Insulin, glucose and hepatocellular carcinoma risk in male hepatitis B carriers: results from 17-year follow-up of a population-based cohort

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This study aimed to investigate the association of fasting insulin and glucose levels with hepatocellular carcinoma (HCC) risk in a case–cohort study within a cohort (1989–2006) of 2903 male government employees chronically infected with hepatitis B virus (HBV) in Taiwan. Insulin, glucose and HBV-related factors were assayed in baseline plasma among 124 HCC cases and a random subcohort of 1084 of the total cohort. After adjustment for demographics and HBV-related factors, including viral load and genotype, the HCC risk was higher for the highest [>6.10 μU/ml, hazard ratio (HR) = 2.36, 95% confidence interval (CI): 1.43–3.90] and lowest (<2.75 μU/ml, HR = 1.57, 95% CI: 0.96–2.58) categories of insulin, compared with insulin of 2.75–4.10 μU/ml. The dose–response relationship between insulin and HCC varied by follow-up time, with stronger association for the HCC cases that occurred ≥8 years after baseline (P for trend <0.0001). The effect of higher insulin on HCC risk remained after adjustment for other metabolic factors, and was fairly consistent across strata of age, body mass index, and HBV genotypic variants. However, it was more profound among those with viral load <4.39 log_{10} copies/ml at recruitment (>6.10 μU/ml, HR = 6.15, 95% CI: 2.48–15.22). Higher insulin was also associated with an increased risk for cirrhosis diagnosed by ultrasonography and elevated alanine aminotransferase. No association with either cirrhosis or HCC was noted for glucose or diabetes after adjusting for insulin. In conclusion, elevated insulin levels are an independent risk factor for HCC among HBV carriers, especially for those with lower viral load.

Introduction

Worldwide, chronic hepatitis B virus (HBV) infection affects >350 million people who are at increased risk for hepatocellular carcinoma (HCC) (1). In Asia-Pacific endemic regions of hepatitis B, HBV-related factors, including prolonged HBV e antigen (HBeAg)-positive phase, high viral load, HBV genotype C infection and the basal core promoter (BCP) double mutations, have been documented as important predictors of HCC (2–8). Except for alcohol abuse (9), however, little is known about the association between modifiable lifestyle factors and the risk of HCC in hepatitis B.

Abbreviations: ALT, alanine aminotransferase; BCP, basal core promoter; BMI, body mass index; CI, confidence interval; GGT, γ-glutamyltransferase; HBeAg, HBV e antigen; HBV, hepatitis B virus; HBsAg, HBV surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; HOMA-IR, homeostasis model assessment-insulin resistance; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

Emerging data indicate that the prevalence of obesity is rising globally (10). Excess body weight increases the risk of non-alcoholic fatty liver disease (NAFLD) (11), and has been associated with risks for HCC and liver cirrhosis of different etiology, including hepatitis B, by multiple epidemiological studies (12–14). Obesity is closely associated with a cluster of metabolic disorders, including glucose intolerance, insulin resistance (IR) and diabetes (11). While IR risk has recently been demonstrated to be increased as a result of chronic hepatitis C virus (HCV) infection with IR itself associated with increased hepatic steatosis and advanced fibrosis risk in hepatitis C patients (15,16), the effect of IR on hepatitis B progression remains unclear. Furthermore, although diabetes or elevated fasting glucose has been associated with HCC incidence or mortality, this association is found primarily in developed countries where HCV is the major cause of HCC (17–21). In areas endemic for hepatitis B, inconsistent results have been reported and previous studies did not incorporate viral factors in the risk analysis of diabetes (12,22–24).

In the present study, we assessed the relative contribution of metabolic factors, including excess body mass index (BMI), fasting levels of insulin and glucose and diabetes, to HCC risk, with a particular focus on the role of insulin and glucose, in a prospective cohort of male HBV carriers. Because information on various viral factors is available, we were able to explore the impact of viral factors on the metabolic factors-related HCC risk.

Subjects and methods

Study design and participants This study was conducted within an ongoing prospective cohort study comprising 2903 unrelated, originally healthy, male HBV surface antigen (HBsAg)-positive government employees aged 30–65 years, who were enrolled during routine free physical examination between August 1989 and June 1992 (12). At baseline, participants completed questionnaires regarding lifestyle habits and medical history. They also underwent a physical examination by physicians, an anthropometric evaluation, and a blood pressure measurement and provided a blood sample following an overnight fast of at least 8 h for serological assays. BMI was calculated using weight divided by height squared (kg/m²).

Participants were followed by medical examinations approximately every year and by computerized data linkage with the profiles on the national registry of cancer and death in Taiwan. HCC was diagnosed by either a histological finding or elevated serum α-fetoprotein level (≥400 ng/ml) combined with at least one positive image on angiography, sonography, and/or computed tomography. All subjects gave written informed consent, and the research ethics committee at the College of Public Health, National Taiwan University approved this study.

In the present study, subjects were recruited from an existing case–cohort study that designed to evaluate the role of longitudinal HBV DNA levels in the development of HCC (5). This case–cohort study was established in 2004, when we chose a random subcohort (n = 1084; including 53 incident HCC cases) from all cohort members meeting the following criteria: (i) antibodies to HCV (anti-HCV) negative, (ii) no prior history of antiviral therapy and (iii) at least two blood samples collected after study entry. All cases (n = 59) not already included who developed HCC by 2005 and met the above criteria were added to complete the case–cohort population (n = 1143). The present study extended the follow-up period to 31 December 2006 and identified 13 additional HCC cases from the subcohort. We excluded one case due to insufficient sample; remaining were 124 cases and 1018 non-cases.

Biomarkers
All subjects were tested for HBsAg, alanine aminotransferase (ALT) and α-fetoprotein at baseline. Their frozen baseline serum samples were retrieved from blood bank to assay for anti-HCV, and frozen

baseline plasma samples were retrieved to assay for glucose, insulin, and HBV-related factors that included HBeAg, viral load, genotype and the BCP double mutations.

Follow-up assessment included ultrasonography and biochemical assay. High-resolution real-time ultrasonography was performed routinely since 1993, serum ALT and aspartate aminotransferase were examined routinely after August 1994, and γ-glutamyltransferase (GGT), cholesterol and triglycerides after August 1996. We retrospectively measured HBV viral load in all the plasma samples (2–12 samples per subject) taken from each subject during follow-up until the date of the case's diagnosis (if before 31 December 2004) or 31 July 2005, as described in our previous study (5). To identify change in diabetes status, plasma samples collected during 2–5 years after entry into the cohort from participants were retrieved to assay for glucose.

HBsAg, anti-HCV and HBeAg were assayed using commercially available immunoassay kits, whereas plasma HBV DNA, viral genotype and the BCP double mutations were assayed by polymerase chain reaction-based methods as described previously (5–7). Plasma insulin concentrations were determined by use of the Siemens Coat-A-Count Insulin radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Glucose concentrations were determined using an automatic dry-chemical analyzer (VITROS 5,1 FS; Johnson & Johnson, New Brunswick, NJ). We used the homeostasis model assessment-insulin resistance (HOMA-IR) index to estimate IR [HOMA-IR index = fasting insulin (μU/ml) X fasting glucose (mmol/l)/22.5] (25).

Statistical analysis

Plasma insulin and IR were categorized according to previous studies (26,27) or in quartiles based on the distribution of subjects. For cross-tabulated data, a Mantel–Haenszel extension of the χ^2 test for trend was used to examine the dose–response relationship across the four insulin groups. Continuous data were compared using analysis of variance or the Kruskal–Wallis test. The Cox proportional hazards model, adapted for the case–cohort design (28), was used to estimate the hazard ratios (HRs) for incident HCC. We estimated HRs according to insulin groups for two categories of time (<8 and ≥8 years) with the cutoff that approximately corresponds to the median of follow-up time of cases. Unconditional logistic regression was used to determine associations with liver abnormalities diagnosed by ultrasonography or biochemical tests; these results are reported as odds ratios and 95% confidence intervals (CIs). In multivariate models, factors known or suspected to influence HCC incidence were considered as covariates. Trend tests were conducted using the category medians as the scores. Effect modification was assessed by stratified analyses and by including cross product terms of the primary factor and the variable of interest in multivariable models. Tests for interaction to identify heterogeneity of the insulin effect were computed based on likelihood ratio tests. A two-sided P value of <0.05 was considered to indicate statistical significance. All analyses were conducted with SAS software 9.1.3 (SAS Institute, Cary, NC).

Results

Baseline characteristics

Only 31 (2.7%) of the 1142 subjects reported a history of diabetes. After adjusting for age, HCC cases were more probably to have a higher prediagnostic value of insulin, HOMA-IR and HBV viral load than those free from HCC during follow-up (all $P < 0.0001$), whereas the

Table I. Baseline characteristics of HCC cases and non-cases

Characteristic	HCC ($n = 124$)		Non-cases ($n = 1018$)		P^a
Demographics					
Age, years, No. (%)					<0.0001
30–39	15	(12.1)	369	(36.3)	
40–49	44	(35.5)	394	(38.7)	
50–59	31	(25.0)	159	(15.6)	
≥60	34	(27.4)	96	(9.4)	
College education or above, No./total (%)	92/124	(74.2)	845/1017	(83.1)	0.0753
Alcohol consumption, No. (%)	29	(23.4)	210	(20.6)	0.7078
Alcohol consumption >140 g/week, No./total (%)	11/122	(9.0)	84/1009	(8.3)	0.5868
Smoking status, No. (%)					0.4211[b]
Never	74	(59.7)	712	(69.9)	
Former	19	(15.3)	89	(8.7)	
Current	31	(25.0)	217	(21.3)	
First-degree family history of HCC, No. (%)	22	(17.7)	64	(6.3)	<0.0001
Metabolic factors					
History of diabetes mellitus, No. (%)	8	(6.5)	23	(2.3)	0.0792
Height, mean (SD), cm	167.6	(5.2)	168.1	(5.8)	0.5107
Weight, mean (SD), kg	67.8	(9.4)	67.1	(8.6)	0.2330
BMI, mean (SD), kg/m^2	24.1	(2.9)	23.7	(2.6)	0.0677
BMI≥25 kg/m^2, No. (%)	49	(39.5)	302	(29.7)	0.0145
Fasting plasma glucose, mean (SD), mg/dL	93.5	(16.0)	88.7	(17.0)	0.9325
Fasting plasma glucose≥126 mg/dL, No. (%)	5	(4.0)	20	(2.0)	0.4362
Fasting plasma insulin, mean (SD), μU/ml	5.68	(5.91)	4.33	(4.32)	<0.0001
IR (HOMA-IR), mean (SD)	1.35	(1.53)	0.98	(1.09)	<0.0001
Clinical and viral features					
ALT >40 U/l, No./total (%)	33/123	(26.8)	64/1015	(6.3)	<0.0001
Plasma HBV DNA, mean (SD), log$_{10}$ copies/ml	5.93	(2.02)	4.37	(1.89)	<0.0001
HBeAg positivity, No./total (%)	20/122	(16.4)	89/993	(9.0)	<0.0001
HBV genotype, total no.	123		1002		<0.0001[b]
B, No. (%)	54	(43.9)	818	(81.6)	
B + C, No. (%)	6	(4.9)	45	(4.5)	
C, No. (%)	63	(51.2)	139	(13.9)	
BCP double mutations, No./total (%)	74/118	(62.7)	295/983	(30.0)	<0.0001

[a]Except for age, significant test was performed by using Cox regression adjusted for age (continuous).
[b]P value was calculated based on the likelihood ratio test.

Table II. Ultrasonographic and biochemical features assayed during follow-up by baseline fasting plasma insulin levels

Characteristic	Fasting plasma insulin levels (µU/ml)				
	<2.75 (n = 395)	2.75–4.10 (n = 265)	4.11–6.10 (n = 259)	>6.10 (n = 223)	P for trend
No. of visits, median (interquartile range)	8 (5–9)	8 (5–9)	7 (5–9)	7 (5–9)	0.4081[a]
Self-reported diabetes, No./total (%)[b]	5/372 (1.3)	6/250 (2.4)	6/240 (2.5)	23/209 (11.0)	<0.0001
Plasma glucose, mean (SD), mg/dl[c]	87.2 (20.4)	87.8 (28.1)	87.2 (27.7)	99.1 (57.4)	0.0002[d]
Plasma glucose≥126 mg/dl, No./total (%)[c]	12/375 (3.2)	7/253 (2.8)	7/241 (2.9)	19/209 (9.1)	0.0044
Newly diagnosed diabetes, No./total (%)[e]	9/368 (2.5)	6/246 (2.4)	7/236 (3.0)	15/194 (7.7)	0.0048
Total cholesterol >200 mg/dl, No./total (%)	187/343 (54.5)	135/221 (61.1)	103/213 (48.4)	107/185 (57.8)	0.8763
Triglycerides >200 mg/dl, No./total (%)	43/343 (12.5)	26/221 (11.8)	32/213 (15.0)	46/185 (24.9)	0.0005
Liver biochemistry					
ALT, total No.	358	242	225	200	
Single elevation, No. (%)	68 (19.0)	44 (18.2)	45 (20.0)	44 (22.0)	
Multiple elevations, No. (%)	68 (19.0)	44 (18.2)	52 (23.1)	57 (28.5)	
ALT elevation ≥1 visit					
Multivariate OR (95% CI)[f]	0.84 (0.57–1.23)	1 (Ref)	1.13 (0.74–1.72)	1.68 (1.09–2.58)	0.0006
Aspartate aminotransferase, total No.	358	242	225	200	
Single elevation, No. (%)	59 (16.5)	37 (15.3)	41 (18.2)	36 (18.0)	
Multiple elevations, No. (%)	47 (13.1)	24 (9.9)	39 (17.3)	33 (16.5)	
Aspartate aminotransferase elevation≥1 visit					
Multivariate OR (95% CI)[f]	1.01 (0.67–1.53)	1 (Ref)	1.39 (0.89–2.17)	1.32 (0.84–2.10)	0.1215
GGT, total No.	342	221	213	185	
Single elevation, No. (%)	35 (10.2)	25 (11.3)	23 (10.8)	24 (13.0)	
Multiple elevations, No. (%)	29 (8.5)	12 (5.4)	18 (8.5)	25 (13.5)	
GGT elevation≥1 visit					
Multivariate OR (95% CI)[f]	0.94 (0.58–1.53)	1 (Ref)	0.96 (0.56–1.65)	1.64 (0.97–2.77)	0.0241
Ultrasound scanning, total No.	372	253	236	214	
Fatty liver, No. (%)	204 (54.8)	182 (71.9)	163 (69.1)	169 (79.0)	
Multivariate OR (95% CI)[f]	0.45 (0.31–0.66)	1 (Ref)	0.87 (0.57–1.32)	1.79 (1.12–2.87)	<0.0001
Liver cirrhosis, No. (%)	41 (11.0)	15 (5.9)	34 (14.4)	34 (15.9)	
Multivariate OR (95% CI)[f]	1.78 (0.88–3.57)	1 (Ref)	2.49 (1.21–5.15)	2.55 (1.22–5.33)	0.0886

Total number of subjects may vary because of missing values due to non-participation in the follow-up examinations.
[a]Statistical significance was examined by the Kruskal–Wallis test.
[b]Self-reported history of diabetes obtained by follow-up questionnaire in 1071 subjects.
[c]Fasting glucose levels were tested in plasma samples collected during 2–5 years after study entry from 1078 subjects.
[d]Significant test by one-way analysis of variance.
[e]Defined by the criteria of self-reported history of diabetes or fasting plasma glucose level of at least 126 mg/dl.
[f]Odds ratios (ORs) and 95% CIs were adjusted for number of visits, age (continuous), smoking, alcohol consumption, a first-degree family history of HCC and baseline viral factors, including HBV viral load (continuous), genotype (C versus non-C), HBeAg status and the BCP double mutations.

fasting glucose levels did not significantly differ between cases and non-cases. Cases also had a higher frequency of BMI ≥ 25, elevated ALT, HBeAg positivity, HBV genotype C infection, BCP double mutations and a first-degree family history of HCC than non-cases (all $P < 0.02$) (Table I). With increasing fasting levels of insulin, weight, BMI, fasting levels of glucose, HOMA-IR, the proportion of subjects reporting a history of diabetes and the prevalence of elevated ALT all increased (all P for trend <0.02). However, insulin levels were not associated with any hepatitis B viral factors (data not shown).

Metabolic factors and follow-up ultrasonographic and biochemical features

In total, 1142 subjects contributed 8052 person-examinations (median, eight per person). The number of visits was not significantly different between insulin groups. Increasing levels of insulin were associated with elevated levels of fasting glucose, triglycerides and the occurrence of diabetes (P for trend <0.005). After adjustment for the number of visits, demographics and viral factors, there was a monotonic rise in the risk for elevated levels of ALT during follow-up with increasing insulin (P for trend = 0.0006). But a J-shaped relation was observed between insulin and the risk of liver cirrhosis detected by ultrasonography, such that both high and low levels of insulin were found to be associated with increased risk (Table II). When the number of visits, demographics and other metabolic factors, including glucose, BMI and a history of diabetes, were included as covariates to adjust their effects, the associations for insulin with elevated ALT (HR for >6.10 versus 2.75–4.10 µU/ml = 1.64, 95% CI: 1.09–2.46, P for trend = 0.0202) and liver

cirrhosis (HR for >6.10 versus 2.75–4.10 µU/ml = 2.84, 95% CI: 1.45–5.57, P for trend = 0.0843) did not materially change. But the associations for insulin levels with the risks of GGT elevation and fatty liver on ultrasonography were greatly attenuated, and the association between insulin and GGT became statistically non-significant (data not shown).

In subjects with a BMI ≥25, the risks for ALT (HR = 1.48, 95% CI = 1.10–2.00) or GGT (HR = 1.66, 95% CI = 1.14–2.39) elevation and fatty liver (HR = 2.46, 95% CI = 1.75–3.47) were significantly higher than in those with a BMI <25 after adjustment for the number of visits, demographics and other metabolic factors including insulin, confirming our previous observation from using the entire cohort (12). However, the risk for cirrhosis related to a BMI ≥25 was greatly attenuated and became statistically non-significant after adjustment for insulin. After adjustment for insulin, we found no association with any liver abnormality for diabetes or fasting levels of glucose determined in plasma samples collected at baseline or during 2–5 years after study entry (data not shown).

Metabolic factors and HCC

We also found a J-shaped relation between insulin or the HOMA-IR index and incident HCC, irrespective of using cutpoints as described previously (26,27) or quartile cutpoints. In a model using insulin of 2.75–4.10 µU/ml as the referent group adjusted for differences among the four insulin groups including the number of visits, demographic characteristics and viral factors, the HR of HCC was 1.57 (95% CI: 0.96–2.58) for the lowest category of insulin (<2.75 µU/ml) and 2.36 (95% CI: 1.43–3.90) for the highest category of insulin

Table III. HRs for incident HCC by metabolic factors assayed at baseline

Metabolic factors	HCC (n=124)		Non-cases (n=1018)		HR[a]	95% CI	P for trend
	No.	(%)	No.	(%)			
Fasting insulin levels, μU/ml							
Category cutpoints							0.0140
<2.75	41	(33.1)	354	(34.8)	1.57	(0.96–2.58)	
2.75–4.10	16	(12.9)	249	(24.5)	1	(Ref)	
4.11–6.10	29	(23.4)	230	(22.6)	1.28	(0.76–2.15)	
>6.10	38	(30.6)	185	(18.2)	2.36	(1.43–3.90)	
Quartile cutpoints							0.0556
<2.27	32	(25.8)	250	(24.6)	1.43	(0.89–2.30)	
2.27–3.60	21	(16.9)	266	(26.1)	1	(Ref)	
3.61–5.36	26	(21.0)	263	(25.8)	0.95	(0.58–1.56)	
>5.36	45	(36.3)	239	(23.5)	1.78	(1.15–2.75)	
Fasting glucose levels, mg/dl							0.0161
<110	108	(87.1)	955	(93.8)	1	(Ref)	
110–125	11	(8.9)	43	(4.2)	1.40	(0.80–2.45)	
≥126	5	(4.0)	20	(2.0)	2.37	(1.12–5.04)	
History of diabetes by questionnaire							
No	116	(93.5)	995	(97.7)	1	(Ref)	
Yes	8	(6.5)	23	(2.3)	2.11	(1.13–3.94)	0.0192[b]
IR (HOMA-IR)							
Category cutpoints							0.0382
<0.70	48	(38.7)	459	(45.1)	1.30	(0.82–2.08)	
0.70–1.02	19	(15.3)	229	(22.5)	1	(Ref)	
1.03–1.53	26	(21.0)	190	(18.7)	1.20	(0.73–1.98)	
>1.53	31	(25.0)	140	(13.8)	1.97	(1.20–3.22)	
Quartile cutpoints							0.0175
<0.46	31	(25.0)	250	(24.6)	1.48	(0.90–2.44)	
0.46–0.77	20	(16.1)	270	(26.5)	1	(Ref)	
0.78–1.22	23	(18.5)	262	(25.7)	0.92	(0.54–1.55)	
>1.22	50	(40.3)	236	(23.2)	1.96	(1.23–3.10)	
BMI ≥25 kg/m²							
No	75	(60.5)	716	(70.3)	1	(Ref)	
Yes	49	(39.5)	302	(29.7)	1.58	(1.16–2.17)	0.0041[b]

[a]HR and 95% CIs were adjusted for number of visits, age (continuous), smoking, alcohol consumption, a first-degree family history of HCC and baseline viral factors, including HBV viral load (continuous), genotype (C versus non-C), HBeAg status and the BCP double mutations.
[b]P values were obtained by the Wald test.

(>6.10 μU/ml) (Table III). This association was largely unaltered by adjustment for demographics and other metabolic factors (>6.10 versus 2.75–4.10 μU/ml, HR = 2.24, 95% CI = 1.37–3.67). Exclusion of men reporting a history of diabetes at baseline did not substantially change the results. In contrast, after adjustment for demographics and other metabolic factors including insulin, the association between a BMI ≥25 and HCC became statistically non-significant (HR = 1.35, 95% CI: 0.98–1.86, P = 0.0648). Also, neither fasting glucose nor a history of diabetes measured at baseline or follow-up was associated with HCC (data not shown).

Insulin and HCC in selected subgroups
The median lag time measured as the interval from blood draw to HCC diagnosis was 7.5 (range, 0.6–16.3) years. High insulin level (≥4.11 μU/ml) was more strongly associated with increased HCC risk among cases with a lag time of at least 8 years, whereas the lowest category of insulin was more strongly associated with HCC among cases with a lag time of <8 years (Table IV).

We also performed analyses stratified according to established risk factors for HCC (3–8,12,13) (Table IV). The effect of an insulin level >6.10 μU/ml (versus 2.75–4.10 μU/ml) in increasing HCC risk was consistent across subgroups of age, BMI, HBV genotype and the presence or absence of the BCP double mutations, but its effect varied by baseline viral load of HBV (P for heterogeneity = 0.0021), with relative risk estimate being larger among men with viral load <4.39 log$_{10}$ copies/ml, the threshold of viral load associated with increased risk for HCC in previous studies (3,5) (HR: 6.15, 95% CI: 2.48–15.22), than among men with a higher viral load (HR: 1.49, 95% CI: 0.84–2.66). According to our previous study (6), there are three

trajectory classes for the 16-year time trends of HBV viral load. The positive association of insulin with HCC was also stronger among men with sustained low viral load in the levels of 3–4 log$_{10}$ copies/ml than among those with sustained high viral load in the levels of 5–6 log$_{10}$ copies/ml or those characterized by extremely high viral load in the levels of 8–9 log$_{10}$ copies/ml at recruitment that declined linearly over time (P for heterogeneity = 0.0629).

Discussion

Fatty liver is common in middle-aged HBV carriers with a prevalence of 30–90%, depending on BMI (12,30,31). Simple fatty liver without inflammation was not a predictor for the risk of HCC among male HBV carriers (12). Inasmuch, it remains of interest to elucidate the interaction between NAFLD and HBV viral factors in the progression of hepatitis B.

Overweight and obesity have been associated with an increased risk to develop HCC worldwide (12,13). IR, which leads to the accumulation of fat within hepatocytes, is linked to both excess BMI and NAFLD. Hepatic fat accumulation produces oxidative stress, resulting in inflammation and fibrosis (11,32). In experimental models, insulin stimulates cellular proliferation in cancer cell lines and promotes tumor growth in mice (33–35). Recent researches have demonstrated higher risk of several malignancies among individuals with higher insulin levels, a consequence of IR (26,27,36,37). However, the role of excess body weight compared with that of higher insulin in inducing HCC and its related conditions remains unknown.

Table IV . Association of insulin with incident HCC stratified by time and selected risk factors

Factor, group		Fasting plasma insulin levels (μU/ml)				*P* for trend
		<2.75	2.75–4.10	4.11–6.10	>6.10	
Follow-up, years						
<8 (*n* = 1082)	HR (95% CI)[a]	2.11 (1.17–3.82)	1 (Ref)	1.34 (0.69–2.61)	1.79 (0.91–3.49)	0.5785
≥8 (*n* = 1061)	HR (95% CI)[a]	1.33 (0.62–2.88)	1 (Ref)	2.32 (1.11–4.85)	4.15 (2.05–8.39)	<0.0001
Age, years						
<50 (*n* = 806)	HR (95% CI)[a]	1.56 (0.83–2.93)	1 (Ref)	0.93 (0.45–1.95)	2.24 (1.17–4.28)	0.1400
≥50 (*n* = 336)	HR (95% CI)[a]	2.25 (1.11–4.53)	1 (Ref)	2.82 (1.40–5.66)	3.13 (1.55–6.30)	0.0436
BMI, kg/m^2						
<25 (*n* = 791)	HR (95% CI)[a]	2.07 (1.19–3.59)	1 (Ref)	1.95 (1.06–3.60)	2.22 (1.17–4.23)	0.6184
≥25 (*n* = 351)	HR (95% CI)[a]	1.43 (0.55–3.71)	1 (Ref)	1.02 (0.44–2.37)	2.12 (0.97–4.64)[b]	0.0306
<23[c] (*n* = 455)	HR (95% CI)[a]	1.63 (0.83–3.24)	1 (Ref)	1.48 (0.65–3.38)	2.70 (1.17–6.23)	0.2269
≥23 (*n* = 687)	HR (95% CI)[a]	2.20 (1.15–4.22)	1 (Ref)	1.83 (0.98–3.43)	2.77 (1.51–5.07)	0.0394
HBV genotype						
C (*n* = 202)	HR (95% CI)[a]	1.28 (0.67–2.45)	1 (Ref)	1.10 (0.56–2.19)	2.28 (1.20–4.34)	0.0223
B or B + C (*n* = 923)	HR (95% CI)[a]	2.46 (1.20–5.02)	1 (Ref)	2.13 (1.01–4.49)	3.38 (1.63–6.98)	0.0747
Baseline HBV viral load, log$_{10}$ copies/ml[d]						
<4.39 (*n* = 666)	HR (95% CI)[a]	1.68 (0.63–4.46)	1 (Ref)	1.85 (0.65–5.25)	6.15 (2.48–15.22)	<0.0001
≥4.39 (*n* = 476)	HR (95% CI)[a]	1.71 (1.00–2.94)	1 (Ref)	1.43 (0.82–2.51)	1.49 (0.84–2.66)	0.7928
Time trend for HBV viral load[e]						
Sustained						
Low (*n* = 686)	HR (95% CI)[a]	3.31 (1.13–9.71)	1 (Ref)	1.90 (0.57–6.34)	5.80 (1.98–17.00)	0.0337
Sustained						
High (*n* = 366)	HR (95% CI)[a]	2.10 (1.13–3.89)	1 (Ref)	1.74 (0.93–3.28)	1.93 (1.02–3.63)	0.7497
Extremely						
High to low (*n* = 90)	HR (95% CI)[a]	0.43 (0.14–1.37)	1 (Ref)	0.87 (0.27–2.86)	0.84 (0.26–2.75)	0.2363
BCP double mutations						
Absence (*n* = 732)	HR (95% CI)[a]	1.74 (0.71–4.27)	1 (Ref)	2.85 (1.20–6.78)	4.12 (1.77–9.60)	0.0007
Presence (*n* = 369)	HR (95% CI)[a]	1.97 (1.10–3.52)	1 (Ref)	1.57 (0.83–2.95)	2.31 (1.25–4.28)	0.3359

[a]HRs and 95% CIs were adjusted for number of visits, age (continuous), smoking, alcohol consumption and a first-degree family history of HCC.
[b]*P* = 0.0607.
[c]Asian BMI criterion of overweight (29).
[d]The cutpoint is the threshold of viral load associated with increased risk for HCC in previous studies (3,5). *P* for heterogeneity of HRs by HBV viral load = 0.0021.
[e]Three patterns of time trend for viral load ('sustained low': having a level of 3–4 log$_{10}$ copies/ml over time; 'sustained high': having a level of 5–6 log$_{10}$ copies/ml over time and 'extremely high to low': having a level of 8–9 log$_{10}$ copies/ml at study entry that declined linearly over time) were defined according to our previous longitudinal viral-load study (6), in which trajectory analysis, a type of latent class analysis which identifies homogeneous groups within a heterogeneous population assumed to contain multiple latent trajectories, was performed with the SAS procedure PROC TRAJ to estimate trajectories of viral load across 16 years of follow-up. *P* for heterogeneity of HRs by time trends for HBV viral load = 0.0629.

To our knowledge, this is the first study to demonstrate a prospective association between fasting insulin levels and IR with the risk of HCC. After a maximum follow-up of 17 years, higher insulin was found to be strongly related to increased risk of incident HCC and related conditions, including elevated liver enzymes and liver cirrhosis detected by ultrasonography, among male HBV carriers. Overall, there was a J-shaped relation between insulin and HCC. A similar pattern was seen with the effect of the HOMA-IR index, which is highly dependent on insulin (25). The highest insulin levels (>6.10 μU/ml) were seen to be associated with an ~2.4-fold risk of HCC when compared with insulin 2.75–4.10 μU/ml. However, the lowest insulin levels (<2.75 μU/ml) also conferred a 1.6-fold risk for HCC. The dose–response effect of insulin appeared to be evident for an increased risk of HCC that occurred at least 8 years after the baseline blood draw.

There was also a J-shaped association between insulin and the risk of liver cirrhosis detected by ultrasonography, which is an important premalignant condition for HCC (12,38), whereas the rise in the risk for ALT elevation with increasing insulin was more monotonic. These findings raise the possibility that liver destruction in certain hepatitis B cirrhotic patients, even in the absence of overt clinical signs, may lead to diminished insulin levels many years before HCC diagnosis. Consistent with this hypothesis, a previous study indicated that liver damage from hepatic tumor is associated with a reduction in circulating levels of insulin-like growth factor-I, a hormone related to insulin (39). In our data, excluding cases diagnosed with HCC during the first 8 years of follow-up revealed that the increased HCC risk was only attenuated among men at the low end of insulin, but, contrary to this finding, the risk among those in the highest category was even greater.

Therefore, the possibility of the elevated risk in the lowest category of insulin due to antecedent disease cannot be excluded, and high insulin may exert its effect on the early stage of HCC development.

In endemic area of hepatitis B, literature on the association of HCC with diabetes is contradictory (12,22–24). A Korean cohort study followed 1.3 million people for up to 10 years and indicated a 1.5-fold increased HCC risk with glucose-defined diabetes (≥126 mg/dl) in men and, to a lesser degree for women (24). However, the study did not include the assessment of HBsAg or anti-HCV. Whether the findings of the Korean study can be generalizable to HBV carriers is unclear. Besides our previous cohort study (12), other two Taiwanese cohort studies have examined the association between diabetes and HCC, and the findings did not suggest an effect of diabetes in HBV carriers (22,23). Most of the HCC cases in this study were non-diabetic at baseline or during follow-up. We observed that adjustment for BMI, glucose and a history of diabetes only slightly attenuated the positive association between higher insulin and HCC. In contrast, despite a significant association observed between HCC and a history of diabetes or glucose-defined diabetes after adjustment for various viral factors, neither diabetes nor glucose was statistically associated with HCC risk after adjusting for insulin. The results suggest a more direct role of elevated fasting insulin than diabetes in the development of HCC in chronic hepatitis B.

The present study extended our previous work that demonstrated overweight as a risk factor for HBV-related HCC (12). However, because a BMI ≥25 was no longer associated with elevated HCC risk after adjusting for insulin, the previously reported association may probably be largely explained by the high levels of insulin in

overweight subjects. Our finding of a relationship between insulin and HCC across BMI groups again strengthens the relative importance of high insulin level versus excess body weight in determining HCC. We showed that BMI was associated with fatty liver and elevation of liver enzymes but not with more advanced liver disease in terms of cirrhosis and HCC after adjusting for insulin, indicating that excess body weight may have a greater role in causing hepatic steatosis and inflammation than following advanced illness.

Among the strengths of the present study is the characterization of various viral factors, which enabled evaluation of potential confounding and effect modification by these important risk factors for HBV-related HCC (2–8). We observed that the elevated HCC risk associated with higher insulin was largely unaltered when controlling various viral factors, and there existed an effect of higher insulin across subgroups categorized by HBV genotype and the presence or absence of the BCP double mutations. However, the HR for HCC associated with higher insulin was greater in male HBV carriers with low viral load than in those with high viral load. Currently, the guidelines for antiviral treatment in hepatitis B are based on HBV viral load $>10^5$ copies/ml and ALT levels more than two times the upper limit of normal (40). However, HBV carriers with normal ALT and viral load $<10^4$ copies/ml are still at a substantial risk of HCC (41). In light of our findings, the further reduction of the HCC burden would be achievable by insulin-lowering approaches, either through lifestyle changes or via pharmacologic approaches.

In conclusion, among male HBV carriers, higher-fasting insulin levels are a risk factor for the development of HCC and its related conditions, including cirrhosis and hepatitis. The analysis suggested little confounding by other metabolic factors, including BMI, fasting glucose and diabetes, as well as various viral factors, but the observed association of HCC risk with higher insulin levels was stronger in low than in high viral load group.

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