IJC
International Journal of Cancer

Metabolic risk factors and primary liver cancer in a prospective study of 578,700 adults

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Initial studies have indicated diabetes and obesity to be risk factors for hepatocellular carcinoma; but the association between other metabolic risk factors and primary liver cancer (PLC) has not been investigated. The metabolic syndrome and cancer project (Me-Can) includes cohorts from Norway, Austria and Sweden with data on 578,700 subjects. We used Cox proportional hazard models to calculate relative risks (RRs) of PLC by body mass index (BMI), blood pressure and plasma levels of glucose, cholesterol and triglycerides as continuous standardized variables (*z*-score with mean = 0 and standard deviation (SD) = 1) and their standardized sum of metabolic syndrome (MetS) *z*-score. RRs were corrected for random error in measurements. During an average follow-up of 12.0 years (SD = 7.8), 266 PLCs were diagnosed among cohort members. RR of liver cancer per unit increment of *z*-score adjusted for age, smoking status and BMI and stratified by birth year, sex and sub-cohorts, was for BMI 1.39 (95% confidence interval (CI) 1.24–1.58), mid blood pressure 2.08 (0.95–4.73), blood glucose 2.13 (1.55–2.94) cholesterol 0.62 (0.51–0.76) and serum triglycerides 0.85 (0.65–1.10). The RR per one unit increment of the MetS *z*-score was 1.35 (1.12–1.61). BMI, glucose and a composite MetS score were positively and cholesterol negatively associated with risk of liver cancer.

Primary liver cancer (PLC) is the sixth most common cancer in the world and is characterized by high mortality. It is estimated to cause over half a million deaths per year worldwide.[1] Owing to its high fatality, the incidence and mortality rates are almost equal with the mortality to incidence ratio being almost a unity.[2] Hepatocellular carcinoma (HCC) is by

Key words: cohort study, epidemiology, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, metabolic syndrome
Abbreviations: 40-y: age 40-programme; BMI: body mass index; CONOR: Cohort of Norway; DCO: death certificate only; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; ICC: intrahepatic cholangiocarcinoma; ICD: International Classification of Diseases; MetS: metabolic syndrome; Me-Can: Metabolic syndrome and Cancer project; Mm_ MAST: Malmo modification of the brief Michigan Alcoholism Screening Test; mmol/L: millimol per liter; MPP: Malmö Preventive Project; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NCS: Norwegian Counties Study; Oslo: Oslo study I; PLC: primary liver cancer; RDR: regression dilution ratio; RR: relative risk; SD: standard deviation; VHM&PP: Vorarlberg Health Monitoring and Prevention Programme; VIP: Västerbotten Intervention Project
Additional Supporting Information may be found in the online version of this article.
Grant sponsor: World Cancer Research Fund International; **Grant number:** 2007/09; **Grant sponsor:** Austrian National Bank; **Grant number:** OENB-12737 (to H.U.)
DOI: 10.1002/ijc.26338
History: Received 17 Feb 2011; Accepted 27 Jun 2011; Online 29 Jul 2011
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far the major histological subtype and accounts for up to 85% of PLCs.[3] Intrahepatic cholangiocarcinoma (ICC) is the second most common PLC, which accounts for up to 20% of the PLC.[3] Although both histological subtypes are characterized by high mortality, there are differences in the risk factors for their development.[1–4]

Chronic hepatitis B and C viral infections are major risk factors for HCC contributing to the high incidence of this cancer in Asia and Sub-Saharan Africa. In European populations, however, the prevalences of hepatitis B and C are low, <7% (even <2% in North and central Europe) and <2%, respectively.[3,5,6] Another well-established risk factor is heavy and prolonged alcohol consumption.[3] In contrast to HCC, the etiology and pathogenesis of cholangiocarcinomas remain poorly understood. Most of these cancers develop in an otherwise normal liver and only about 10% of cases are preceded by chronic inflammatory disease processes such as primary sclerosing cholangitis, hepatic fluke infestations and hepatholithiasis.[7]

The incidence and mortality of PLC is increasing in the US and several (but not all) European countries.[6,8–14] Approximately, half of the increase is attributable to hepatitis C virus infection,[9,12] but other major contributors to the rise in the disease burden remain to be determined.

The metabolic syndrome (MetS) is a constellation of factors related to insulin resistance including obesity, impaired glucose tolerance, dyslipidemia and hypertension and has consistently been associated with an increased risk of cardiovascular diseases and also recently to risk of cancer at some sites.[15,16] There are evidences that obesity and type 2-diabetescould be directly associated with risk of PLC indicating that metabolic alterations may play a role.[17–19] However, there is little data on the association between PLC and MetS factors other than obesity and diabetes and the MetS as an entity.

The aim of this prospective study was to investigate the association between metabolic risk factor components, namely body mass index (BMI), blood pressure, glucose, cholesterol and triglycerides (individually and combined) and PLC risk.

Material and Methods

Study population

The Metabolic syndrome and Cancer project (Me-Can) includes cohorts with 578,700 participants from Norway (the Oslo study I cohort—Oslo, the Norwegian Counties Study—NCS, the Cohort of Norway—CONOR and the Age 40-programme—40-y), Austria (the Vorarlberg Health Monitoring and Prevention Programme—VHMPP) and Sweden (the Västerbotten Intervention Project—VIP and the Malmö Preventive Project—MPP). A detailed description of Me-Can, and inclusion criteria for participants in this study, has been previously described.[20] In these cohorts, health examinations were performed in 1972 or later, from which data are available on height, weight, blood pressure, blood levels of glucose, total cholesterol, triglycerides, smoking status and alcohol consumption (available only in the MPP cohort).

The study project was approved by research ethical committees in Norway, Austria and Sweden.

Follow-up and endpoints

In all three countries, incident and fatal cases of PLC (International Classification of Diseases, seventh revision (ICD-7): 155.0) were identified through linkages with national cancer registries and the respective National Cause of Death Register, and in Norway and Sweden, data on emigration were available through linkages to the Registers of the Total Population and Population Changes.

In our data, PLC was defined as histologically confirmed HCC or ICC. Adenocarcinomas were considered ineligible for the study as the vast majority of them are metastatic lesions. PLC events also included death certificate cases only (DCO) that are events where the only source of information about the case was a death certificate. To reduce the possible role of reverse causation, follow-up started 1 year after the baseline examination. Follow-up ended at the date of the first cancer diagnosis, emigration, death or December 31, 2003 (Austria), 2005 (Norway) and 2006 (Sweden).

Statistical analysis

Cox proportional hazards regression models with age as the time variable were fitted to obtain hazard ratios, denoted as relative risks (RRs), for PLC events with 95% confidence intervals (95% CI).

We calculated RR for quintiles of exposure, for exposures transformed to standard scores (z-scores), as well as for predefined categories according to the World Health Organisation (WHO).[21–23]

Quintile cut-off points for the exposure variables were calculated within each sub-cohort and sex. For glucose, cholesterol and triglycerides, cut-offs were additionally specific for fasting time before blood sampling (>8 hr, fasting or ≤8 hr, non-fasting). Consequently, the differences in the measured variables across sub-cohorts, sex and fasting status are accounted for. In the analysis, the models were stratified for seven sub-cohorts, sex and year of birth (five categories; ≤1929, 1930–39, 1940–49, 1950–59 and ≥1960), and adjusted for age, smoking status (three categories; never, former and current smokers) and further adjusted for BMI in a second model. To test for trend across quintiles, we used mean levels within sub-cohorts and fasting time specific quintiles of the exposure variables.

In order to assess the dose-response relationship, further analysis was undertaken with the variables on a continuous scale. To convert the exposures to the same scale, we transformed the original values to standardized variables (z-scores) with 0 as mean and 1 as standard deviation. The z-score was calculated as: $z = (x - \mu)/\sigma$, where μ is the mean, σ is the standard deviation and x is the actual level of the exposure. A composite blood pressure variable was computed as the

Table 1. Baseline characteristics of study participants in the metabolic syndrome and Cancer project (Me-Can)

	Men	Women
Cohort (year of baseline measurement), *n* participants (%)		
Oslo (1972–73)	16 760 (6)	
NCS (1974–83)	25 952 (9)	25 072 (9)
CONOR (1995–2003)	52 181 (18)	57 687 (20)
40-y (1994–99)	60 676 (21)	68 211 (23)
VHM&PP (1988–2002)	73 213 (25)	86 671 (30)
VIP (1985–2005)	38 843 (13)	40 669 (14)
MPP (1974–92)	22 241 (8)	10 524 (4)
Total (1972–2005)	289 866	288 834
Baseline age, years		
Mean (SD)	43.9 (11.1)	44.1 (12.3)
Categories, *n* (%)		
<30	27 244 (9)	33 067 (11)
30 to <45	157 145 (54)	154 462 (54)
45 to <60	76 623 (27)	67 689 (23)
60-	28 854 (10)	33 616 (12)
Fasting time, hours, *n* (%)[1]		
<4	120 510 (41)	122 319 (42)
4–8	30 769 (11)	26 802 (9)
>8	138 587 (48)	139 713 (49)
Smoking status, *n* (%)		
Never smoker	113 496 (39)	144 815 (50)
Ex-smoker	86 086 (30)	72 600 (25)
Current smoker	89 419 (31)	70 721 (25)
Missing	865 (0)	698 (0)
BMI (kg/m^2)		
Mean (SD)	25.7 (3.5)	24.9 (4.4)
Categories, *n* (%)		
<25	131 167 (45)	170 535 (59)
25 to <30	127 846 (44)	82 869 (29)
30-	30 853 (11)	35 430 (12)
Follow-up, years		
Mean (SD)	12.8 (8.6)	11.3 (6.8)
Categories, *n* (%)		
<5	36 755 (13)	35 451 (12)
5 to <15	178 968 (62)	199 151 (69)
15 to <25	24 971 (8)	29 751 (10)
25-	48 172 (17)	24 481 (9)

[1]Proportion of participants with a fasting time >8 h: 5% in the Norwegian cohorts, 90% in the VIP and 100% in the VHM&PP and MPP.Abbreviations: Oslo, Oslo study I; NCS, Norwegian Counties Study; CONOR, Cohort of Norway; 40-y, age 40-programme; VHM&PP, Vorarlberg Heath Monitoring and Prevention Programme; VIP, Västerbotten Intervention Project; MPP, Malmö Preventive Project; SD, standard deviation; BMI, body mass index.

mean of the systolic and diastolic measurements. The variables were standardized separately across sub-cohorts and fasting time as in the quintile classification. As glucose and triglycerides were skewed and had outliers, they were logarithmically transformed prior to standardization. A score for the MetS, constructed by adding the individual *z*-scores, were also standardized to a *z*-score variable with mean = 0 and SD = 1, with the same stratification as above. As in the quintile analyses, the models were stratified for sub-cohorts, sex and year of birth, and adjusted for age at measurement, smoking status and in a further model also for BMI. In one of the Swedish sub-cohorts (MPP) a sub-analysis was done with further adjustment for alcohol consumption. Alcohol consumption was assessed by a scoring system based on a modified version of the Michigan Alcoholism Screening Test, previously referred to as the "Malmö modification of the brief Michigan Alcoholism Screening Test" (Mm-MAST).[24,25] Based on this scoring system, individuals were classified into "low," "intermediate" and "high" risk.

We also estimated risks in groups according to cut-offs as defined by WHO for obesity (BMI $\geq$ 30 kg/m^2) and hypertension (systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg). We assessed the proportion of subjects who had glucose levels defined as impaired glucose tolerance (fasting glucose 6.0–6.9 mmol/l) and diabetes (fasting glucose $\geq$ 7.0 mmol/l), hypertriglyceridemia (fasting triglycerides $\geq$1.7mmol/l) and hypercholesterolemia (fasting total cholesterol $\geq$ 6.2mmol/l) among subjects who had fasted > 8 hr prior to blood draw.[21–23]

All risk estimates were adjusted for random error in exposure measurements and within-person variability by use of methods based on regression dilution ratio (RDR). The calculations are based on data from 133,820 participants who have undergone repeated measurements in Me-Can and for whom two or more observations with the same fasting time before measurements were available, in total 406,364 observations. The mean time between the baseline measurement and repeated measurements was 6.9 years (SD = 3.9). RRs derived from quintile and standardized *z*-score analyses were corrected by dividing the regression coefficient in the Cox model by the estimated RDR of exposure.[26,27] RRs from the *z*-scores analyses which include two or more individual metabolic factors in one model were corrected by regression calibration by which each original *z*-score in the Cox model is replaced with its conditional expected value.[27] This allows for correction of random error in measurement also for covariates. Analyses of RDR and regression calibration were based on linear mixed effect models, as described by Wood *et al.*[27,28]

We did our analyses with both sexes combined as the number of PLC cases are few (n_{men} = 195 and n_{women} = 71). However, we checked for interaction between sex and each of the metabolic risk factors as continuous *z*-scores by including a product term of sex and the standardized factors. For significant statistical interaction, results are presented for men and women separately. For time-to-event analysis, all

Table 2. Risk of primary liver cancer in relation to quintiles of metabolic factors

		Primary liver cancer (n = 266)			
Exposures	Quintile level[1]	Mean (SD)	n, cases	Model 1[2]	Model 2[3]
BMI (kg/m^2)	1	20.7 (1.5)	36	1.00	
	2	23.0 (1.1)	38	0.91 (0.55–1.51)	
	3	24.7 (1.0)	45	0.97 (0.59–1.57)	
	4	26.8 (1.0)	53	1.02 (0.63–1.64)	
	5	31.3 (3.2)	94	1.92 (1.23–2.96)	
	p trend			0.001	
Mid BP (mmHg)	1	88.2 (5.7)	29	1.00	1.00
	2	97.0 (4.1)	40	1.40 (0.59–3.38)	1.33 (0.55–3.16)
	3	102.7 (3.8)	41	1.25 (0.52–3.04)	1.13 (0.47–2.74)
	4	109.8 (4.1)	57	1.51 (0.65–3.48)	1.25 (0.53–2.95)
	5	124.5(10.4)	99	2.80 (1.27–6.17)	2.08 (0.95–4.73)
	p trend			0.006	0.07
Glucose (mmol/l)	1	4.1 (0.5)	47	1.00	1.00
	2	4.7 (0.3)	41	0.60 (0.14–2.51)	0.55 (0.13–2.38)
	3	5.1 (0.3)	52	1.43 (0.38–5.61)	1.30 (0.33–5.05)
	4	5.5 (0.4)	44	0.75 (0.18–3.14)	0.65 (0.16–2.71)
	5	6.7 (1.9)	82	3.88 (1.11–13.5)	2.78 (0.78–9.96)
	P trend			0.02	0.08
Cholesterol (mmol/l)	1	4.2 (0.5)	62	1.00	1.00
	2	5.0 (0.3)	42	0.34 (0.18–0.62)	0.33 (0.18–0.61)
	3	5.6 (0.3)	48	0.32 (0.18–0.58)	0.31 (0.17–0.54)
	4	6.2 (0.3)	55	0.29 (0.17–0.52)	0.27 (0.15–0.47)
	5	7.4 (0.8)	59	0.26 (0.14–0.46)	0.23 (0.14–0.41)
	p trend			<0.001	<0.001
Triglycerides[4] (mmol/l)	1	0.7 (0.2)	41	1.00	1.00
	2	1.0 (0.2)	45	0.84 (0.33–2.06)	0.76 (0.31–1.91)
	3	1.3(0.3)	42	0.55 (0.22–1.40)	0.48 (0.19–1.23)
	4	1.8 (0.4)	69	1.35 (0.55–3.09)	1.02 (0.44–2.43)
	5	3.1 (1.5)	63	0.90 (0.37–2.13)	0.59 (0.24–1.43)
	p trend			0.70	0.51

[1]Quintile levels grouped by cohort and sex and for glucose, cholesterol and triglycerides further by fasting time. [2]RR were estimated from Cox regression models with attained age as time scale adjusted for smoking status and age at baseline, stratified by cohort, categories of birth year and sex. RRs are corrected for regression dilution bias by use of the regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; log(glucose), 0.28; cholesterol, 0.66; log(triglycerides), 0.51. Glucose and triglycerides were logarithmically transformed. [3]RR were further adjusted for quintiles levels of BMI (except in BMI analysis), [4]value missing for six casesAbbreviation: RR, relative risk; SD, standard deviation; BMI, body mass index; Mid BP, mean blood pressure; RDR, regression dilution ratio.

incident and DCO (n = 56) cancers were combined to form a PLC event.

Statistical analyses were performed in Stata (version 10.0, StataCorp LP, College Station, TX) and R (version 2.7.2, used for random error calculation).

Results

Mean age at baseline was 43.9 years (SD = 11.1) in men and 44.1 years (SD = 12.3) in women (Table 1). Men were followed on average for 12.8 years (SD = 8.6) and women for 11.3 years (SD = 6.8). The prevalence of overweight or obesity (BMI 25 kg/m^2 or higher) was 55% in men and 41% in women. The prevalence of blood glucose level $\geq$ 6 mmol/l among fasting individuals was 11.3%. Among participants with a follow-up time longer than one year 266 PLC events were diagnosed. The mean age at diagnosis was 53.0 years (SD = 10.9).

Increasing quintile levels of BMI and lower levels of cholesterol quintiles were significantly associated with increases in risk of PLC event (Table 2). The RR for the highest *versus* lowest quintile in models (model 2) adjusted for age,

Table 3. Relative risk (95% CI) of primary liver cancer, by *z*-scores of metabolic factors, and of the MetS score

	Primary liver cancer (n = 266)		
Exposure	**Model**[1]	**Model**[2]	**Model**[3]
BMI	1.39 (1.24–1.58)		1.08 (1.00–1.42)
Mean blood pressure	1.29 (1.06–1.60)	1.08 (0.86–1.36)	1.00 (0.79–1.26)
Glucose[4]	2.38 (1.76–3.14)	2.13 (1.55–2.94)	2.20 (1.55–3.12)
Cholesterol	0.67 (0.56–0.82)	0.62 (0.51–0.76)	0.65 (0.52–0.81)
Triglycerides[4]	1.09 (0.84–1.43)	0.85 (0.65–1.10)	0.91 (0.67–1.24)
MetS	1.35 (1.12–1.61)		

[1]Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age and smoking status and were corrected for regression dilution bias by use of regression dilution ratio (RDR); conversion into uncorrected RR = exp(log(RR)*RDR). RDR: BMI, 0.90; mean blood pressure, 0.54; log(glucose), 0.28; cholesterol, 0.66; log(triglycerides), 0.51. [2]RRs were further adjusted for BMI except for MetS score analysis. In addition, *z*-scores, derived from original values, were corrected for regression dilution bias by calibration. [3]Relative risks were further adjusted for all the individual z-scores (except in MetS score analysis). In addition, *z*-scores, derived from original values, were calibrated, [4]Glucose and triglycerides were logarithmically transformed.Abbreviations: CI, confidence interval; MetS, metabolic syndrome; BMI, body mass index; RR, relative risk.

Table 4. Relative risk (95% CI) of primary liver cancer, by *z*-scores of metabolic factors and of the MetS score in the Malmö Preventive Project (MPP) with and without adjustment for alcohol consumption

	Primary liver cancer (n = 55)	
Exposure	**Model 1**[1]	**Model 2**[2]
BMI	1.71 (1.34–2.17)	1.70 (1.34–2.16)
Mean blood pressure	1.31 (0.83–2.08)	1.32 (0.84–2.09)
Glucose[3]	1.01 (0.41–2.51)	1.13 (0.46–2.80)
Cholesterol	0.90 (0.59–1.38)	0.87 (0.55–1.34)
Triglycerides[3]	0.96 (0.55–1.66)	0.98 (0.56–1.69)
MetS	1.68 (1.16–2.41)	1.70 (1.18–2.43)

[1]Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age, smoking status and BMI. In addition, *z*-scores, derived from original values, were corrected for regression dilution bias by calibration (except BMI and MetS score analyses). [2]Relative risks were further adjusted for alcohol consumption. [3]Glucose and triglycerides were logarithmically transformed.Abbreviations: CI, confidence interval; MetS, metabolic syndrome; BMI, body mass index; RR, relative risk.

smoking status and BMI; stratified by birth years, sex and sub-cohorts and corrected for RDR, was 1.92 (95% CI 1.23–2.96) for BMI, and 0.23 (0.14–0.41) for cholesterol.

In analyses according to *z*-scores adjusted for age, smoking status and BMI; and stratified by birth years, sex and sub-cohorts and corrected for RDR, significant associations were found between PLC event and a unit increment of BMI (RR = 1.39 95% CI (1.24–1.58), blood glucose 2.13 (1.55–2.94) and cholesterol 0.62 (0.51–0.76). The RR per unit increment of the MetS *z*-score was 1.35 (1.12–1.61). In a further analysis where all the metabolic risk factors were calibrated and adjusted for each other, the association persisted only for BMI and cholesterol (Table 3).

In analyses of the exposures in dichotomized categories according to the WHO, increases in risk were found for obesity (BMI above *versus* below 30 kg/m^2) with an RR = 2.07 (1.51–2.83), for impaired glucose metabolism (fasting glucose above *versus* below 6 mmol/l) 1.79 (1.12–2.87) and for diabetes (fasting serum glucose above *versus* below 7.2 mmol/l) 2.07 (1.51–2.83). On the contrary, hypercholesterolemia was shown to be inversely associated with increased risk of PLC with RR for fasting serum cholesterol level above *versus* below 6.2 mmol/l is 0.61 (0.41–0.85) (Supporting Information Table 1).

We also checked for possible reverse causation in the association between total serum cholesterol and PLC through a lag-time analysis that excluded the first 5 years of follow-up after baseline. In the analysis according to *z*-scores, the result showed that the significant inverse association persists (RR = 0.74 (0.59–0.92)). A similar analysis was done to examine whether PLC precedes glucose intolerance and eventually overt diabetes[18,29] by excluding the first 5 years of follow-up after baseline measurement of blood glucose levels. The results from our data show that the significant association between blood glucose and PLC persists [(2.13, 1.55–2.94) and (1.96, 1.34–2.78) with and without exclusion of the first 5 years, respectively].

Because of the strong association between alcohol consumption and liver cancer,[3,4] and a potential association between alcohol consumption and the metabolic factors investigated in our study,[30] we examined alcohol consumption as a potential confounder on our studied associations. Detailed data on alcohol consumption were available in the MPP cohort, and sub-analyses in this cohort showed that adjustment of alcohol consumption did not influence risk estimates of metabolic factors and PLC (Table 4).

Results of sub analyses of risk according to *z*-scores of morphological subtypes, HCC and ICC, are presented in Figure 1. The results for HCC were largely similar to that of PLC but the RRs for the HCC were stronger. ICC showed borderline significant association with BMI and glucose levels.

There was no significant statistical interaction between sex and *z*-scores of the metabolic risk factors except for blood glucose ($p_{\text{interaction}}$ = 0.02). Separate analyses of risk for men

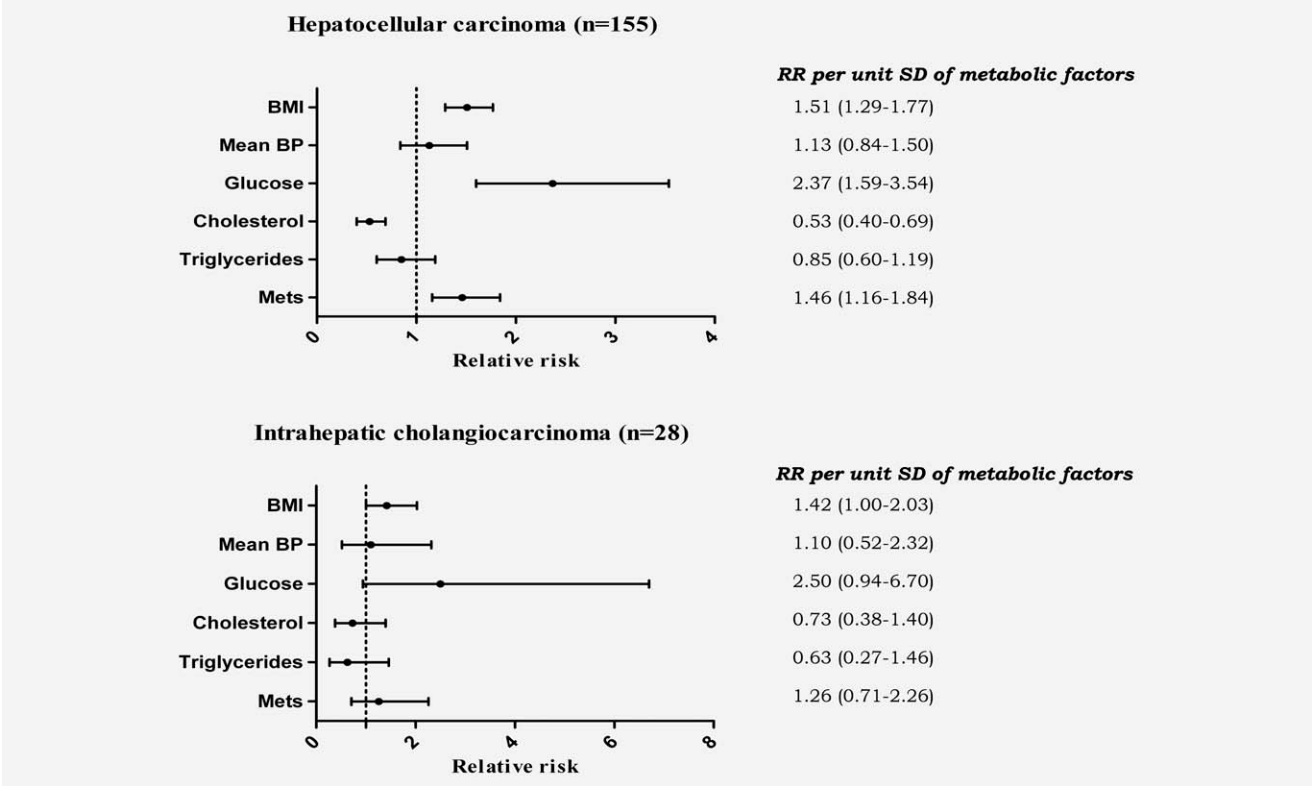

Figure 1. Risk of morphological subtypes of primary liver cancer by *z*-scores of metabolic factors, and of the MetS score. Relative risk were estimated from Cox regression models, with attained age as time scale, stratified by cohort, birth year and sex; adjusted for baseline age, smoking status and BMI. In addition, *z*-scores, derived from original values, were corrected for regression dilution bias by calibration (except BMI and MetS score analyses). Glucose and triglycerides were logarithmically transformed. HCC and ICC do not sum up to 266 as 83 cases of PLC are not classified to their morphological subtypes. Abbreviations: MetS, metabolic syndrome; BMI, body mass index; RR, relative risk; SD, standard deviation; Mean BP, mean blood pressure.

and women showed RRs of 2.67 (1.98–3.60) and 1.04 (0.85–1.29), respectively.

Discussion

In this large pooled European cohort study comprising 578,700 subjects and 266 PLC cases, a MetS score, based on BMI, blood pressure and circulating concentrations of glucose, total cholesterol and triglycerides, was significantly associated with PLC risk. Further analysis of single metabolic risk factors revealed that BMI and glucose were significantly associated with increased risk of PLC.

Our findings for BMI are in accordance with results of most previous prospective reports that have shown the risk of liver cancer to be twice as high among obese subjects as in non-obese controls.[31–34] Furthermore, the current study showed that the association between BMI and PLC could be an independent effect as the RR of PLC remained statistically significant even after adjusting the model for the rest of MetS factors. Considering studies which reported that up to 90% obese individuals have some degree of fatty changes in the liver, the observed association between excess body weight and increased risk of PLC in our study, appears to be supportive of reports that liver cancer in obese individuals may be mediated through the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In addition, NASH predisposes to lipid peroxidation and excess free radical activity with the potential risk of genomic mutations.[35]

The role of high blood pressure in cancer incidence and mortality is unclear. A meta-analysis on the association between hypertension and cancer indicated that hypertension is associated with an increased risk of cancer mortality.[36] The underlying mechanisms however have not been defined, although increase in cell proliferation has been proposed.[37] We found a 2.8-fold higher risk in the top quintile of mean blood pressure compared to the lowest quintile; however, the association became non-significant after adjustment for BMI. These observations do not support an independent role of blood pressure as risk factor for PLC.

Our study suggests that high blood glucose level is associated with PLC in men. Similar results have been reported from several other cohort or case–control studies.[38] Further evidence comes from studies which have shown diabetes to be associated with a spectrum of liver diseases ranging from NAFLD, NASH and cirrhosis.[39,40] According to a recent meta-analysis on the association between HCC and diabetes, the majority of the studies showed a significantly increased risk with a pooled risk ratio of 2.5 (95% CI 1.9–3.2).[38] In several of these studies information on alcohol consumption, smoking or viral hepatitis was available, but adjustment

for these factors resulted in either no or minimal change in the risk estimates.[41–44] Similarly, few studies had accounted for BMI in their analyses, and found that BMI had no effect on the association between diabetes and liver cancer.[42,45] In our study, blood glucose showed a positive association with risk also after adjustment for BMI as well as for the rest of metabolic risk factors as shown in Table 3. We cannot explain why the association between blood glucose and PLC is seen only in men with significant statistical interaction. Several previous epidemiological studies have also reported risk estimates which were somewhat stronger for men than for women.[38,41–43,45] In future, substantially larger studies could determine if a real difference in the association of diabetes/glucose with PLC by sex exists.

Our study revealed a strong significant inverse association between total cholesterol level and PLC risk. A recent study on total serum cholesterol and cancer incidence revealed that the reverse association may be largely due to preclinical effects of cancer on total serum cholesterol; however, the study failed to prove this effect on cancers of the gastrointestinal tract.[46] There are experimental studies which showed that cholesterol is increasingly accumulated in hepatic tumor cells because the cells consume this lipid for their growth[47,48] eventually decreasing the serum level. Lag-time analysis in our study, however, showed that the significant inverse association persisted even after excluding the first 5 years of follow-up after baseline measurement.

We found a significantly increased risk of PLC with the composite MetS score. There was no indication of a synergetic effect between factors in the MetS on risk of PLC. The fact that cholesterol is negatively associated with PLC may underrate the risk estimate. Excluding cholesterol from the composite MetS score variable shows that the risk estimate rises slightly (data not shown).

The RRs of HCC by exposure variables were slightly higher than the risk seen with both subtypes combined. This may indicate that metabolic factors as risk for PLC are more relevant for the occurrence of HCC. On the other hand, the lack of significant associations between ICC and metabolic risk factors is likely due to the small number of ICC cases ($n = 28$) in our study. However, despite the small number of cases, the borderline significant association between blood glucose and ICC is worthy of note. There are no studies, to date, which indicated the possible role of high blood glucose as risk factor for ICC except for a speculative report on diabetes, insulin resistance and ICC.[49]

The main strength of our study is the large data set from seven prospective cohorts with high quality cancer registries with almost complete coverage of cases and data from cause of death registries which gave us high power to detect even quite modest associations as well as associations in lag time analyses. In addition, the large number of repeated measurements allowed us to correct for random error in exposure variables.

Limitations of the study are lack of data on hepatitis virus infection status, which, in some studies, is assumed to be a potential confounder. However, this assumption is controversial and it is unlikely that hepatitis viral infection is related to metabolic risk factors and thus, unlikely to affect the findings of this study.[33,50] Moreover, several case–control and cohort studies showed no or minimal changes in risk after further adjustment for hepatitis infection on the association between obesity or diabetes and liver cancer.[33,37] Another limitation of the study is that there is a slight difference in measurement methods in the sub-cohorts.[20] However, in our analyses, we tried to overcome this problem by using cohort specific cut-points in the analysis of exposures by quintiles and by standardization with *z*-scores. Additionally, we stratified for cohorts in all the analyses. The fact that there is a relatively high DCO rate may also be a limitation; however, we were able to show persistent significant associations after excluding the DCO cases (Fig. 1 and Supporting Information Table 2).

In summary, our study showed that major metabolic risk factors are significantly associated with risk of PLC. Whereas, BMI and glucose are associated with increased risk, cholesterol showed an inverse association. The finding of an inverse association between serum cholesterol and PLC in our study could not be explained merely by a preclinical effect of liver cancer on cholesterol level, indicating a need for further investigation. Beyond the individual factors, the results of our study show, for the first time, that the MetS as an entity presents a significant risk constellation for the occurrence of liver cancer. This suggests that preventive measures that reduce the risk of MetS may also contribute to the long-term reduction in PLC.

Acknowledgements

Authors thank: in Norway, the screening team at the former National Health Screening Service of Norway, now the Norwegian Institute of Public Health, the services of CONOR, the contributing research centres delivering data to CONOR and all study participants; in the VHM&PP, Elmar Stimpfl, data base manager, Karin Parschalk at the cancer registry, and Elmar Bechter and Hans-Peter Bischof, physicians at the Health Department of the Vorarlberg State Government; in the VIP, Åsa Ågren, database manager at the Medical Biobank, Umeå University, Sweden; and in the MPP, Anders Dahlin, data base manager. Authors also thank World Cancer Research Fund International 2007/09 and the Austrian National Bank Grant OENB-12737 (to H.U).

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