Association of Physical Activity and Risk of Hepatobiliary Cancers: A Multinational Cohort Study

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ABSTRACT

Background & Aims: Evidence on the association between physical activity and risk of hepatobiliary cancers is inconclusive. We examined this association in the European Prospective Investigation into Cancer and Nutrition cohort (EPIC).

Methods: We identified 275 hepatocellular carcinoma (HCC) cases, 93 intrahepatic bile duct cancers (IHBC), and 164 non-gallbladder extrahepatic bile duct cancers (NGBC) among 467,336 EPIC participants (median follow-up 14.9 years). We estimated cause-specific hazard ratios (HRs) for total physical activity and vigorous physical activity, performed mediation analysis, and secondary analyses to assess robustness to confounding (e.g., due to hepatitis virus infection).

Results: In the EPIC cohort, the multivariable-adjusted HR of HCC was 0.55 (95% confidence intervals (CI) 0.38-0.80) comparing active and inactive individuals. Regarding vigorous physical activity, for those reporting >2 hours/week compared to those with no vigorous activity, the HR for HCC was 0.50 (0.33-0.76). Estimates were similar in sensitivity analyses for confounding. Total and vigorous physical activity were unrelated to IHBC and NGBC. In mediation analysis, waist circumference explained about 40% and body mass index 30% of the overall association of total physical activity and HCC.

Conclusions: Findings suggest an inverse association between physical activity and risk of HCC, which is potentially mediated by obesity.

Lay summary: In a pan-European study of 467,336 men and women, we found that physical activity is associated with a reduced risk of developing liver cancers over the next decade. This risk was independent of other liver cancer risk factors, and did not vary by age, gender, smoking status, body weight, and alcohol consumption.

Graphical abstract

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Highlights

- Liver cancer rates are increasing in Western countries, possibly due to increases in obesity, diabetes, and physical inactivity.
- Previous evidence was not convincing to support an effect of physical activity on liver cancer.
- We found that physical activity reduced the risk of hepatocellular carcinoma by about 45%.

Abbreviations: BMI, body mass index; CI, confidence interval; DNA; deoxyribonucleic acid; EPIC, European Prospective Investigation into Cancer and Nutrition cohort; EPIC-PAQ, European Prospective Investigation into Cancer and Nutrition cohort physical activity questionnaire; g/d, grams per day; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HR, hazard ratio; IHBC, intrahepatic bile duct cancers; MEDLINE, Medical Literature Analysis and Retrieval System Online; NGBC, non-gallbladder extrahepatic bile duct cancers; RR, relative risk; SD, standard deviation; US, United States of America; WCRF, World Cancer Research Fund International.

Introduction

Liver cancer was the fourth leading cause of cancer death in 2015 [1]. Liver cancer is responsible for around 47,000 deaths per year in the European Union [2]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer derived from hepatocytes and it accounts for 85-90% of all primary liver cancers worldwide. It is the fifth most common cancer in men and the seventh most common cancer in women [1]. The distribution of HCC varies greatly according to geographic location and it is more common in low- and middle-income countries than in developed countries. HCC more frequently occurs in Asia and Africa than in Europe and the US. The strongest risk factor for HCC is cirrhosis, a condition that is related to Hepatitis B virus (HBV), Hepatitis C virus (HCV), excessive consumption of alcohol, and exposure to aflatoxin B1 [1]. The geographic variability of HCC incidence has been widely associated to the different distribution of HBV and HCV infections [1, 3]. In high-income countries, the main risk factors for HCC are smoking, alcoholic cirrhosis, diabetes, obesity, and non-alcoholic hepatic steatosis [1, 4, 5]. The recent increase in HCC incidence is thought to be caused by increases in obesity, diabetes, and physical inactivity [6, 7]. The Physical Activity Collaboration of the National Cancer Institute's Cohort Consortium performed a pooled analysis of 10 prospective US and European cohorts and found that high compared to low leisure-time physical activity was associated with a 27% lower risk of liver cancer incidence [8]. Other prospective studies from the US and East Asian countries support an association of physical activity and lower risk of hepatobiliary cancers [8-13]. However, the World Cancer Research Fund International judged that the evidence was not convincing to support an effect of physical activity on liver cancer [14]. Similarly, an umbrella review provided limited evidence for an association with liver cancer [15]. We report results from the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort to provide additional evidence on the relationship between physical activity and HCC and other hepatobiliary cancers.

Methods

Study Population and Data Collection

The EPIC is a multinational prospective cohort study designed to investigate the link between diet, lifestyle and environmental factors with cancer risk and other chronic diseases. Detailed information on the study design, rationale, and methods of the EPIC cohort has been described previously [16]. Briefly, between 1992 and 2000, >520 thousand men and women, aged 25-70 years, were recruited from 23 centers throughout 10 countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom). Data on physical activity, education, smoking, alcohol consumption, coffee intake, anthropometric measurements and medical history were collected at baseline, before disease onset or diagnosis. All cohort members provided written informed consent. Ethics approval was obtained from the International Agency for Research on Cancer review board (Lyon, France) and participating centers. A total of 467,336 participants were included in the main analyses for total physical activity and hepatobiliary cancer risk after the following exclusions: 25,184 participants with prevalent cancer other than non-melanoma skin cancer; 20 subjects with missing date of diagnosis; and 4,128 individuals without follow-up. Four EPIC study centers (Naples, Umea, South-East of Norway, North-West of Norway) did not measure vigorous physical activity. Thus, the analysis of vigorous physical activity and hepatobiliary cancer risk was limited to 341,533 participants for whom data on this exposure were available.

In a subset [17] of the EPIC cohort as of 2006, sera samples for HBV (ARCHITECT HBsAg, Abbott Diagnostics, France) and HCV (anti-HCV chemiluminescent microparticle immunoassays, Abbott Diagnostics, France) serologic tests were available: 115 HCC cases were matched using incidence density sampling to 230 controls based on age at blood collection, sex, study center, time of the day at blood collection, fasting status at blood collection; among women, additionally by menopausal status, and hormone replacement therapy use at time of blood collection. These data were used in nested case-control analyses to examine potential confounding by viral hepatitis status for the association of physical activity and HCC.

Follow-up of Study Population and Case Ascertainment

Incident first primary hepatobiliary cancer cases and vital status were ascertained through record linkage with cancer and death registries in most centers [16]. In France, Germany and Greece, ascertainment was done using a combination of methods including health insurance records, pathology registries and active follow-up through mailed questionnaires/telephone interviews [16]. Incident cancers were subsequently verified through medical records, pathology reports and discharge diagnosis [16]. In all centers, cancer diagnosis required confirmation through comprehensive pathology review [16]. A detailed protocol entitled 'Guidelines for Collection of End-point Data in the EPIC study' for the collection and standardization of clinical and pathological data for each cancer site was prepared by a special EPIC working group [16]. Cancer incidence was coded according to the International Classification of Diseases-Oncology-2. HCC was defined as C22.0. Cancer of the intrahepatic bile duct (IHBC) was defined as C22.1. Non-gallbladder extrahepatic bile duct tract cancer (NGBC) was defined as tumors in the extrahepatic bile duct (C24.0), Ampulla of Vater (C24.1) or overlapping lesions of the biliary tract (C24.8), and the biliary tract not specified (C24.9). We did not consider cancers of the gallbladder (C23.9) as an endpoint because we assumed different underlying mechanisms [10].

Assessment of Physical Activity

The validated EPIC physical activity questionnaire (EPIC-PAQ) was used to assess recreational, household and occupational physical activity during the past year in all EPIC centers, except in the Norwegian centers [18-20]. Recreational physical activity was assessed by querying about the amount of time in hours per week during the winter and summer spent with cycling and other physical exercises (e.g., jogging, swimming) and was summarized into four groups: inactive, moderately inactive, moderately active, and active [21, 22]. Participants reported their level of occupational physical activity as either sedentary, standing, manual work or heavy manual work. They were also asked whether engaging in household and recreational activities had caused them to experience increases in sweating or heartbeat, and, if so, how many hours per week they dedicated to these vigorous activities. We derived measures of total physical activity and vigorous physical activity from the EPIC-PAQ. The Cambridge Index was used as a measure of

total physical activity by combining recreational physical activity and occupational physical activity [20, 22]. The Cambridge Index was developed [22] and validated [19] by comparing the EPIC-PAQ with objective measures of cardiorespiratory fitness and physical activity energy expenditure. The Spearman correlation between the Cambridge Index and physical activity energy expenditure was 0.33 (95% confidence interval: 0.28 to 0.38) [19]. The Norwegian EPIC centers measured total physical activity using a scale that ranged from 1 to 10 [23]; and the Cambridge Index for the Norwegian centers was derived as described previously [21]. Vigorous physical activity was categorized into 0, ≤2 (below the median), or >2 (above the median) hours per week [21, 24].

Statistical Analysis

Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using cause-specific Cox proportional hazard models, with age as the underlying time metric. Time of study entry was age at recruitment and exit time was age at cancer diagnosis or the last date at which follow-up was considered complete in each center. Models were stratified by center and sex to minimize departure from proportionality and to control for differences between centers, such as follow-up procedures and questionnaire design. Trend tests across exposure groups were performed by modeling the categorical physical activity variables as continuous covariables. We estimated cumulative incidence functions, adjusted for baseline confounders, accounting for competing risk of death from causes other than hepatobiliary cancer using a Fine-Gray subdistribution hazard model. The basic multivariable models were adjusted for education (no school degree, primary school, technical/professional/secondary, university), smoking status and intensity (never, current [1 to 15, 16 to 25, or ≥26 cigarettes/day], or former [≤10 or >10 years]; current pipe, cigar or occasional smoking), current alcohol consumption (grams per day (g/d) modeled continuously using restricted cubic splines), lifetime alcohol use patterns (never, former, >0 – 6 [men]/> 0 – 3 [women], >6-12 [men]/>3-12 [women], >12-24, >24 – 60, > 60 g/d), and daily number of cups of coffee (1 cup was defined as 150 mL). For covariates with missing data (see Table 1), multiple imputation of covariates by fully conditional specification with accommodation of the substantive model [25] and 25 sets of imputed data was used. We examined multiplicative effect modification

by testing interaction terms of physical activity variables with sex, age (continuous), waist circumference (continuous), body mass index (continuous), baseline alcohol consumption (continuous) and lifetime alcohol consumption (categorical) using likelihood ratio tests; for continuous covariates a procedure based on fractional polynomials was used [26].

Because obesity and diabetes may be potential intermediates [4, 27, 28], our primary multivariable model did not control for them. Causal mediation analysis methods, as described for survival data [29], were used to examine the proportions of the association of physical activity with hepatobiliary cancer risk that was mediated by waist circumference, body mass index, and diabetes. These mediators were selected a priori based on subject knowledge [4, 27, 28] and were assessed using multiple linear regression (waist circumference, body mass index) and logistic regression (diabetes) for the mediator models and accelerated failure time models with Weibull distribution for time to event [29, 30]. Proportion mediated was calculated as indirect natural effect divided by the sum of the direct and indirect natural effect [29] and 500 simulations were used to derive quasi-Bayesian CI [30]. To facilitate the interpretation of mediation analyses, the categories ‘active’ vs. ‘inactive’ of the Cambridge Index and ‘>2 hours/week’ vs. ‘no’ vigorous physical activity were compared. The mediation method assumes no unmeasured confounding in the exposure-outcome, mediator-outcome, and exposure-mediator relations, and no effect of the exposure on confounders of the mediator-outcome relation. We did not detect any exposure-mediator interactions.

We conducted several sensitivity analyses to test the robustness of our primary models. First, to minimize the influence of reverse causation, we excluded hepatobiliary cancer events that occurred during the first two years of follow-up. Second, although our primary analysis assumed that obesity and diabetes mediate the association of physical activity and hepatobiliary cancer risk, it is also plausible to hypothesize that overweight/obesity and diabetes render physical activity difficult (i.e., confound the association) [31]. Accordingly, we performed secondary analyses with additional adjustment for waist circumference and diabetes. Third, we assessed the robustness of observed associations to unmeasured confounding. Specially, we calculated E-Values [32], which indicate the minimum strength of association than an unmeasured

confounder would need to have with the exposure and the outcome on the risk ratio scale to fully account for an observed exposure-outcome association, above and beyond the measured covariates. Additionally, we used data from the EPIC nested case-control study [17] to adjust associations for HBV/HCV status. Odds ratios (OR) for HCC were derived from multivariable conditional logistic regression, adjusted for matching variables, age, sex, smoking status, current alcohol use, and coffee intake. Analysis of the nested case-control subset was performed among all subjects with additional adjustment for HBC/HCV; and among HBC/HCV negative individuals. Fourth, as an alternative to the stratified Cox model, we modeled unobserved heterogeneity across centers using a Cox model with a shared frailty. Fifth, due to different assessment of total physical activity in the Norwegian centers, we re-estimated our Cox models for total physical activity after excluding data from the Norwegian centers. Sixth, we performed complete cases analysis when covariates had missing values. P values < 0.05 are reported as statistically significant. Analyses were performed using R (version 3.5.1), SAS (version 9.4), and Stata (version 15.1).

Results

EPIC Study

Characteristics of Participants

Among the 467,336 participants in the EPIC study, the mean (SD) age was 51.3 (9.9) years, and 70.2% were women. During a median follow-up time of 14.9 years, participants contributed 6,508,182 person years, and 275 HCC, 93 IHBC, and 164 NGBC cancer cases occurred. Age-adjusted baseline characteristics of the analytical sample are provided in Table 1.

Physical Activity and Hepatobiliary Cancer Risk

Total physical activity and vigorous physical activity were inversely associated with HCC but not with IHBC and NBGC. The adjusted HR for HCC comparing ‘active’ and ‘inactive’ individuals was 0.55 (95% CI: 0.38 to 0.80, P for Trend < 0.001) (Table 2). The adjusted HR of HCC for ‘>2 hours/week’ of vigorous activity vs. no vigorous activity was 0.50 (95% CI: 0.33 to 0.76, P for

Trend <0.001) for HCC (Table 3). The adjusted cumulative incidence functions indicate that the physically inactive group showed excess HCC incidence compared to more active groups (Figure 1). The relations of total physical activity and vigorous physical activity with outcomes were not modified by sex, age, waist circumference, body mass index, smoking, current alcohol consumption or lifetime alcohol consumption (all P for interaction >0.1).

Mediation of the Association between Physical Activity and HCC Risk

We used mediation analysis to estimate the proportions of the associations with HCC that were mediated by waist circumference, body mass index, and diabetes (Table 4). Waist circumference explained 40% and body mass index 30% of the overall association of total physical activity and HCC. The proportions of the total effect of vigorous physical activity on HCC mediated by waist circumference and body mass index were 17% and 12%, respectively. Diabetes did not seem to mediate the observed associations.

Sensitivity Analyses

In sensitivity analyses, the associations of total physical activity and vigorous physical activity with HCC, IHBC and NBGC were virtually unchanged when events occurring during the first two years of follow-up were excluded (Supplementary Tables 1 and 2). In models additionally adjusted for waist circumference and diabetes, the HR for HCC were attenuated but remained statistically significant. In the Cox model for total physical activity and HCC, for an unmeasured confounder to explain the HR estimate of 0.55, the unmeasured confounder would have to increase the likelihood of physical activity and decrease the likelihood of HCC by 3.0-fold, above and beyond the measured confounders. For an unmeasured confounder to bring up the upper confidence limit of 0.80 for this estimate to above 1.0, the unmeasured confounder would still have to both increase the likelihood of physical activity and decrease the likelihood of HCC by 1.8-fold, conditional on the measured covariates. Similarly, an unobserved confounder would need to be associated with a RR of 3.4 with vigorous physical activity and HCC to explain the estimated HR of 0.50 and a RR of 1.9 to move the upper confidence limit above 1.0, conditional on the measured covariates. We used the EPIC nested case-control study to perform additional

adjustment for HBV/HCV. The results of these analyses were similar in direction and magnitude to those reported for the entire cohort, but they were not statistically significant, due to small sample size (Supplementary Table 3). However, the data from the case-control dataset provide further support for the notion that additional confounding by HBV/HCV might not be sufficient to explain away the observed association of physical activity and HCC. Estimates from frailty models to account for between-center heterogeneity were similar those from the stratified Cox models. After exclusion of Norwegian centers and in complete case analyses, HR were almost identical to the primary analysis. The HR and CI from the complete case analyses were similar to those from primary models employing multiple imputation (Supplement Tables 1 and 2).

Discussion

In this analysis of a multinational European cohort, higher total physical activity and vigorous physical activity were associated with lower risk of HCC. We observed a 45% lower risk of HCC when comparing high and low levels of total physical activity. The highest level of vigorous physical activity was associated with a 50% lower risk for HCC. Moreover, we observed that inverse associations of total physical activity and vigorous physical activity with HCC did not differ substantially between subgroups based on gender, lifestyle, and anthropometric variables. Findings from the sensitivities analyses suggest that the association of physical activity and HCC might be robust to reverse causation and unobserved confounding (e.g., by hepatitis virus infection). Our study also explored the roles of obesity and diabetes in physical activity's association with HCC. Our findings indicate that waist circumference mediated about 40% and BMI about 30% of the overall association of total physical activity and HCC. In contrast, diabetes did not seem to play an important role as a mediating factor.

These findings are in line with a pooled analysis of 10 cohorts with a total of 1,384 cases that reported a 27% lower risk of liver cancer comparing high and low levels of leisure time physical activity [8]. In the NIH-AARP Diet and Health Study, high versus no vigorous physical activity was related to a 44% lower risk of HCC [10]. Similar to our study, no association between physical activity and biliary tract cancer was shown in a previous analysis of NIH-AARP Diet and Health Study [10].

Several biological mechanisms might explain the inverse association between physical activity and hepatobiliary cancer, including systemic and local effects [28, 33]. The interrelated mechanisms most extensively studied are changes in whole-body and visceral fatness, metabolic dysregulation (e.g., insulin, glucose, insulin-like growth factors), adipokines (e.g., leptin, adiponectin), sex hormones (e.g., estrogen, testosterone), chronic low-grade inflammation, oxidative stress causing DNA damage and gene mutations (e.g., tumor suppression genes), impaired immune function, diluting effects on carcinogenic bile acids, and decreased intestinal transit time [33-35]. Evidence from prospective observational studies and randomized controlled trials suggests that the most relevant mechanism by which physical activity positively affects liver cancer risk is lowering body weight [27, 36-38]. The present study systematically explored the role of markers of overall adiposity (BMI), indirect measures of central obesity (waist circumference) and metabolic dysregulation (diabetes) in the overall association between physical activity and HCC. We found that central obesity might account for a large proportion of the direct effect of physical activity on HCC. The mechanisms underlying the association between central obesity and hepatobiliary cancer, particularly HCC, may occur through accumulation of excessive liver fat that increases pro-inflammatory molecules, leptin, and adiponectin [27].

The analysis of this large multinational European cohort provided sufficient events to examine the association of physical activity with hepatobiliary cancers. The cohort study also provided first insights into the relative importance of different intensities of physical activity. We performed sensitivity analyses to address potential selection bias, differences in case ascertainment between centers, and additional unobserved confounding. Although HBV and HCV are considered among the strongest risk factors for HCC [3], previous studies [8-13, 37] were unable to adjust for HBV and HCV. In the EPIC nested case control study the size and direction of the effect size for the association of physical activity and HCC was similar to that of the entire EPIC cohort; however, it was not statistically significant. Our sensitivity analyses for unobserved confounding using E-Values [32] further support the notion that any unmeasured confounding would need to be substantial to explain the inverse association of physical activity and HCC. The study had additional limitations. We were not able to adjust for other potentially important confounding factors (e.g., pleiotropic effects of statins) and to examine the role of intermediate

phenotypes (non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, cirrhosis). Further, compared to the general population, women were overrepresented in our sample, although men have higher risk of HCC [39]. Another limitation is that we were not able to examine in detail the type, intensity and amount of physical activity needed to reduce HCC risk. Physical activity and anthropometric measures were assessed only once at baseline. Repeated measurements of physical activity, anthropometric measures, and other potential biological intermediates over time would have strengthen our understanding of the underlying mechanisms. A recent analysis of the NIH-AARP Diet and Health Study [9] revealed that consistent participation in physical activity throughout the life course might be needed to reduce the risk of liver cancer incidence. We performed mediation analysis for indirect effects acting through general and central obesity, but we were unable to study trajectories of physical activity and body weight that could help to better separate the role of obesity as a confounder and mediator of the association of physical activity and risk of hepatobiliary cancer [8].

In conclusion, our analysis suggests that physical activity reduces risk of HCC. Studies with more detailed and objectively measured physical activity assessed at multiple time points throughout the life course are warranted to confirm our findings and may help establish the optimal dose, type, intensity, and timing of physical activity that is needed to prevent HCC.

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Supplementary material

Supplementary Tables can be found in the online version of the article.

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Table 1 Age-adjusted Baseline Characteristics of the EPIC Cohort by Total Physical Activity (n = 467,336)

		Total physical activity (Cambridge Physical Activity Index)			
	Total N	Inactive	Moderately inactive	Moderately active	Active
Vigorous Physical Activity (%)					
None	182,178	55.9	42.5	30.0	28.5
≤2 hours/week	88,245	18.1	19.9	19.0	18.7
>2 hours/week	71,110	11.2	14.6	17.0	17.1
Missing	125,803	14.8	23.0	34.0	35.7
Sex (%)					
Men	139,168	26.8	27.7	27.5	40.4
Women	328,168	73.2	72.3	72.5	59.6
Education (%)					
No school degree/ unknown	20,859	7.3	3.7	3.6	4.2
Primary school	120,284	35.7	23.1	23.1	25.8
Technical/professional/secondary	198,720	40.0	43.7	43.8	43.6
University	112,121	15.6	26.3	26.8	24.1
Missing	10,658	1.3	2.6	2.6	2.3
Smoking (%)					
Never	20,2567	48.6	43.5	40.9	40.5
Current					
<15 cigarettes/day	53,680	10.2	11.1	12.1	12.9
≥15 cigarettes/day	37,534	9.4	7.7	7.4	7.9
Current pipe, cigar or occasional smoking	40,040	7.3	9.6	9.4	6.8
Former					
<10 years	44,584	8.2	9.4	9.9	10.8
≥10 years	75,403	13.6	16.0	16.9	18.4
Missing	13,528	2.6	2.7	3.4	2.8

Baseline alcohol consumption (g/d)		3.1	5.8	5.4	7.3
Average Lifetime alcohol consumption (g/d)					
Non-drinkers	28,146	8.7	6.4	4.3	4.6
Former	17,026	5.1	3.9	2.7	2.9
>0 – 6 (M)/> 0 – 3 (W)	93,442	25.4	21.3	16.3	17.2
>6-12(M)/>3-12(W)	110,070	24.6	24.3	22.2	22.6
>12-24	63,487	12.0	13.4	14.3	14.2
>24 – 60	41,822	7.2	8.6	10.1	9.8
>60	8,977	1.5	1.8	2.2	2.2
Missing	104,366	15.4	20.2	27.8	26.4
Coffee (ml/d)		179.3	281.1	316.9	409.4
Waist circumference (cm)		87.2	83.3	82.9	84.2
Missing	108,439				
Body mass index (kg/m²)					
Missing	82,692	26.4	25.1	24.8	24.9
Diabetes (%)		5.4	2.6	2.0	1.9
Missing	36,517				

EPIC, European Prospective Investigation into Cancer and Nutrition. Entries are adjusted medians for continuous variables and adjusted percentages for categorical variables. Adjustment for age using median regression (continuous covariates), binary logistic regression (dichotomous covariates), ordinal logistic regression (ordered categorical covariates), multinomial logistic regression (unordered categorical covariates)

Table 2 Association of Total Physical Activity and Hepatocellular Carcinoma (HCC), Intrahepatic Bile Duct Cancers (IHBC) and Non-Gallbladder Biliary Tract Cancer (NGBC) Risk in the EPIC cohort (n = 467,336)

	Total Physical Activity (Cambridge Index)				*P Value for Trend*
	Inactive (Reference)	Moderately inactive	Moderately active	Active	
HCC *(n)*	*91*	*83*	*48*	*53*	
HR (95% CI)	1.00	0.65 (0.48-0.89)	0.49 (0.34-0.71)	0.55 (0.38-0.80)	<0.001
IHBC *(n)*	*26*	*27*	*21*	*19*	
HR (95% CI)	1.00	0.72 (0.41-1.26)	0.66 (0.36-1.21)	0.82 (0.43-1.53)	0.477
NGBC *(n)*	*39*	*46*	*36*	*43*	
HR (95% CI)	1.00	0.67 (0.43-1.05)	0.67 (0.42-1.08)	0.88 (0.55-1.39)	0.761

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). IHBC, intrahepatic bile duct cancers (C22.1). Non-gallbladder extrahepatic bile duct tract cancer (NGBC, C24.0, C24.1, C24.8, C24.9). HR (cause-specific hazard ratio) from center-and sex stratified Cox proportional hazards model, age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, coffee. Missing covariate data was imputed using multiple imputation.

Table 3 Association of Vigorous Physical Activity and Hepatocellular Carcinoma (HCC), Intrahepatic Bile Duct Cancers (IHBC) and Non-Gallbladder Biliary Tract Cancer (NGBC) Risk in the EPIC cohort (n = 341,533)

HR (95% CI)	Vigorous Physical Activity			*P Value for Trend*
	None (Reference)	≤2 hours/week	>2 hours/week	
HCC *(n)*	*122*	*33*	*32*	
HR (95% CI)	1.00	0.50 (0.33-0.75)	0.50 (0.33-0.76)	<0.001
IHBC *(n)*	*46*	*11*	*14*	
HR (95% CI)	1.00	0.52 (0.26-1.06)	0.75 (0.39-1.44)	0.271
NGBC *(n)*	64	26	24	
HR (95% CI)	1.00	0.78 (0.47- 1.30)	0.80 (0.48-1.35)	0.368

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). IHBC, intrahepatic bile duct cancers (C22.1). Non-gallbladder extrahepatic bile duct tract cancer (NGBC, C24.0, C24.1, C24.8, C24.9). HR (cause-specific hazard ratio) from center-and sex stratified Cox proportional hazards model, age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, coffee. Missing covariate data was imputed using multiple imputation.

Table 4 Mediation Analysis for the Association of Total Physical Activity and Vigorous Physical Activity and Hepatocellular Carcinoma (HCC) in the EPIC cohort

Mediator	Total Physical Activity (Cambridge Index) (n = 363,228)		Vigorous Physical Activity (n = 275,433)	
	Proportion Mediated, %	P Value for Indirect Effect	Proportion Mediated, %	P Value for Indirect Effect
Waist Circumference	40.0	0.02	16.7	0.01
Body Mass Index	29.7	0.02	11.9	<0.01
Diabetes	4.2	0.21	0.6	0.23

EPIC, European Prospective Investigation into Cancer and Nutrition. HCC, hepatocellular carcinoma (C22.0). Adjusted for age, sex, education, smoking, baseline alcohol consumption, lifetime alcohol consumption, and coffee intake. Complete-case analysis was used for mediation analysis.

Figure 1 Cumulative Incidence of HCC according to Total Physical Activity and Vigorous Physical Activity

Adjusted cumulative incidence from a Fine-Gray model, with age as time metric, adjusted for education, smoking, baseline alcohol consumption, lifetime alcohol consumption, and coffee.