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ARTICLE

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Epidemiology

Associations between serum glucose, insulin, insulin resistance and the risk of incident primary liver cancer or chronic liver disease mortality: a nested case–control study

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BACKGROUND: To evaluate the associations between pre-diagnostic levels of serum insulin, glucose and insulin resistance (HOMA-IR) and future risk of incident primary liver cancer (PLC) or chronic liver disease (CLD)-related mortality.
METHODS: We used a nested case-control design to evaluate subjects over 22 years of follow-up. Glucose, insulin, and three markers of hepatitis B virus (HBV) and hepatitis C virus were measured in fasting baseline serum from 119 incident PLCs, 157 CLD-death cases and 512 matched controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression to estimate the associations between insulin, glucose, HOMA-IR and the risk of PLC or CLD death.
RESULTS: Compared with the lowest quartile of insulin, multivariable adjusted models showed that subjects in the highest quartile had elevated odds of developing PLC ($OR_{Q4/Q1} = 2.42$, 95% CI = 1.26–4.75, $P_{trend} = 0.007$), particularly in HBV-positive subjects ($P_{interaction} = 0.040$), and of CLD death ($OR_{Q4/Q1} = 1.80$, 95% CI = 1.02–3.21, $P_{trend} = 0.018$). For glucose, in the HBV-positive group, subjects in the fourth quartile had an increased risk of PLC ($OR_{Q4/Q1} = 2.18$, 95% CI = 1.07–4.60, $P_{trend} = 0.009$), and of CLD mortality ($OR_{Q4/Q1} = 1.75$, 95% CI = 0.95–3.28, $P_{trend} = 0.019$). Subjects with the highest HOMA-IR values had a threefold risk of developing PLC ($OR_{Q4/Q1} = 2.94$, 95% CI = 1.54–5.87, $P_{trend} = 0.001$), and a twofold risk of CLD death ($OR_{Q4/Q1} = 2.20$, 95% CI = 1.25–3.94, $P_{trend} = 0.005$).
CONCLUSIONS: We found that serum insulin and HOMA-IR could potentially be risk factors for PLC or CLD death.

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INTRODUCTION

Chronic liver disease (CLD) has poor long-term clinical outcomes and is a major cause of illness and death worldwide [1]. Liver cancer is the second most common cause of cancer death in China [2], where there are 388,800 new cases and 336,400 liver cancer deaths each year [3]. Chronic infections of hepatitis B (HBV) and hepatitis C (HCV) viruses are regarded as the predominant causes of primary liver cancer (PLC) [4] and strong risk factors for CLD [5, 6] in China.

In the liver and skeletal muscles, glucose is stored as glycogen, while in adipocytes, it is stored as triglycerides. Insulin, a peptide hormone secreted by pancreatic islet beta cells, regulates and controls the circulation of glucose in the blood and energy metabolism. In response to increased concentrations of circulating glucose, insulin is rapidly secreted into the systemic circulation, which increases the uptake of glucose and an anabolic state by activating cell-membrane insulin receptors [7].

In addition to its metabolic actions, insulin has a potent mitogenic effect, which promotes proliferation in normal or malignant cells [8–10]. Insulin is also a growth factor and effectively stimulates liver cancer cell growth in vitro or in vivo [11], and excessive insulin is thought to be a cancer-promoting factor in patients [12]. On the other hand, high glucose levels are capable of accelerating tumorigenesis by its damage to DNA [13, 14], and glucose catabolism can promote the proliferation of cancer cells [15, 16].

In addition, several epidemiologic studies have reported positive associations between serum insulin or glucose levels and chronic liver disease [12, 17], including PLC [12, 18, 19].To date, however, few prospective studies have directly examined serum insulin and glucose levels as risk factors for PLC or CLD-related death. Because the physiologic effects of insulin and glucose are so interrelated, it is important to evaluate both their individual and joint effects (insulin resistance) on the risk of PLC or CLD death in the same study.

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In a prospective case–control study nested in the Linxian Nutrition Intervention Trial (NIT) cohorts in China, we evaluated the associations between prediagnostic serum insulin, glucose and insulin resistance (IR), and the risk of incident PLC and CLD-related mortality.

MATERIALS AND METHODS

Study population and data acquisition

The present study was a prospective case–control study nested in the Linxian Nutrition Intervention Trial (NIT) cohorts in China. The populations in these cohorts were deficient in many nutrients [20], and they had extremely high rates of oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma [21]. The purpose of these trials was to evaluate whether vitamin/mineral supplements in physiologic doses could reduce the rates of these cancers in this nutrient-deficient population [22]. Subjects were selected from both the Dysplasia Trial cohort and the General Population Trial cohort. The population and detailed design of the Linxian NITs have been previously described [22–24]. Briefly, the Dysplasia Trial enrolled individuals between the ages of 40 and 69 years who were cytologically diagnosed with oesophageal dysplasia and lived in three communes located in northern Linxian from August to October of 1984. A total of 3318 residents were randomised and received either a daily multiple mineral/vitamin supplement (12 minerals and 14 vitamins), or matching placebo for 6 years, from May 1985 to April 1991. The General Population Trial enrolled individuals between 40 and 69 years old from the general population of four communes in Linxian from March to May of 1985. A total of 29,584 healthy adults were randomised and received one of four daily mineral/vitamin supplement combinations for 5.25 years from March 1986 to May 1991 in a one-half replicate of a 2 [4] fractional factorial experimental design [23]. Individuals with cancer, debilitating disease including liver disease or those who required daily medications were excluded from both trials.

At the baseline exams conducted between August 1984 and May 1985, all subjects were interviewed using a structured questionnaire recording data on age, smoking, alcohol consumption, etc., given a physical examination, and had a 10 mL blood sample drawn before either intervention started. These samples were stored on ice for 3–6 h during transportation to the field station lab. Then they were centrifuged and aliquoted into 1 mL vials, frozen and stored at −85 °C for long-term storage until thawed for the current laboratory measurements.

Follow-up and identification of outcome events

During the trial (1985–1991) and post-trial follow-up periods (after 1991), follow-up was performed, and incident cancer cases were identified by several methods to ensure essentially complete ascertainment of events. Village health workers visited each participant monthly and a panel of Chinese experts confirmed new cancer diagnoses by reviewing medical records from the local hospitals. Most incident primary liver cancers were diagnosed by combined evidence from biochemical assays, clinical examination, ultrasound, and computed tomography scan.

Nested case–control design and subject selection

A total of 255 incident PLC cases and 310 CLD-related deaths occurring were identified from baseline through the end of 2007. In this study, we included all of the of PLC cases and CLD-related deaths cases with sufficient available serum for testing of glucose and insulin, respectively. There were no significant differences (all $P > 0.05$) in characteristics (age, gender, smoking, BMI and HBV) between the included cases and excluded cases. Incidence density and frequency-matched controls (2:1) were selected for both case groups by age at baseline (±3 years), gender and trial, and were NIT participants who were alive and free of cancer at diagnosis time. Finally, we included 119 primary liver cancer cases (Dysplasia Trial = 29; General Population Trial = 90), and 157 CLD deaths cases (Dysplasia Trial = 29; General Population Trial = 127) in the current analysis. Since the controls for the primary liver cancer cases and the controls for the CLD deaths were not significantly different in age, sex or trial, we used the entire set of 512 controls (Dysplasia Trial = 131; General Population Trial = 381) in all current analyses, in order to increase statistical power.

Laboratory measurements

Each specimen's tube was labelled with a previously assigned unique serial number, and all laboratory technicians were blinded to case–control status and identification information. Each serum sample was tested for serum glucose concentration, insulin concentration, hepatitis B virus surface antigen (HBsAg), antibody to hepatitis B virus core antigen (anti-HBc) and antibody to hepatitis C virus (anti-HCV). Serum glucose or insulin were measured using the Glucose Test Kit or Insulin Test Kit on the Cobas c501 automatic biochemistry system (Roche Diagnostic Corp., Germany). HBsAg was analysed using the Bio-Rad Genetic Systems HBsAg EIA 3.0 kit (Bio-Rad Laboratories, Hercules, CA, USA); anti-HBc was analysed using the HBc (recombinant) ORTHO ELISA Test System (Ortho-Clinical Diagnostics, Raritan, NJ, USA); and anti-HCV was analysed using the ORTHO HCV version 3.0 enzyme-linked immunosorbent assay (Ortho-Clinical Diagnostics, Raritan, NJ, USA). All tests were performed according to the instructions of the reagent manufacturers. Every 36 samples were accompanied by three pooled serum samples as internal controls. Pooled samples were made from 70 NIT serum samples that had undergone the same storage conditions as the study samples but were not included in the present study. The coefficients of variation (CV) of 65 blinded quality control (QC) samples for serum glucose and insulin measurements were 1.7% and 3.7%, respectively.

Statistical analysis

Fasting glucose ≥6.99 mmol/L was defined biochemically as diabetes mellitus. The homoeostasis model assessment of insulin resistance (HOMA-IR) [fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5], was calculated as described previously [12, 25]. Non-normally distributed continuous variables are presented as median (interquartile range) and compared using Wilcoxon's rank-sum test. Categorical variables are presented as number (percentages) and are compared using the chi-square test. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Fully adjusted models were adjusted for age (continuous), sex, smoking, drinking alcohol, BMI (continuous), Trial, HBsAg, anti-HBc and anti-HCV. We used two different metrics of serum glucose, insulin and HOMA-IR to evaluate the independent association between glucose, insulin, or insulin resistance and risk of liver cancer incidence and CLD mortality: (1) as a continuous variable, scaled to one-half the interquartile range (0.85 mmol/L for glucose, 1.26 μU/mL for insulin, and 0.331 for HOMA-IR); and (2) as quartiles (Glucose, Q1: ≤ 3.78; Q2: 3.78 ~ 4.65; Q3: 4.65 ~ 5.48; Q4: > 5.48 mmol/L; Insulin, Q1: ≤ 1.63; Q2: 1.63 ~ 2.67; Q3: 2.67 ~ 4.15; Q4: > 4.15 μU/mL; and HOMA-IR, Q1: ≤ 0.28; Q2: 0.28 ~ 0.53; Q3: 0.53 ~ 0.95; Q4: > 0.95) in the control population who did not report clinical diabetes at baseline.

Multivariable analyses were adjusted for other known liver disease factors and potential confounders, including age at baseline (continuous), gender (female or male), BMI (continuous), smoking (yes: lifetime smoking ≥6 months, or no: lifetime smoking <6 months or no smoking), drinking (yes: any alcohol consumption in the last 12 months, or no: no alcohol consumption in the last 12 months), trial (Dysplasia Trial or General Population Trial), HBsAg (negative or positive), anti-HBc (negative or positive) and anti-HCV (negative or positive). Subgroup analyses were performed to evaluate the possible impact of residual confounding or effect modification. To evaluate whether the preclinical disease may have influenced the results, we conducted sensitivity analyses by excluding the subjects with baseline biochemical diabetes mellitus or/and cases diagnosed during the first 2 and 5 years of follow-up. P values for interactive effects were calculated by including the appropriate two variables multiplied together in the model. For each fully adjusted logistic regression model, statistical significance and goodness-of-fit were evaluated using likelihood tests and Hosmer and Lemeshow tests, respectively. All statistical analyses were conducted with R version 3.5.3 or SPSS version 17.0. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

We included incident 119 PLCs, 157 CLD-related deaths, and 512 controls. None of them had a clinical diagnosis of diabetes at baseline. The baseline and demographic characteristics of the subjects are shown and compared in Table 1. The median age of participants was 55 years old. There were no statistically significant differences between cases (PLCs or CLD deaths) and controls in terms of BMI, smoking history, alcohol consumption or trial.

Table 1. Baseline characteristics of subjects involved in the case–control study nested in the Linxian Nutritional Intervention Trials cohort, China, 1984–1985.

Characteristics	Control ($n = 512$)	Case ($n = 276$)			
		Primary liver cancer ($n = 119$)	*P*	Chronic liver disease death ($n = 157$)	*P*
Age, M (IQR)	55 (50–60)	55 (49–62)	0.657	55 (49–61)	0.488
Gender, *n* (%)			0.332		0.431
Female	236 (46.1)	49 (41.2)		78 (45.3)	
Male	276 (53.9)	70 (58.8)		79 (54.7)	
BMI, kg/m^2, M (IQR)	21.5 (20.0–23.1)	21.8 (20.0–23.0)	0.847	21.6 (20.3–23.6)	0.537
Trial, *n* (%)			0.784		0.068
Dysplasia	131 (25.6)	29 (24.4)		29 (18.5)	
General population	381 (74.4)	90 (75.6)		128 (81.5)	
Smoking ≥ last 6 months, *n* (%)			0.660		0.730
No	333 (65.2)	75 (63.0)		104 (66.7)	
Yes	178 (34.8)	44 (37.0)		52 (33.3)	
Any alcohol consumption in last 12 months, *n* (%)			0.551		0.416
No	404 (79.1)	97 (81.5)		128 (82.1)	
Yes	107 (20.9)	22 (18.5)		28 (17.9)	
HBsAg, *n* (%)			**<0.001**		**<0.001**
Negative	489 (95.7)	94 (79.0)		116 (73.9)	
Positive	22 (4.3)	25 (21.0)		41 (26.1)	
Anti-HBc, *n* (%)			**0.040**		**0.001**
Negative	220 (43.1)	39 (32.8)		45 (28.7)	
Positive	291 (56.9)	80 (67.2)		112 (71.3)	
Anti-HCV, *n* (%)			**0.008**		**0.004**
Negative	479 (93.7)	103 (86.6)		136 (86.6)	
Positive	32 (6.3)	16 (13.4)		21 (13.4)	
Glucose, mmol/L, M (IQR)	4.65 (3.78–5.48)	4.81 (4.06–5.66)	0.077	4.94 (4.02–5.91)	**0.013**
Insulin, μU/mL, M (IQR)	2.67 (1.63–4.15)	2.94 (2.06–4.67)	**0.017**	3.10 (1.96–4.75)	**0.014**
HOMA-IR	0.53 (0.28–0.95)	0.65 (0.37–1.09)	**0.013**	0.68 (0.39–1.19)	**0.007**

Bold values indicate statistical significance.
M (IQR) median (interquartile range), *BMI* body mass index, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibody to hepatitis B core antigen, *anti-HCV* antibody to hepatitis C virus, *HOMA-IR* homoeostasis model assessment of insulin resistance.

As expected, compared with controls, PLCs and CLD cases had a higher prevalence of HBsAg, anti-HBc and anti-HCV positivity (all $P < 0.05$). The median fasting serum glucose and insulin concentrations were higher in those who went on to develop PLC (4.81 mmol/L and 2.94 μU/mL) or CLD-related mortality (4.94 mmol/L and 3.10 μU/mL) than that in the control group (4.65 mmol/L and 2.67 μU/mL), respectively.

Associations for the concentration of serum glucose, insulin and HOMA-IR with PLC or CLD death

Table 2 shows the associations between serum glucose levels, PLC and CLD death. In the fully adjusted models, there was an increasing risk of PLC ($P_{trend} = 0.038$) and CLD mortality ($P_{trend} = 0.030$) with increasing glucose quartile. Among HBV-positive participants, those with the highest serum glucose quartile, as compared with those in the lowest quartile, had an increased risk of PLC (OR = 2.18, 95% CI = 1.07–4.60, $P_{trend} = 0.009$), and we also found an increasing trend for CLD death ($P_{trend} = 0.019$).

Subjects in the highest quartile of insulin levels, compared with the lowest quartile, had a nearly 2.5-fold increased risk of PLC ($OR_{Q4/Q1} = 2.42$, 95% CI = 1.26–4.75, $P_{trend} = 0.007$), and had an 80% higher risk of CLD deaths ($OR_{Q4/Q1} = 1.80$, 95% CI = 1.02–3.21, $P_{trend} = 0.018$) (Table 3). These increased risks were especially prominent in HBV-positive participants ($OR_{Q4/Q1} = 6.03$, 95% CI = 2.48–16.10, $P_{trend} < 0.001$ for PLC; $OR_{Q4/Q1} = 2.59$, 95% CI = 1.30–5.26, $P_{trend} = 0.004$ for CLD), and in participants in the General Population Trial ($OR_{Q4/Q1} = 2.34$, 95% CI = 1.09–5.24, $P_{trend} = 0.024$ for PLC; $OR_{Q4/Q1} = 1.71$, 95% CI = 0.91–3.23, $P_{trend} = 0.033$ for CLD). Moreover, there was a synergistic interaction between insulin and HBV infection that contributed to the development of PLC ($P_{interaction} = 0.040$), but such an interaction was not found in CLD deaths ($P_{interaction} = 0.128$).

HOMA-IR (Table 4) was also positively associated with both incident PLC ($OR_{Q3/Q1} = 2.63$, 95% CI = 1.37–5.21; $OR_{Q4/Q1} = 2.94$; 1.54–5.87; $P_{trend} = 0.001$), and CLD death ($OR_{Q4/Q1} = 2.20$, 95% CI = 1.25–3.94, $P_{trend} = 0.005$), with stronger associations observed in HBV-positive participants ($OR_{Q4/Q1} = 6.39$; 2.66–17.32; $P_{trend} < 0.001$ for PLC; and $OR_{Q4/Q1} = 2.91$, 95% CI = 1.47–5.98, $P_{trend} = 0.005$ for CLD death), but there was no interaction between HBV and HOMA-IR (both $P > 0.05$).

Only 29 (24.4%) PLCs and 29 (18.5%) CLD deaths were from the Dysplasia Trial population. Thus, the associations for serum glucose, insulin and HOMA-IR with PLC or CLD death were not as robust in this Trial population. In addition, due to the fact that a very low proportion of study participants drank alcohol (CLD: ~20.2%, PLC: ~20.4%), were overweight (CLD: BMI > 28, ~0.1%, BMI > 25, 8.8%, PLC: BMI > 28, ~0.8%, BMI > 25, 7.4%,) or tested positive for anti-HCV (CLD: ~7.9%, PLC: ~7.5%), we also did not find statistically significant effects of these variables on PLC incidence or CLD death (data not shown).

Table 2. Associations of baseline serum glucose with primary liver cancer incidence and chronic liver disease death by categories in the Linxian Nutritional Intervention Trials Cohort.

	Continuous[a]	Quarters									
		Q1		Q2		Q3		Q4		P_{trend}	$P_{interaction}$
	OR (95% CI)	n^b/n^c	Ref.	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)		
Primary liver cancer											
Crude	**1.16 (1.02–1.31)**	23/129	1	24/128	1.05 (0.56–1.97)	37/128	1.62 (0.92–2.91)	35/127	1.55 (0.87–2.79)	0.063	–
Age and gender-adjusted	**1.16 (1.02–1.32)**	23/129	1	24/128	1.03 (0.55–1.93)	37/128	1.61 (0.91–2.90)	35/127	1.54 (0.86–2.79)	0.063	–
Fully adjusted[d]	**1.17 (1.03–1.34)**	23/129	1	24/128	1.06 (0.56–2.01)	37/128	1.69 (0.95–3.06)	35/127	1.66 (0.92–3.03)	**0.038**	–
HBV[d]											0.328
Positive	**1.26 (1.07–1.48)**	15/80	1	14/76	0.98 (0.43–2.20)	24/66	1.98 (0.96–4.18)	27/72	**2.18 (1.07–4.60)**	**0.009**	
Negative	0.98 (0.76–1.25)	8/49	1	10/52	0.99 (0.34–2.91)	13/61	1.16 (0.44–3.22)	8/55	0.79 (0.25–2.45)	0.185	
Trial[d]											0.559
Dysplasia	**1.38 (1.08–1.79)**	3/32	1	5/30	1.91 (0.41–10.38)	10/28	**4.70 (1.22–23.59)**	11/41	3.49 (0.94–16.99)	0.050	
General population	1.10 (0.94–1.29)	20/97	1	19/98	0.97 (0.48–1.98)	27/100	1.35 (0.70–2.64)	24/86	1.43 (0.73–2.85)	0.192	
Chronic liver disease death											
Crude	**1.18 (1.05–1.33)**	34/129	1	30/128	0.89 (0.51–1.54)	40/128	1.19 (0.71–2.00)	53/127	1.58 (0.97–2.62)	**0.034**	–
Age and gender adjusted	**1.18 (1.04–1.33)**	34/129	1	30/128	0.89 (0.51–1.54)	40/128	1.18 (0.70–1.99)	53/127	1.55 (0.94–2.57)	**0.044**	–
Fully adjusted[d]	**1.18 (1.05–1.34)**	34/129	1	30/128	0.92 (0.53–1.61)	40/128	1.21 (0.72–2.07)	53/127	1.65 (1.00–2.78)	**0.030**	–
HBV[d]											0.128
Positive	**1.21 (1.05–1.40)**	24/80	1	18/76	0.80 (0.40–1.60)	33/66	1.67 (0.90–3.13)	38/72	1.75 (0.95–3.28)	**0.019**	
Negative	1.16 (0.92–1.46)	10/49	1	12/52	1.04 (0.38–2.83)	7/61	0.53 (0.18–1.54)	15/55	1.48 (0.57–4.00)	0.665	
Trial[d]											0.928
Dysplasia	1.17 (0.85–1.61)	5/32	1	6/30	1.31 (0.33–5.41)	7/28	1.39 (0.36–5.63)	11/41	1.74 (0.51–6.61)	0.387	
General population	1.10 (0.94–1.29)	29/97	1	24/98	0.86 (0.46–1.59)	33/100	1.14 (0.64–2.06)	42/86	1.68 (0.95–3.00)	**0.047**	

Bold values indicate statistical significance.
[a]ORs for continuous glucose were scaled to one half the interquartile range (0.85 mmol/L) of control.
[b]Number of subjects in the case group.
[c]Number of subjects in the control group.
[d]Adjusted for age, sex, smoking, drinking, BMI, Trial, HBsAg, anti-HBc and anti-HCV.

Table 3. Associations of baseline serum insulin with primary liver cancer incidence and chronic liver disease death by categories in the Linxian Nutritional Intervention Trials Cohort.

	Continuous[a]	Quarters									
		Q1		Q2		Q3		Q4		P_{trend}	$P_{interaction}$
	OR (95% CI)	n^b/n^c	Ref.	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)		
Primary liver cancer											
Crude	**1.09 (1.01–1.18)**	19/129	1	29/129	1.53 (0.82–2.90)	34/127	1.82 (0.99–3.41)	37/127	**1.98 (1.09–3.68)**	**0.025**	–
Age and gender-adjusted	**1.10 (1.02–1.19)**	19/129	1	29/129	1.68 (0.89–3.22)	34/127	**2.08 (1.12–3.95)**	37/127	**2.41 (1.29–4.64)**	**0.006**	–
Fully adjusted[d]	**1.11 (1.02–1.21)**	19/129	1	29/129	1.62 (0.85–3.13)	34/127	**2.08 (1.10–4.01)**	37/127	**2.42 (1.26–4.75)**	**0.007**	–
HBV[d]											**0.040**
Positive	**1.17 (1.05–1.32)**	8/74	1	20/83	**2.61 (1.09–6.81)**	23/73	**3.69 (1.55–9.64)**	29/64	**6.03 (2.48–16.10)**	**<0.001**	
Negative	1.02 (0.85–1.17)	11/55	1	9/45	1.01 (0.36–2.81)	11/54	1.00 (0.34–2.92)	8/63	0.54 (0.17–1.68)	**0.315**	
Trial[d]			1								0.899
Dysplasia	1.09 (0.88–1.33)	6/39	1	7/29	2.13 (0.57–8.08)	6/32	1.83 (0.48–7.06)	10/31	3.23 (0.90–12.47)	0.109	
General population	**1.12 (1.02–1.23)**	13/90	1	22/100	1.58 (0.75–3.47)	28/95	**2.23 (1.07–4.83)**	27/96	**2.34 (1.09–5.24)**	**0.024**	
Chronic liver disease death											
Crude	**1.10 (1.02–1.18)**	30/129	1	31/129	1.03 (0.59–1.81)	42/127	1.42 (0.84–2.43)	54/127	**1.83 (1.11–3.07)**	**0.008**	–
Age and gender-adjusted	**1.09 (1.01–1.18)**	30/129	1	31/129	1.04 (0.59–1.85)	42/127	1.44 (0.84–2.50)	54/127	**1.86 (1.08–3.22)**	**0.011**	–
Fully adjusted[d]	**1.08 (1.01–1.17)**	30/129	1	31/129	0.95 (0.53–1.70)	42/127	1.39 (0.80–2.45)	54/127	**1.80 (1.02–3.21)**	**0.018**	–
HBV[d]											0.836
Positive	**1.13 (1.03–1.24)**	20/74	1	25/83	1.12 (0.57–2.23)	29/73	1.60 (0.81–3.22)	39/64	**2.59 (1.30–5.26)**	**0.004**	
Negative	0.93 (0.74–1.11)	10/55	1	6/45	0.64 (0.19–2.05)	13/54	0.87 (0.30–2.53)	15/63	0.73 (0.24–2.24)	0.733	
Trial[d]											0.369
Dysplasia	1.10 (0.88–1.33)	4/39	1	8/29	2.72 (0.63–13.03)	7/32	2.06 (0.48–9.77)	10/31	3.03 (0.72–14.47)	0.256	
General population	1.09 (0.99–1.19)	26/90	1	23/100	0.75 (0.39–1.42)	35/95	1.30 (0.70–2.41)	44/96	1.71 (0.91–3.23)	**0.033**	

Bold values indicate statistical significance.
[a]ORs for continuous insulin were scaled to one-half the interquartile range (1.26 μU/mL) of control.
[b]Number of subjects in the case group.
[c]Number of subjects in the control group.
[d]Adjusted for age, sex, smoking, drinking, BMI, Trial, HBsAg, anti-HBc and anti-HCV.

Table 4. Associations of baseline HOMA-IR with primary liver cancer incidence and chronic liver disease death by categories in the Linxian Nutritional Intervention Trials Cohort.

	Continuous[a]	Quarters									
		Q1		Q2		Q3		Q4		P_{trend}	$P_{interaction}$
	OR (95% CI)	n^b/n^c	Ref.	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)		
Primary liver cancer											
Crude	**1.12 (1.02–1.22)**	16/128	1	28/128	1.75 (0.91–3.46)	36/128	**2.25 (1.21–4.36)**	39/128	**2.44 (1.32–4.69)**	**0.004**	–
Age and gender-adjusted	**1.13 (1.03–1.24)**	16/128	1	28/128	1.84 (0.95–3.65)	36/128	**2.54 (1.34–4.97)**	39/128	**2.82 (1.50–5.54)**	**0.001**	–
Fully adjusted[d]	**1.14 (1.03–1.25)**	16/128	1	28/128	1.94 (0.99–3.89)	36/128	**2.63 (1.37–5.21)**	39/128	**2.94 (1.54–5.87)**	**0.001**	–
HBV[d]											0.062
Positive	**1.26 (1.12–1.42)**	7/73	1	19/83	**2.76 (1.12–7.56)**	24/74	**4.27 (1.75–11.67)**	30/64	**6.39 (2.66–17.32)**	**<0.001**	
Negative	0.92 (0.72–1.12)	9/55	1	9/44	1.38 (0.49–3.97)	12/54	1.26 (0.45–3.64)	9/64	0.83 (0.26–2.55)	0.726	
Trial[d]											0.875
Dysplasia	1.21 (0.98–1.49)	3/37	1	9/28	**5.33 (1.35–27.14)**	5/29	3.44 (0.72–19.24)	12/37	**6.07 (1.57–30.73)**	**0.038**	
General population	**1.10 (1.02–1.19)**	13/91	1	19/100	1.47 (0.68–3.30)	31/99	**2.47 (1.21–5.31)**	27/91	**2.50 (1.18–5.52)**	**0.008**	
Chronic liver disease death											
Crude	**1.12 (1.04–1.22)**	26/128	1	35/128	1.35 (0.77–2.38)	41/128	1.58 (0.92–2.76)	55/128	**2.12 (1.26–3.63)**	**0.004**	–
Age and gender-adjusted	**1.12 (1.03–1.22)**	26/128	1	35/128	1.37 (0.78–2.43)	41/128	1.58 (0.91–2.81)	55/128	**2.14 (1.25–3.74)**	**0.005**	–
Fully adjusted[d]	**1.12 (1.02–1.21)**	26/128	1	35/128	1.32 (0.74–2.37)	41/128	1.56 (0.88–2.80)	55/128	**2.20 (1.25–3.94)**	**0.005**	–
HBV[d]											0.846
Positive	**1.16 (1.04–1.29)**	17/73	1	28/83	1.49 (0.75–3.02)	29/74	1.82 (0.90–3.74)	39/64	**2.91 (1.47–5.98)**	**0.002**	
Negative	1.00 (0.83–1.19)	9/55	1	7/44	0.86 (0.27–2.63)	12/54	0.91 (0.32–2.67)	16/64	1.07 (0.38–3.07)	0.841	
Trial[d]											0.784
Dysplasia	1.19 (0.96–1.48)	4/37	1	6/28	2.07 (0.47–9.94)	8/29	2.49 (0.6–11.53)	11/37	2.55 (0.66–11.42)	0.226	
General population	**1.07 (1.01–1.16)**	22/91	1	29/100	1.22 (0.65–2.33)	33/99	1.42 (0.76–2.70)	44/91	**2.23 (1.19–4.26)**	**0.011**	

Bold values indicate statistical significance.
[a]ORs for continuous HOMA-IR were scaled to one-half the interquartile range (0.331) of control.
[b]Number of subjects in the case group.
[c]Number of subjects in the control group.
[d]Adjusted for age, sex, smoking, drinking, BMI, Trial, HBsAg, anti-HBc and anti-HCV.

Table 5. Sensitivity analysis of for associations of baseline glucose, insulin, and HOMA-IR, and primary liver cancer incidence and chronic liver disease death after exclusion of cases occurring in 2 or 5 years of follow-up or participants with diabetes mellitus.

	Continuous[a]	Quartile								
		Q1		Q2		Q3		Q4		P_{trend}
	OR (95% CI)	n^b/n^c	Ref.	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	
Primary liver cancer										
Cases that occurred within 2 years excluded										
Insulin[d]	**1.10 (1.01–1.20)**	19/129	1	28/129	1.55 (0.81–3.00)	34/127	**2.04 (1.08–3.93)**	35/127	**2.22 (1.15–4.39)**	**0.015**
Glucose[d]	**1.17 (1.02–1.34)**	22/129	1	24/128	1.12 (0.59–2.13)	37/128	1.77 (0.99–3.23)	33/127	1.64 (0.90–3.04)	**0.046**
HOMA-IR[d]	**1.13 (1.02–1.24)**	16/128	1	27/128	1.87 (0.96–3.77)	36/128	**2.6 (1.36–5.16)**	37/128	**2.75 (1.42–5.49)**	**0.002**
Cases that occurred within 5 years excluded										
Insulin[d]	**1.10 (1.01–1.21)**	12/129	1	22/129	1.81 (0.85–3.99)	32/127	**2.93 (1.43–6.35)**	31/127	**2.96 (1.40–6.57)**	**0.003**
Glucose[d]	**1.22 (1.06–1.42)**	18/129	1	17/128	0.99 (0.48–2.04)	33/128	**2.00 (1.07–3.86)**	29/127	1.91 (0.99–3.75)	**0.012**
HOMA-IR[d]	**1.10 (1.02–1.19)**	11/128		20/128	1.89 (0.87–4.33)	32/128	**3.15 (1.53–6.96)**	34/128	**3.62 (1.74–8.02)**	**0.001**
Participants with baseline glucose ≥6.99 mmol/L excluded										
Insulin[d]	**1.11 (1.01–1.22)**	18/128	1	28/128	1.6 (0.83–3.14)	32/118	**2.10 (1.09–4.13)**	32/117	**2.24 (1.13–4.53)**	**0.015**
Glucose[d]	1.11 (0.95–1.31)	23/129	1	24/128	1.08 (0.57–2.04)	37/128	1.7 (0.95–3.08)	26/106	1.52 (0.81–2.88)	0.078
HOMA-IR[d]	**1.12 (1.01–1.25)**	16/127	1	27/128	1.86 (0.95–3.76)	35/126	**2.50 (1.30–4.98)**	32/110	**2.71 (1.37–5.54)**	**0.003**
Participants with baseline glucose ≥6.99 mmol/L and cases that occurred within 2 years excluded										
Insulin[d]	1.10 (0.99–1.21)	18/128	1	27/128	1.53 (0.79–3.01)	32/118	**2.06 (1.07–4.06)**	31/117	**2.11 (1.06–4.30)**	**0.025**
Glucose[d]	1.12 (0.95–1.32)	22/129	1	24/128	1.14 (0.60–2.17)	37/128	1.78 (0.99–3.26)	25/106	1.53 (0.80–2.94)	0.077
HOMA-IR[d]	1.12 (0.99–1.24)	16/127	1	26/128	1.79 (0.91–3.63)	35/126	**2.48 (1.29–4.95)**	31/110	**2.58 (1.30–5.29)**	**0.005**
Participants with baseline glucose ≥6.99 mmol/L and cases that occurred within 5 years excluded										
Insulin[d]	1.09 (0.97–1.22)	12/128	1	21/128	1.41 (0.65–3.18)	30/118	**2.47 (1.18–5.46)**	27/117	**2.34 (1.08–5.33)**	**0.015**
Glucose[d]	1.19 (0.99–1.43)	18/129	1	17/128	1.06 (0.50–2.22)	33/128	**2.11 (1.10–4.16)**	22/106	1.76 (0.86–3.66)	**0.030**
HOMA-IR[d]	1.08 (0.98–1.19)	11/127	1	20/128	1.89 (0.87–4.35)	31/126	**2.97 (1.42–6.59)**	28/110	**3.27 (1.53–7.43)**	**0.001**
Chronic liver disease death										
Cases that occurred within 2 years excluded										
Insulin[d]	**1.09 (1.01–1.17)**	29/129	1	31/129	0.98 (0.55–1.76)	39/127	1.32 (0.75–2.36)	52/127	**1.81 (1.02–3.24)**	**0.022**
Glucose[d]	**1.20 (1.06–1.35)**	32/129	1	30/128	0.98 (0.56–1.73)	40/128	1.29 (0.76–2.22)	49/127	1.64 (0.98–2.80)	**0.036**
HOMA-IR[d]	**1.19 (1.02–1.22)**	24/128	1	35/128	1.42 (0.79–2.59)	39/128	1.60 (0.89–2.92)	53/128	**2.33 (1.31–4.23)**	**0.004**
Cases that occurred within 5 years excluded										
Insulin[d]	**1.08 (1.01–1.18)**	26/129	1	30/128	1.04 (0.57–1.90)	31/127	1.14 (0.62–2.11)	49/127	**1.88 (1.04–3.45)**	**0.029**
Glucose[d]	**1.19 (1.04–1.35)**	30/129	1	27/128	0.95 (0.53–1.70)	38/128	1.34 (0.77–2.33)	41/127	1.49 (0.86–2.60)	0.082
HOMA-IR[d]	**1.08 (1.01–1.15)**	22/128	1	33/128	1.43 (0.78–2.66)	33/128	1.45 (0.78–2.71)	48/128	**2.29 (1.26–4.25)**	**0.009**
Participants with baseline glucose ≥6.99 mmol/L excluded										
Insulin[d]	1.09 (0.99–1.19)	28/128	1	29/128	0.96 (0.53–1.74)	39/118	1.48 (0.83–2.66)	47/117	**1.84 (1.01–3.37)**	**0.018**
Glucose[d]	1.14 (0.99–1.33)	34/129	1	30/128	0.92 (0.53–1.61)	40/128	1.21 (0.71–2.06)	39/106	1.45 (0.84–2.53)	0.123
HOMA-IR[d]	**1.11 (1.01–1.23)**	25/127	1	34/128	1.35 (0.75–2.45)	39/126	1.56 (0.87–2.84)	45/110	**2.20 (1.22–4.07)**	**0.009**

Table 5. continued

	Continuous[a]	Quartile								
		Q1		Q2		Q3		Q4		P_{trend}
	OR (95% CI)	n^b/n^c	Ref.	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	n^b/n^c	OR (95% CI)	
Participants with baseline glucose ≥6.99 mmol/L and cases that occurred within 2 years excluded										
Insulin[d]	1.09 (1.00–1.19)	27/128	1	29/128	0.99 (0.54–1.8)	36/118	1.40 (0.77–2.54)	45/117	**1.84 (1.01–3.40)**	**0.024**
Glucose[d]	1.15 (0.99–1.33)	32/129	1	30/128	0.98 (0.56–1.73)	40/128	1.29 (0.75–2.21)	35/106	1.41 (0.80–2.49)	0.152
HOMA-IR[d]	**1.12 (1.01–1.24)**	23/127	1	34/128	1.46 (0.8–2.69)	37/126	1.60 (0.88–2.97)	43/110	**2.33 (1.27–4.37)**	**0.008**
Participants with baseline glucose ≥6.99 mmol/L and cases that occurred within 5 years excluded										
Insulin[d]	1.09 (0.99–1.19)	24/128	1	28/128	1.05 (0.57–1.97)	29/118	1.23 (0.66–2.34)	42/117	**1.93 (1.03–3.65)**	**0.030**
Glucose[d]	1.13 (0.96–1.32)	30/129	1	27/128	0.95 (0.53–1.71)	38/128	1.33 (0.77–2.32)	28/106	1.23 (0.67–2.24)	0.298
HOMA-IR[d]	1.07 (0.99–1.16)	21/127	1	32/128	1.47 (0.80–2.78)	31/126	1.45 (0.77–2.76)	39/110	**2.32 (1.23–4.45)**	**0.015**

Bold values indicate statistical significance.
[a]ORs for continuous glucose (0.85 mmol/L), insulin (1.26 μU/mL) or HOMA-IR (0.331) were scaled to one half the interquartile range of control.
[b]Number of subjects in the case group.
[c]Number of subjects in the control group.
[d]Adjusted for age, sex, smoking, drinking, BMI, Trial, HBsAg, anti-HBc and anti-HCV.

Sensitivity analyses

In sensitivity analyses, excluding cases that were diagnosed within 2 or 5 years of baseline and/or baseline diabetes mellitus defined biochemically (≥6.99 mmol/L) did not alter the results of the main analyses for insulin or HOMA-IR, but the glucose results were attenuated and lost their significant quartile trends for both PLC and CLD endpoints when participants with biochemically defined diabetes mellitus were excluded (Table 5).

DISCUSSION

To the best of our knowledge, this is one of the first study to prospectively examine associations between pre-diagnostic serum glucose, insulin, and HOMA-IR and the risk of incident PLC or CLD mortality in the Chinese population. We found an association between higher fasting serum glucose, insulin, and HOMA-IR and an increased risk of developing PLC and CLD-related mortality during 22 years of follow-up, with stronger associations observed in subjects with HBV infection.

Glucose, PLC and CLD

Diabetes is an independent risk factor for PLC [26] and CLD death [27]. In this study, although we observed a positive association between biochemically defined diabetes and PLC, it was not statistically significant because of the few participants with high glucose (Supplementary Table S1).

In East Asia, especially in China, HBV is the most important risk factor for PLC and CLD death. In our study, HBV-positive individuals had a marginally higher risk of PLC (OR = 1.42, 95% CI: 0.92–2.19) and a significantly higher risk of CLD death (OR = 1.79, 95% CI: 1.20–2.67) than HBV-negative individuals. We also found that higher glucose had increased risks of PLC ($P_{trend} = 0.009$) and CLD deaths ($P_{trend} = 0.019$) in HBV-positive subjects. However, there was no statistical interaction between glucose levels and HBV infection status. Other previous studies also did not observe different risk between glucose and PLC and CLD death by HBV status [18, 28]. In Feng et al.'s study, elevated glucose was associated with risk of PLC in HBV-negative subjects but not in HBV-positive subjects, and no interaction was found between glucose levels and HBsAg infection status [18]. In the prospective China Kadoorie Biobank cohort, which recruited 0.5 million adults with 10 years of follow-up, there was a positive association of baseline glucose levels with risk of liver cancer, with each 1 mmol/L higher baseline plasma glucose being associated with an adjusted hazard ratio of 1.04 (95% CI: 1.03–1.06), but the association did not differ by HBV infection [28].

Insulin, PLC and CLD

Our study observed an increased risk of PLC and CLD mortality associated with high serum insulin levels, especially in subjects with HBV infection. Several previous studies have had similar results. Chao et al. found a positive association between serum insulin and the risk of PLC among HBV carriers [29]. A study of Finnish men also suggested higher insulin levels were associated with increased risk of incident PLC ($OR_{Q4/Q1} = 3.41$) and CLD mortality ($OR_{Q4/Q1} = 2.51$) [12]. Excluding the participants with clinical diabetes mellitus, there were also positive associations between insulin levels and PLC or CLD death in this Finnish population [12], which is consistent with our findings, indicating that higher fasting serum insulin may contribute to carcinogenesis in the absence of diabetes.

Overwhelming evidence suggests a strong role of HBV infection in causing or exacerbating the development of PLC [30]. We found a statistically significant positive interaction between HBV infection and insulin levels in increasing the risk of developing PLC ($P_{interaction} = 0.040$). The underlying biological mechanism may be explained as follows. The oncogenic properties of HBV have been linked to transactivation of cellular signalling pathways via the HBV X protein (HBx). Insulin receptor substrate 1 (IRS-1), an

important molecule in the insulin signal transduction pathway, has been associated with the development of liver cancer [31]. One study used an HBV-related double transgenic murine model and showed that overexpression of both HBx and IRS-1 could stimulate cell proliferation in the liver sufficient to promote hepatocellular carcinoma (HCC) development and progression [32].

HOMA-IR, PLC and CLD

In this study, the greatest risk of PLC or CLD death was found among the highest quartiles of HOMA-IR. IR is often a precursor to type 2 diabetes [33]. HOMA-IR may be an earlier indication of evolving hyperglycaemia or/and hyperinsulinemia [34]. Chronic and prolonged hyperglycaemia leads to hepatocellular damage, changes the structure and function of pancreatic β-cells and causes IR, hence inducing and accelerating the occurrence and progression of non-alcoholic fatty liver disease [35] and various cancers, including lung, breast and colon cancers [36, 37]. Previous epidemiologic results have also found an association between higher HOMA-IR and a higher risk of PLC and CLD death [12], which is consistent with our findings.

In this study, we also observed stronger findings for glucose, insulin, and HOMA-IR with PLC than with CLD death. It is possible that higher glucose and insulin levels may be more strongly associated with subsequent PLC than fatal noncancer liver disease endpoints [12]. In support of this, some studies have suggested that high glucose and insulin concentrations may promote the growth of liver tumour [38], although the observed differences could also be due to chance.

Alternatively, associations with glucose and insulin levels could reflect reverse causality. However, excluding the subjects with biochemical diabetes mellitus or/and cases diagnosed within 2 or 5 years of baseline from the analyses did not substantially alter odds ratios for insulin or HOMA-IR. On the other hand, excluding subjects with baseline biochemical diabetes attenuated the glucose results. Thus, serum insulin and HOMA-IR were more stable and reliable in the absence of diabetes than glucose to evaluate the risk of PLC incidence or CLD death.

Strengths and limitations

Our study had several strengths. Chief among them was the use of a prospective design (serum glucose and insulin were measured in serum collected before the onset of the disease). The serum used for testing was collected at baseline, before interventions, diseases or other possible confounders could affect the interpretation of the associations. The questionnaire information was also collected at the beginning of the cohort, by face-to-face interviews, avoiding bias in data collection. We also had high-quality follow-up, and the lost to follow-up rate was <1%. Furthermore, our study design and analysis considered the major risk factors for liver disease (HBV and HCV infection) in order to isolate the relationship of concern in this study.

There were also several limitations in this study. Our sample size was limited for detecting modest associations and for examining stratifications. A second limitation of this study was that a large proportion of the primary liver cancer cases (90%) were not diagnosed based on histological evidence, but by the combined evidence from biochemical assays, clinical examination, ultrasound and CT scan, which could not exclude the possibility of misclassification. However, if that were the case, our reported results would likely be attenuated in magnitude. Future studies are needed to address these issues.

In conclusion, we have provided the first prospective evaluation of the associations of serum insulin, glucose and HOMA-IR with the risk of incident PLC or CLD mortality. High serum insulin and HOMA-IR contributed to increased risk for incident PLC and CLD-related death. These results may be of potential scientific and clinical significance for PLC or CLD prevention and control. Further studies are needed to confirm these findings in other populations and to elaborate on underlying mechanisms.

DATA AVAILABILITY

The data generated in this study are available upon request from the corresponding author.

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AUTHOR CONTRIBUTIONS

Study concepts and study design: JY, WC and Y-LQ; data acquisition: JY, L-YY, Y-WL, HY, J-HF, J-FC, BL, NDF and SMD; quality control data and algorithms: J-HF and WC; data analysis, interpretation and statistical analysis: JY; manuscript preparation: JY; manuscript editing: YJ and WC; manuscript review: WC, SMD, NDF, CCA, PRT and Y-LQ; all authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was performed according to the guidelines of the Helsinki declaration. All consent procedures, including human specimen collection, were approved by the Institutional Review Boards of the U.S. National Institutes of Health and the Chinese Academy of Medical Sciences (Beijing, China), and all participants provided written informed consent.

CONSENT TO PUBLISH

Not applicable.

ADDITIONAL INFORMATION

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