registry system, the proportion of cases for which information was available from death certificates only was 4.7%.

Selection of cases and controls

From the 18,628 participants, we selected those with either hepatitis B virus (HBV) or HCV infection at baseline ($n = 1{,}544$). Plasma samples were screened for HBsAg by reversed passive hemagglutination with a commercial kit (Institute of Immunology Co., Ltd.) and for anti-HCV with a third-generation immunoassay (Lumipulse II Ortho HCV; Ortho-Clinical Diagnosis K.K.; ref. 19). In this study, positivity for HBsAg was regarded as indicating HBV infection, and positivity for anti-HCV as indicating HCV infection (20). Up to the end of the study period after blood collection, we identified 91 new cases of primary liver cancer among the 1,544 participants. For each patient newly diagnosed with liver cancer, we selected two controls at random from among the participants with no history of liver cancer when the diagnosis was made. Controls were matched to each patient in terms of age (within 5 years), sex, public health center area, fasting status at blood collection, baseline menopausal status (for women), and hepatitis virus infection status (HBV/HCV). No appropriate matched controls were found for 1 patient, and only 1 matched control for each of 3 other patients, so a total of 90 patients and 177 controls were included in the present analysis.

Laboratory assay for adiponectin

Circulating adiponectin levels have been reported to be stable over time and to have high reliability (21–23), so this biologic marker is likely to be useful in epidemiologic studies. From the blood samples collected at baseline, plasma levels of total adiponectin and high-molecular-weight (HMW) adiponectin, which is considered to be the active form of the hormone (10), were analyzed with a Human Adiponectin ELISA Kit for Total and Multimers (Sekisui Medical, Co., Ltd.) by the ELISA method. Cases and matched controls were assayed in the same batch. The minimum detection level was 0.39 μg/mL for both total and HMW adiponectin. For the assays, inter-assay and intra-assay coefficients of variation from the company's quality control samples were ≤3.9% and ≤5.7% for total adiponectin, and ≤3.3% and ≤6.2% for HMW adiponectin, respectively. All samples were analyzed at a single laboratory (Mitsubishi Chemical Medience Corporation) by technicians blinded to case–control status.

Statistical analysis

All analyses were performed with STATA version 11 (STATA Corporation). All P values reported are two-sided, and significance level was set at $P < 0.05$.

Comparisons of the baseline characteristics between the cases and controls were made by the χ^2 or Mann–Whitney test, as appropriate. Because adiponectin levels are reportedly low in obese people and in patients with type 2 diabetes (9, 24), we confirmed whether the relation between plasma levels of total/HMW adiponectin and BMI/diabetes in the controls did not contradict existing knowledge. Spearman's rank correlation coefficients were calculated for adiponectin levels and BMI. Comparative adiponectin levels between the participants with diabetes and those without were evaluated by the Mann–Whitney test. In this study, diabetes was defined as a self-reported history of diabetes, and/or antidiabetic medication use, and/or blood glucose $\geq$5.55 mmol/L (100 mg/dL) fasting or $\geq$7.77 mmol/L (140 mg/dL) nonfasting (20).

For total and HMW adiponectin, participants were classified into sex-specific tertiles according to the frequency of distribution among the controls. Using a conditional logistic regression model, we calculated ORs and 95% confidence intervals (CI) of primary liver cancer for plasma levels of total and HMW adiponectin. Dummy variables were created for the categories of plasma adiponectin levels, and the lowest category was used as the reference category. To investigate whether adiponectin was associated with liver cancer through the pathway for insulin resistance (24), the ORs were adjusted for BMI (<18.5, 18.5–21.9, 22.0–24.9, and $\geq$25.0 kg/m^2) and diabetes (yes or no). We also adjusted for the following variables previously associated with liver cancer risk (20, 25, 26): alcohol consumption (never, past, <150, 150 to <450, $\geq$450 g/week ethanol), coffee consumption (almost never, 1 time/week to <1 cup/day, $\geq$1 cup/day), and serum alanine aminotransferase (ALT) levels (<30, 30–69, $\geq$70 IU/L). Because adjustment for smoking status (a suspected risk factor for liver cancer) and vegetable and fish intake [factors previously associated with liver cancer risk (27, 28)] produced ORs that were almost identical to those without adjustment, results for these variables are not presented in this article. Linear trends for ORs were tested using the exposure categories as ordinal variables. An earlier study demonstrated that high adiponectin levels might be a proxy marker increasing the likelihood of subsequent liver cancer development (14). Therefore, we performed additional analyses after excluding 17 cases of liver cancer diagnosed in the first 3 years after blood collection because of checking for temporality. In addition, subgroup analyses were performed for 71 cases of liver cancer death where cancer development was likely to have been observed in the early follow-up period, and after limiting analysis to or excluding 53 cases of liver cancer diagnosed in the first 7 years after blood collection.

In addition, we investigated whether the association between adiponectin levels and primary liver cancer risk was modified by sex, hepatitis virus infection status (HBV/HCV), BMI (<25.0, $\geq$25.0 kg/m^2), and diabetes (yes or no). For these stratified analyses, participants were divided into two groups based on median plasma levels of total and HMW adiponectin in the controls. We used unconditional logistic regression for BMI and diabetes, and included matching factors in the multivariable models. Statistical interaction between adiponectin levels and stratified variables was tested with a likelihood ratio test.

Results

Table 1 shows the baseline characteristics of the case and control groups. The prevalence of overweight, diabetes, and high ALT levels was tended to be higher and that of daily coffee consumption was lower in the case group than in the control group. Participants in the control group tended to be light (<150 g/week ethanol) to moderate (150 to <450 g/week ethanol) drinkers. The median values of total adiponectin were statistically higher among the cases than the controls (6.16 vs. 5.11 μg/mL; $P = 0.03$) and those of HMW adiponectin were marginally higher in the case group (2.66 vs. 2.06 μg/mL; $P = 0.06$; Fig. 1). In the control group, Spearman's rank correlation coefficients of BMI with total and HMW adiponectin were -0.40 ($P < 0.01$) and -0.25 ($P < 0.01$), respectively. Total and HMW adiponectin levels were lower among the participants with diabetes than those without (median: 3.45 vs. 5.34 μg/mL, $P = 0.02$ for total adiponectin; 1.26 vs. 2.25 μg/mL, $P = 0.04$ for HMW adiponectin).

Table 2 presents the conditional logistic regression results for the association between total and HMW plasma adiponectin levels and the risk of primary liver cancer. After adjustment for BMI and diabetes, we found a statistically significant positive association between plasma adiponectin and liver cancer risk ($P_{trend} < 0.01$ for both total and HMW adiponectin). The ORs for the highest tertile of total and HMW adiponectin levels versus the lowest tertile were 3.30 (95% CI, 1.45–7.53) and 3.41 (95% CI, 1.50–7.73), respectively. The positive association remained significant for total adiponectin ($P_{trend} = 0.04$) and marginal for HMW adiponectin ($P_{trend} = 0.06$) when further variables (alcohol and coffee consumption, ALT levels) were added to the model. However, this association was attenuated after we excluded 17 cases diagnosed in the first 3 years (multivariate $P_{trend} = 0.10$ for total and 0.16 for HMW adiponectin). When we restricted analysis to the 71 cases of liver cancer death, the positive association remained (multivariate $P_{trend} = 0.01$ for both total and HMW adiponectin). Adiponectin levels were also associated with an increased risk of liver cancer development in the first 7 years after blood collection (multivariate $P_{trend} = 0.01$ for both total and HMW adiponectin), but not liver cancer development after the first 7 years.

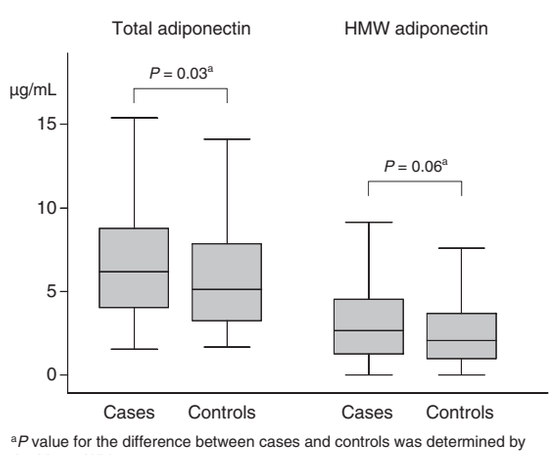
For stratified analyses, plasma levels of adiponectin were dichotomized at the median values for two groups, high and low (Table 3). The multivariable ORs for the high versus low group were 3.08 (95% CI, 1.62–5.86) for total adiponectin and 2.03 (95% CI, 1.09–3.78) for HMW adiponectin. The magnitudes of association between plasma

Table 1. Selected baseline characteristics of cases and controls

Variables		Cases ($n = 90$) Prevalence (%)	Controls ($n = 177$) Prevalence (%)	P[a]
Age, y	40–49	2.2	3.4	Matching variable
	50–59	27.8	28.8	
	60–69	70.0	67.8	
Sex	Men	68.9	68.9	Matching variable
	Women	31.1	31.1	
Hepatitis virus infectious status[b]	HBV	12.2	11.9	Matching variable
	HCV	87.8	88.1	
Alcohol consumption	Never	51.1	39.0	0.08
	Past	11.1	7.3	
	<150 g/wk ethanol	17.8	30.5	
	150 to <450 g/wk ethanol	14.4	19.8	
	≥450 g/wk ethanol	5.6	3.4	
Smoking status, current smoker		37.8	35.6	0.58
BMI, ≥25 kg/m^2		37.8	17.5	<0.01
Diabetes, yes[c]		33.3	23.0	0.05
Coffee consumption, ≥1 cup/d		23.3	39.0	0.01
ALT level, ≥70 IU/L		46.0	6.0	<0.01
Vegetable intake, g/d[d]		44 (24–69)	48 (27–75)	0.33
Fish intake, g/d[d]		45 (28–67)	43 (27–66)	0.78
Time from blood draw to diagnosis for the cases, y[d]		6.1 (3.6–9.5)		

[a]Calculated using the χ^2 and Mann–Whitney test.
[b]Positive for HBsAg was regarded as indicating HBV infection and positive for anti-HCV antibody as indicating HCV infection.
[c]Diabetes was defined as a self-reported history of diabetes, and/or antidiabetic medication use, and/or blood glucose ≥5.55 mmol/L (100 mg/dL) fasting or ≥7.77 mmol/L (140 mg/dL) nonfasting.
[d]Median (interquartile range).

adiponectin levels and primary liver cancer risk tended to be greater in women than in men. The P value for interaction between HMW adiponectin and sex was borderline significant ($P = 0.05$). Analyses stratified according to hepatitis virus infection status, BMI, and diabetes showed no remarkable differences between the two strata for either total or HMW adiponectin.



[a]P value for the difference between cases and controls was determined by the Mann–Whitney test.

Figure 1. Box and whisker plot for plasma adiponectin levels of cases and controls.

Discussion

One strength of the present study of the association between plasma adiponectin levels and the risk of primary liver cancer development is that blood samples were collected from a nested case–control cohort of approximately 1,500 adults with hepatitis virus infection, a subset derived from a large-scale population-based prospective cohort. Because the measurements preceded the onset of outcomes, we were able to clarify the temporal association between the putative exposure and the hypothesized outcome. In addition, the cases and controls were selected from the same cohort, minimizing the possibility of the selection bias inherent to case–control studies. This study is thus an important contribution to the research into the role of adiponectin in liver carcinogenesis.

We did not find an inverse association between plasma levels of adiponectin and primary liver cancer development risk, despite cumulating evidence that adiponectin plays a protective role in carcinogenesis. However, we do not believe that the results of our study contradict earlier findings. No epidemiologic studies

Table 2. ORs and 95% CIs of primary liver cancer based on plasma adiponectin levels

	Tertile			
Plasma level	**Low**	**Middle**	**High**	***P*[a]**
Total adiponectin				
Range, μg/mL	≤3.40 for men and ≤5.32 for women	3.41–5.68 for men and 5.33–8.50 for women	>5.68 for men and >8.50 for women	
No. cases/controls	17/60	36/56	37/61	
OR (95% CI)	1.00 (reference)	2.23 (1.14–4.38)	2.25 (1.11–4.55)	0.03
Multivariable OR (95% CI)[b]	1.00 (reference)	2.59 (1.22–5.50)	3.30 (1.45–7.53)	<0.01
Multivariable OR (95% CI)[c] + alcohol and coffee consumption, ALT level	1.00 (reference)	2.44 (0.60–9.95)	3.76 (1.06–13.42)	0.04
HMW adiponectin				
Range, μg/mL	≤1.05 for men and ≤2.37 for women	1.06–2.56 for men and 2.38–4.30 for women	>2.56 for men and >4.30 for women	
No. cases/controls	17/60	37/58	36/59	
OR (95% CI)	1.00 (reference)	2.39 (1.18–4.86)	2.38 (1.15–4.93)	0.03
Multivariable OR (95% CI)[b]	1.00 (reference)	2.77 (1.26–6.06)	3.41 (1.50–7.73)	<0.01
Multivariable OR (95% CI)[c] + alcohol and coffee consumption, ALT level	1.00 (reference)	2.89 (0.76–11.07)	3.85 (0.99–15.05)	0.06

[a]Linear trends were tested using the exposure categories as ordinal variables.

[b]Adjusted for BMI (<18.5, 18.5–21.9, 22.0–24.9, ≥25.0 kg/m^2) and diabetes (yes or no).

[c]Further adjusted for alcohol consumption (never, past, <150, 150 to <450, ≥450 g/wk ethanol), coffee consumption (almost never, 1 time/wk to <1 cup/d, ≥1 cup/d), and ALT level (<30, 30–69, ≥70 IU/L).

have actually shown an inverse association between circulating levels of adiponectin and the occurrence of liver cancer: earlier studies have shown either a positive association or no association. One cohort study in Japan indicated that patients with chronic hepatitis C and high serum adiponectin levels had a higher risk of developing hepatocelluar carcinoma; our results support this finding (14). In another cohort study in France, serum levels of adiponectin measured in 248 patients with compensated HCV cirrhosis were found to be unassociated with hepatocelluar carcinoma occurrence (15). This study differed from ours, however, in that the subjects were Caucasian, and all of them had been clinically diagnosed with cirrhosis. A nested case–control study in Japan of 59 patients with liver cancer and 334 controls showed no statistically significant association between serum levels of total, HMW, middle-molecular weight, and low-molecular weight adiponectin and the incidence of or mortality from liver cancer (16). However, this study did not include hepatitis virus infection status as a matching variable and treated anti-HCV as a confounding factor. Although *in vitro* and *in vivo* studies have indicated that adiponectin exerts antitumor effects against hepatocelluar carcinoma by inducing apoptosis or suppressing tumor angiogenesis (12, 13), it is not unusual for the results of epidemiologic studies to contradict those of experimental studies. In addition, the insulin-sensitizing properties of adiponectin do not explain the connection with liver cancer. After adjustment and stratification for BMI and diabetes (determinants of insulin resistance), we observed an association between adiponectin and primary liver cancer. Thus, the association seems to be independent of BMI and diabetes, even though adiponectin has been seen as a mediator of obesity in cancer development (7, 8). It does not seem likely, therefore, that the link between adiponectin and liver cancer can be explained by the potential mechanisms underlying the protective role of adponectin in carcinogenesis.

The observed positive association between adiponectin and primary liver cancer risk may lead to adiponectin being regarded as a risk marker for primary liver cancer. Some studies have shown that circulating adiponectin levels are higher in subjects with liver cirrhosis (29–32), and that they increase in line with fibrosis stage (33, 34). Tietge and colleagues reported that adiponectin levels in cirrhosis correlated negatively with the parameters of hepatic protein synthesis capacity (including serum albumin levels and blood-coagulating factors), and positively with the parameters of hepatic hemodynamics (including portal pressure, hepatic vascular resistance, and decreased hepatic blood flow; ref. 31), thus demonstrating that liver function determined circulating adiponectin levels. In one animal study, common bile duct ligation was performed in mice, which led rapidly to an accumulation of serum adiponectin (32), demonstrating that biliary excretion is involved in the clearance of adiponectin. To summarize, impaired liver function or biliary secretion

Table 3. ORs and 95% CIs of primary liver cancer associated with plasma adiponectin levels by subgroups

		Total adiponectin		HMW adiponectin	
		Low	**High**	**Low**	**High**
	Range, μg/mL	≤4.33 for men and ≤6.12 for women	>4.33 for men and >6.12 for women	≤1.59 for men and ≤2.74 for women	>1.59 for men and >2.74 for women
All participants					
	No. cases/controls	30/89	60/88	36/89	54/88
	Mutivariable OR (95% CI)[a]	1.00 (reference)	3.08 (1.62–5.86)	1.00 (reference)	2.03 (1.09–3.78)
Sex					
Men	No. cases/controls	24/61	38/61	30/61	32/61
	Mutivariable OR (95% CI)[a]	1.00 (reference)	2.43 (1.11–5.34)	1.00 (reference)	1.27 (0.60–2.65)
Women	No. cases/controls	6/28	22/27	6/28	22/27
	Mutivariable OR (95% CI)[a]	1.00 (reference)	9.90 (1.96–50.14)	1.00 (reference)	9.93 (1.97–50.18)
	P[b]	0.27		0.05	
Hepatitis virus infectious status					
HBV	No. cases/controls	5/11	6/10	6/11	5/10
	Mutivariable OR (95% CI)[a]	1.00 (reference)	1.46 (0.18–11.48)	1.00 (reference)	1.15 (0.12–10.70)
HCV	No. cases/controls	25/78	54/78	30/78	49/78
	Mutivariable OR (95% CI)[a]	1.00 (reference)	3.18 (1.58–6.39)	1.00 (reference)	2.13 (1.08–4.18)
	P[b]	0.87		0.97	
BMI					
<25.0 kg/m^2	No. cases/controls	15/66	41/80	20/68	36/78
	Mutivariable OR (95% CI)[c]	1.00 (reference)	3.01 (1.42–6.38)	1.00 (reference)	1.95 (0.97–3.89)
≥25.0 kg/m^2	No. cases/controls	15/23	19/8	16/21	18/10
	Mutivariable OR 95% CI)[c]	1.00 (reference)	3.88 (1.10–13.70)	1.00 (reference)	2.60 (0.72–9.44)
	P[b]	0.41		0.47	
Diabetes					
No	No. cases/controls	17/63	43/75	21/64	39/74
	Mutivariable OR (95% CI)[c]	1.00 (reference)	3.14 (1.45–6.80)	1.00 (reference)	1.98 (0.96–4.06)
Yes	No. cases/controls	13/26	17/13	15/25	15/14
	Mutivariable OR (95% CI)[c]	1.00 (reference)	3.28 (0.89–12.08)	1.00 (reference)	1.90 (0.52–6.96)
	P[b]	0.73		0.84	

[a]Adjusted for BMI and diabetes using a conditional logistic model.

[b]Statistical interaction between adiponectin levels and stratified variables was tested with a likelihood ratio test.

[c]We used an unconditional logistic model adjusted for age, sex, public health center area, fasting status at blood collection, hepatitis virus infectious status, baseline menopausal status, BMI, and diabetes except for stratified variable.

due to liver disease (including cirrhosis) seems to lead to hyperadiponectinemia. In our study, therefore, the adiponectin levels observed in participants with hepatitis virus infection might have been a reflection of the progression of virus-related liver disease. This hypothesis is supported by the positive association between adiponectin and liver cancer observed in our subgroup analysis restricted to cases of liver cancer death and liver cancer cases diagnosed in the first 7 years after blood collection, and by the null association between adiponectin and liver cancer revealed when we excluded 17 cases diagnosed in the first 3 years after blood collection. We believed, therefore, that circulating adiponectin is probably just a risk marker of primary liver cancer caused by hepatitis virus infection and not a causal factor. If this is the case, future studies are needed to investigate whether adiponectin

measurement in patients with hepatitis virus infection is a valid marker in predicting the risk of primary liver cancer development.

Like Arano and colleagues, we found that the association between adiponectin levels and primary liver cancer risk was particularly pronounced in women (14). Circulating adiponectin levels are influenced by sex hormones such as testosterone. Testosterone lowers circulating levels of adiponectin (35), which might account for the weaker association between adiponectin and liver cancer in men. However, the number of liver cancer cases in our study was relatively small, so the interpretability of our results might be limited. Further investigations are needed to clarify the sex-specific association between adiponectin levels and primary liver cancer occurrence.

A potential limitation of our study is that we had no information on the clinical severity and later progress of hepatitis or about the treatment the participants with HBV or HCV infection received before or during the study period. However, if plasma adiponectin levels reflect the progression of virus-related liver disease, the participants in the high adiponectin group are more likely to have received treatment than those in the low group, which might have led to underestimation of liver cancer occurrence and ORs in the high adiponectin group. Therefore, our finding of a positive association between plasma adiponectin levels and liver cancer risk should remain true. In addition, although JPHC cohort II participants were selected from the general population, the participants in our study were limited to those who responded to the questionnaire and provided a blood sample. Our findings may, therefore, lack generalizability (36).

In conclusion, the present nested case–control study provides epidemiologic evidence that higher plasma levels of total and HMW adiponectin are independently associated with an increased risk of primary liver cancer in middle-aged Japanese adults with hepatitis virus infection. Our findings also indicate that circulating adiponectin levels may be a risk marker for primary liver cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Michikawa, M. Inoue, M. Iwasaki, S. Tsugane
Development of methodology: T. Michikawa, M. Inoue, M. Mizokami
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Sawada, M. Iwasaki, T. Shimazu, S. Tsugane
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Michikawa, M. Inoue, N. Sawada, M. Iwasaki, T. Yamaji, S. Tsugane
Writing, review, and/or revision of the manuscript: T. Michikawa, M. Inoue, S. Sasazuki, M. Iwasaki, T. Shimazu, T. Yamaji, S. Tsugane
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Inoue, Y. Tanaka, S. Tsugane
Study supervision: M. Inoue, S. Tsugane

Acknowledgments

The authors thank all staff members in each study area and in the central offices for their cooperation and technical assistance. The authors are indebted to the Aomori, Iwate, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for providing their incidence data.

Members of the JPHC Study (principal investigator: S. Tsugane) Group are: S. Tsugane, S. Sasazuki, M. Iwasaki, N. Sawada, T. Shimazu, T. Yamaji, and T. Hanaoka, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cerebral and Cardiovascular Center, Osaka; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, Y. Tanaba, and H. Sato, Iwate Prefectural Ninohe Public Health Center, Iwate; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, N. Nagai, Y. Ito, and Y. Roppongi, Akita Prefectural Yokote Public Health Center, Akita; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, M. Machida, and K. Kobayashi, Nagano Prefectural Saku Public Health Center, Nagano; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, T. Fujieda, and S. Yamato, Ibaraki Prefectural Mito Public Health Center, Ibaraki; K. Matsui, T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Niigata; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Kochi; H. Sueta, H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Goto, Nagasaki Prefectural Kamigoto Public Health Center, Nagasaki; H. Sakiyama, N. Onga, H. Takaesu, M. Uehara, and T. Nakasone, Okinawa Prefectural Miyako Public Health Center, Okinawa; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Osaka; Y. Tsubono, Tohoku University, Miyagi; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya, University of Tsukuba, Ibaraki; M. Kabuto, National Institute for Environmental Studies, Ibaraki; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; M. Akabane, Tokyo University of Agriculture, Tokyo; T. Kadowaki and M. Inoue, The University of Tokyo, Tokyo; M. Noda and T. Mizoue, National Center for Global Health and Medicine, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; Y. Takashima and Y. Yoshida, Kyorin University, Tokyo; K. Nakamura, Niigata University, Niigata; S. Matsushima and S. Natsukawa, Saku General Hospital, Nagano; H. Shimizu, Sakihae Institute, Gifu; H. Sugimura, Hamamatsu University School of Medicine, Shizuoka; S. Tominaga, Aichi Cancer Center, Aichi; N. Hamajima, Nagoya University, Aichi; H. Iso and T. Sobue, Osaka University, Osaka; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Chiba Prefectural Institute of Public Health, Chiba; E. Maruyama, Kobe University, Hyogo; M. Konishi, K. Okada, and I. Saito, Ehime University, Ehime; N. Yasuda, Kochi University, Kochi; S. Kono, Kyushu University, Fukuoka; S. Akiba, Kagoshima University, Kagoshima.

Grant Support

This study was supported by National Cancer Center Research and Development Fund (to M. Inoue, N. Sawada, S. Sasazuki, M. Iwasaki, T. Shimazu, T. Yamaji, and S. Tsugane), A Grant-in-Aid for Cancer Research (to M. Inoue and S. Tsugane), Research on Hepatitis (to M. Inoue), and by the Third-Term Comprehensive Control Research for Cancer from the Ministry of Health, Labour, and Welfare, Japan (to S. Tsugane).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 8, 2013; revised August 8, 2013; accepted August 26, 2013; published OnlineFirst September 17, 2013.

References

1. Saunders D, Seidel D, Allison M, Lyratzopoulos G. Systematic review: the association between obesity and hepatocellular carcinoma—epidemiological evidence. Aliment Pharmacol Ther 2010; 31:1051–63.

2. Larsson SC, Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. Br J Cancer 2007;97:1005–8.
3. Ikeda N, Saito E, Kondo N, Inoue M, Ikeda S, Satoh T, et al. What has made the population of Japan healthy? Lancet 2011;378:1094–105.
4. Tanaka H, Imai Y, Hiramatsu N, Ito Y, Imanaka K, Oshita M, et al. Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. Ann Intern Med 2008;148:820–6.
5. Tanaka K, Tsuji I, Tamakoshi A, Matsuo K, Ito H, Wakai K, et al. Obesity and liver cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. Jpn J Clin Oncol 2012;42:212–21.
6. Inoue M, Kurahashi N, Iwasaki M, Tanaka Y, Mizokami M, Noda M, et al. Metabolic factors and subsequent risk of hepatocellular carcinoma by hepatitis virus infection status: a large-scale population-based cohort study of Japanese men and women (JPHC Study Cohort II). Cancer Causes Control 2009;20:741–50.
7. Inoue M, Tsugane S. Insulin resistance and cancer: epidemiological evidence. Endocr Relat Cancer 2012;19:F1–8.
8. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 2005;42: 987–1000.
9. Kamada Y, Takehara T, Hayashi N. Adipocytokines and liver disease. J Gastroenterol 2008;43:811–22.
10. Barb D, Williams CJ, Neuwirth AK, Mantzoros CS. Adiponectin in relation to malignancies: a review of existing basic research and clinical evidence. Am J Clin Nutr 2007;86:s858–66.
11. Wieser V, Moschen AR, Tilg H. Adipocytokines and hepatocellular carcinoma. Dig Dis 2012;30:508–13.
12. Man K, Ng KT, Xu A, Cheng Q, Lo CM, Xiao JW, et al. Suppression of liver tumor growth and metastasis by adiponectin in nude mice through inhibition of tumor angiogenesis and downregulation of Rho kinase/IFN-inducible protein 10/matrix metalloproteinase 9 signaling. Clin Cancer Res 2010;16:967–77.
13. Saxena NK, Fu PP, Nagalingam A, Wang J, Handy J, Cohen C, et al. Adiponectin modulates C-jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. Gastroenterology 2010;139:1762–73.
14. Arano T, Nakagawa H, Tateishi R, Ikeda H, Uchino K, Enooku K, et al. Serum level of adiponectin and the risk of liver cancer development in chronic hepatitis C patients. Int J Cancer 2011;129:2226–35.
15. Nkontchou G, Bastard JP, Ziol M, Aout M, Cosson E, Ganne-Carrie N, et al. Insulin resistance, serum leptin, and adiponectin levels and outcomes of viral hepatitis C cirrhosis. J Hepatol 2010;53:827–33.
16. Kotani K, Wakai K, Shibata A, Fujita Y, Ogimoto I, Naito M, et al. Serum adiponectin multimer complexes and liver cancer risk in a large cohort study in Japan. Asian Pac J Cancer Prev 2009;10(Suppl):87–90.
17. Tsugane S, Sobue T. Baseline survey of JPHC study—design and participation rate. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001;11: S24–9.
18. World Health Organization. International classification of diseases for oncology. 3rd ed. Geneva, Switzerland: World Health Organization; 2000.
19. Abdel-Hamid M, El-Daly M, El-Kafrawy S, Mikhail N, Strickland GT, Fix AD. Comparison of second- and third-generation enzyme immunoassays for detecting antibodies to hepatitis C virus. J Clin Microbiol 2002; 40:1656–9.
20. Michikawa T, Inoue M, Sawada N, Iwasaki M, Tanaka Y, Shimazu T, et al. Development of a prediction model for 10-year risk of hepatocellular carcinoma in middle-aged Japanese: The Japan Public Health Center–based Prospective Study Cohort II. Prev Med 2012; 55:137–43.
21. Pischon T, Hotamisligil GS, Rimm EB. Adiponectin: stability in plasma over 36 hours and within-person variation over 1 year. Clin Chem 2003; 49:650–2.
22. Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu D, et al. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. Cancer Epidemiol Biomarkers Prev 2007;16:2464–70.
23. Kaplan RC, Ho GY, Xue X, Rajpathak S, Cushman M, Rohan TE, et al. Within-individual stability of obesity-related biomarkers among women. Cancer Epidemiol Biomarkers Prev 2007;16:1291–3.
24. Marra F, Bertolani C. Adipokines in liver diseases. Hepatology 2009;50: 957–69.
25. Ishiguro S, Inoue M, Tanaka Y, Mizokami M, Iwasaki M, Tsugane S. Serum aminotransferase level and the risk of hepatocellular carcinoma: a population-based cohort study in Japan. Eur J Cancer Prev 2009;18:26–32.
26. Inoue M, Kurahashi N, Iwasaki M, Shimazu T, Tanaka Y, Mizokami M, et al. Effect of coffee and green tea consumption on the risk of liver cancer: cohort analysis by hepatitis virus infection status. Cancer Epidemiol Biomarkers Prev 2009;18:1746–53.
27. Kurahashi N, Inoue M, Iwasaki M, Tanaka Y, Mizokami M, Tsugane S. Vegetable, fruit and antioxidant nutrient consumption and subsequent risk of hepatocellular carcinoma: a prospective cohort study in Japan. Br J Cancer 2009;100:181–4.
28. Sawada N, Inoue M, Iwasaki M, Sasazuki S, Shimazu T, Yamaji T, et al. Consumption of n-3 fatty acids and fish reduces risk of hepatocellular carcinoma. Gastroenterology 2012;142:1468–75.
29. Sadik NA, Ahmed A, Ahmed S. The significance of serum levels of adiponectin, leptin, and hyaluronic acid in hepatocellular carcinoma of cirrhotic and noncirrhotic patients. Hum Exp Toxicol 2012;31: 311–21.
30. Liu CJ, Chen PJ, Lai MY, Liu CH, Chen CL, Kao JH, et al. High serum adiponectin correlates with advanced liver disease in patients with chronic hepatitis B virus infection. Hepatol Int 2009;3:364–70.
31. Tietge UJ, Boker KH, Manns MP, Bahr MJ. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. Am J Physiol Endocrinol Metab 2004;287:E82–9.
32. Tacke F, Wustefeld T, Horn R, Luedde T, Srinivas Rao A, Manns MP, et al. High adiponectin in chronic liver disease and cholestasis suggests biliary route of adiponectin excretion *in vivo*. J Hepatol 2005;42: 666–73.
33. Hui CK, Zhang HY, Lee NP, Chan W, Yueng YH, Leung KW, et al. Serum adiponectin is increased in advancing liver fibrosis and declines with reduction in fibrosis in chronic hepatitis B. J Hepatol 2007;47: 191–202.
34. Kaser S, Moschen A, Kaser A, Ludwiczek O, Ebenbichler CF, Vogel W, et al. Circulating adiponectin reflects severity of liver disease but not insulin sensitivity in liver cirrhosis. J Intern Med 2005;258: 274–80.
35. Page ST, Herbst KL, Amory JK, Coviello AD, Anawalt BD, Matsumoto AM, et al. Testosterone administration suppresses adiponectin levels in men. J Androl 2005;26:85–92.
36. Iwasaki M, Yamamoto S, Otani T, Inoue M, Hanaoka T, Sobue T, et al. Generalizability of relative risk estimates from a well-defined population to a general population. Eur J Epidemiol 2006;21:253–62.