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Composite Score of Healthy Lifestyle Factors and Risk of Hepatocellular Carcinoma: Findings from a Prospective Cohort Study

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Abstract

Background.—While the associations between individual lifestyle factors and risk of hepatocellular carcinoma (HCC) have been previously described, their combined impact on HCC risk is unknown.

Methods.—The association of a composite score of healthy lifestyle factors, including body mass index, alcohol consumption, cigarette smoking, alternative Mediterranean diet, and sleep duration, and HCC risk was examined in the Singapore Chinese Health Study, an on-going prospective cohort study of 63,257 Chinese. Cox proportional hazard regression method was used to estimate hazard ratio (HR) and its 95% confidence interval (CI). Conditional logistic regression method was used to evaluate this composite lifestyle score-HCC risk association among a subset

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DISCLOSURE

No conflict of interest was declared by any authors.

of individuals who tested negative for hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibody.

Results.—After a mean follow-up of 17.7 years, 561 participants developed HCC. Individuals with higher composite scores representing healthier lifestyles (range 0–8) were at significantly lower risk of HCC. Compared to the lowest composite score category (0–4), the HRs (95% CIs) for the composite scores of 5, 6, 7, and 8 were 0.67 (0.62–0.85), 0.61 (0.48–0.77), 0.49 (0.37–0.65), and 0.13 (0.06–0.30), respectively (P_{trend}<0.0001). A similar inverse association was observed in participants with negative HBsAg and anti-HCV negative serology (HR=0.38, 95% CI: 0.19–0.79; for the highest versus the lowest category of the composite scores (P_{trend}=0.001).

Conclusion.—Healthy lifestyles protects against HCC development, especially for individuals without hepatitis B virus and hepatitis C infections.

Impact.—Our current study highlight the importance of a comprehensive lifestyle modification strategy for HCC primary prevention.

Keywords

Composite score of healthy lifestyle factors; hepatocellular carcinoma risk

INTRODUCTION

Primary liver cancer was ranked the 6th most commonly diagnosed cancer and 4th leading cause of cancer deaths worldwide in 2018[1]. Hepatocellular carcinoma (HCC) accounts for 85–90% of all primary liver cancers[2]. The established major risk factors for HCC are chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), excessive alcohol use, and dietary exposure to aflatoxin[3,4]. Approximately two-third of HCC cases are attributable to hepatitis B and/or C[5]. In the United States, the incidence rate of liver cancer has been increasing by 3–4% per year since the mid-1970's and was three times higher in the 2012–2016 period than in the 1975–1979 period[6]. With the implementation of effective universal hepatitis B vaccination program and curative therapy for hepatitis C, it is expected that non-infectious modifiable risk factors, including obesity, alcohol intake, cigarette smoking, unhealthy diet and/or physical inactivity, will become increasingly important in the development of HCC.

Prior studies have shown that unhealthy lifestyle factors including high intake of alcohol, higher body mass index (BMI), cigarette smoking, a diet with high red/processed meat and low fiber, and physical inactivity are individually associated with higher risk of primary liver cancer or HCC[7–9]. A recent analysis reported that longer duration of sleep was associated with moderately increased risk of primary liver cancer[10]. Longer duration of sleep was also associated with an increased risk of non-alcoholic fatty liver disease (NAFLD), an emerging risk factor for HCC[11,12]. No study has examined the aggregated effect of these modifiable risk factors on the risk of developing HCC. Such data are important to support the development of comprehensive lifestyle modification programs for primary prevention of HCC. Therefore, we evaluated the association between the composite score of healthy lifestyle factors and the risk of HCC incidence in a population-based prospective cohort of more than 60,000 participants with up to 25 years of follow-up.

METHODS

Study Population

Data for the current analysis was obtained from the Singapore Chinese Health Study, which was described in details elsewhere[13]. Briefly, the Singapore Chinese Health Study is an on-going population-based prospective cohort study that recruited 63,257 Chinese men and women, aged 45–74 from two main dialect groups of Chinese in Singapore (i.e., Hokkiens and Cantonese) who resided in the government-built housing estates between April 1993 and December 1998. The Hokkiens and Cantonese were originated from the Fujian and Guangdong provinces in southern China, respectively. All study participants provided written informed consent. The Singapore Chinese Health Study is approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh.

During the 1994–1999 period of recruitment, blood and urine samples were collected from a 3% random sample of the cohort. Between July 1999 and December 2003, all surviving study participants were re-interviewed by phone to update information on alcohol use, tobacco smoking, medical history, current physical activity, and body weight. Subsequently, they were asked to donate a blood (if declined, a mouthwash sample was collected instead) and urine sample for research use. Of all the subjects that we re-contacted successfully, 28,346 subjects (approximately 57% of eligible participants) provided blood samples.

Assessment of Lifestyle Risk Factors

At baseline, study participants were interviewed at home by trained interviewers who used a structured questionnaire to collect information on demographics, body weight and height, lifetime use of tobacco (cigarettes and water-pipe), current physical activity, occupational exposure, medical history, family history of cancer, menstrual and reproductive history (for women only), and dietary habits during the past 12 months. For cigarette smoking, subjects were first asked their smoking status (i.e., never, current, and former smokers). Former and current smokers were further asked for information on: 1) age of starting and age of quitting smoking, 2) number of cigarette per day and 3) number of years of smoking[14]. For physical activity, the questionnaire listed the following categories: never, 0.5–1, 2–3, 4–6, 7–10, 11–20, 21–30, and 31 hours or more per week, for each of 3 physical activity categories: 1) strenuous sports (i.e., jogging, bicycling on hills, tennis, squash, swimming laps, or aerobics); 2) vigorous work (i.e., moving heavy furniture, loading or unloading trucks, shoveling, or equivalent manual labor), and 3) moderate activities (i.e., brisk walking, bowling, bicycling on level ground, tai chi, and chi kung)[15]. Sleep duration was evaluated using the following question: *"On average, during the last year, how many hours in a day did you sleep?"* with a response choice of 6 pre-defined categories: ≤5, 6, 7, 8, 9 and ≥10 hours/day.

Dietary intake was assessed using semi-quantitative food frequency questionnaire (FFQ), which had been developed for and validated in the study population against a series of 24-hour dietary recall interviews[16] and selected biomarkers studies[17,18]. The dietary questionnaire comprised 165 commonly consumed food and beverage items by Chinese

Singaporeans. Study participants were asked how frequently they consumed the food or food groups in 8 pre-defined categories, from "*never or hardly ever*" to "*two or more times a day*", followed by predetermined portion sizes assisted with the illustration of food photos in an album. For alcohol consumption, each study participant was asked about his/her drinking frequency during the past year of four types of alcoholic beverages, beer, wine, Western hard liquor, and Chinese hard liquor, with a response choice of 8 pre-defined categories for each type of beverage: never or hardly, once a month, 2–3 times a month, once a week, 2–3 times a week, 4–6 times a week, once a day, and ≥2 times a day. The portion size for each type of alcoholic beverage was defined as follows: 1) Beer: ≤1 small bottle (375 mL), 2 small bottles or 1 large bottle (750 mL), and ≥3 large bottles; 2) Wine: ≤1 glass (118 mL), 2, 3 and ≥4 glasses; 3) Western or Chinese hard liquor: ≤1 shot (30 mL), 2, 3, and ≥4 shots. Between April 1994 and March 1997, the FFQ was validated against two 24-hour dietary recalls (24-HDR), one on a weekday and the other on a weekend that was approximately two month apart, among a random sample of 810 participants of the SCHS. The correlation coefficients between the FFQ and 24-HDR for the majority of calorie-adjusted nutrients ranged from 0.24 to 0.79[16].

Classification of the Composite Score of Healthy Lifestyle Factors

The classification of the composite score of healthy lifestyle factors was based on their independent association with liver cancer or HCC[7–9,19,20]. The lifestyle factors are alcohol consumption, cigarette smoking, dietary intake, and sleep duration, in addition to BMI. Alcohol consumption was categorized by the number of alcoholic drinks: one drink was defined as 375 mL of beer (13.6 grams of ethanol), 118 mL of wine (11.7 grams of ethanol), and 30 mL of Western or Chinese hard liquor (10.9 grams of ethanol)[21]. BMI was calculated as the current weight in kilograms divided by height in meters squared (kg/m^2).

Alternative Mediterranean diet (aMED) score.—The previous studies have found an inverse association between dietary habits of Mediterranean populations and the risk of chronic diseases[22,23]. It originally included 9 items[24]. In our analysis, we included eight components: 1) vegetables, 2) fruit and nuts, 3) cereals, 4) dairy, 5) legumes, 6) fish, 7) the ratio of monounsaturated fatty acid (MUFA) over saturated fatty acids (SFA), and 8) meat and meat products after excluding alcohol (an independent risk factor[9]). Each component was assigned a score of 0 or 1 according to the consumption level (below or above the study population specific median) and healthy/unhealthy status of the component. The summed score of aMED ranged from 0 to 8.

For BMI, we used the cut-off of 23.0 kg/m^2, following both World Health Organization and the Asia-Pacific recommendations[25,26]. Individuals with BMI<23.0 kg/m^2 were assigned a lifestyle score of one, otherwise a score of zero. For alcohol consumption, zero was assigned to heavy drinker, defined as men who drank ≥15 drinks/week and women who drank ≥8 drinks/week, and one otherwise (i.e., to both non-drinker and non-heavy drinker group). For cigarette smoking, zero was assigned to heavy smokers (defined as those who started smoking before 15 years of age and smoked 13 or more cigarettes per day), one to the remaining light smokers and two to never-smokers[27]. For aMED, we divided all study participants into 3 groups by their aMED scores: zero was assigned to the lowest quartile of

aMED score, one to quartiles 2 and 3, and two to quartile 4. For sleep duration, one was assigned to those with 6–8 hours of sleep per day and zero otherwise. The sum of the five individual scores constituted the overall combined lifestyle index score, ranging from 0 to 8, of which higher scores represented healthier lifestyle.

Ascertainment of Hepatocellular Carcinoma Cases

Incident HCC cases and deaths were identified by annual linkage analysis of all surviving cohort participants with the national databases of the Singapore Cancer Registry and the Singapore Birth and Death Registry, respectively. The International Classification of Diseases-Oncology 2nd Edition Codes C22.0 were used to determine HCC cases[28]. To date, only 56 (<0.1%) of the entire cohort participants are known to be lost to follow-up due to migration out of Singapore. Thus, the ascertainment of incident cancer cases and deaths among the cohort participants was virtually complete. As of December 2015, with an average 17.7 years of follow-up, 561 study participants developed HCC among 61,321 participants who were free of cancer at enrollment after excluding 1,936 participants with a history of cancer at baseline.

Case-Control Study of HCC

We also constructed a case-control study of HCC within the Singapore Chinese Health Study. All 197 incident HCC cases diagnosed before 12/31/2015 who donated a baseline blood sample were eligible for the present study. For each case, we randomly selected 2–3 control subjects who donated a baseline blood sample and were alive and free of cancer at the date of diagnosis of the index case. Controls were individually matched to the index case by age at enrollment (±2 years old), gender, dialect groups (i.e., Hokkien, Cantonese), and date of baseline blood collection (± 6 months).

Serum samples of all subjects included in the case-control study of HCC were tested for serological markers of HBV and HCV exposures using standard assays as described previously[9,20,29]. The case/control status of the test samples was blinded to laboratory personnel. Briefly, the presence of HBsAg was determined on the first 302 samples (80 cases vs. 222 controls) using a commercially available test kit (AUSRIA, Abbott, Laboratories, North Chicago, IL, USA). Negative samples were further tested for the presence of antibodies to hepatitis core antigen (anti-HBc) and antibodies to hepatitis surface antigen (anti-HBs) using standardized test kit (Corab and Ausab, Abbott Laboratories, North Chicago, IL, USA). For the remaining 360 serum samples (117 cases vs. 243 controls), only the HBsAg status was determined using the same assay. We also tested for the presence of antibodies to the hepatitis C virus (anti-HCV) on the first 302 samples (80 cases and 222 controls) using ELISA (version 2.0) test kit (Ortho Diagnostic System) and confirmation of positive samples were performed using RIBA (version 2.0) test kit (Chiron, Emeryville, CA). Given the relatively low prevalence of anti-HCV in our study population (1.4%), we stopped testing anti-HCV for the remaining HCC cases and controls.

Statistical Analysis

Means and standard deviation (SD) were calculated for continuous variables while counts and proportions were computed for categorical variables. The *t*-test and χ^2 test were used to

compare the distributions of continuous and categorical variables, respectively, between cases and non-cases. Analysis of variance (ANOVA) was used for the comparisons across different groups of the composite score of healthy lifestyle factors. Person-years at risk for each participant was calculated from the date of interview at entry into the cohort to the date of HCC diagnosis, death, migration out of Singapore, or December 31, 2015, whichever occurred first.

The Cox proportional hazard regression method was used to evaluate the association between the individual and composite score of healthy lifestyle factors and the risk of developing HCC. Hazard ratios (HRs) and their corresponding 95% CIs of HCC associated with higher individual and composite score of healthy lifestyle factors were derived from the Cox proportional hazard regression models. Proportional hazard assumption was tested using Schoenfeld residuals test, and no violation was found. An ordinal variable was used for the linear trend test for the index score of combined lifestyle factors and HCC risk. Potential confounders included in the multivariable Cox proportional hazards models were age, gender, dialect group (i.e., Hokkien or Cantonese), level of education (i.e., no formal education, primary school, secondary or higher education), year of enrollment (i.e., 1993–1995 and 1996–1998), history of type 2 diabetes (yes versus no) and total energy intake (Kcal/d). We also performed stratified analysis by gender and sensitivity analysis by excluding HCC cases and person-years observed within the first 2 years after the baseline interview. Additional sensitivity analyses were also performed for composite score of healthy lifestyle factors without sleep and aMED variables.

We used conditional logistic regression method in analysis for the entire dataset of the nested case-control study to evaluate the association between composite score of healthy lifestyle factors and the risk of HCC with additional adjustment for HBsAg and anti-HCV serological statuses. For those with missing anti-HCV (117 cases and 243 controls), a separate indicator variable was created and included in the logistic models. In the stratified analysis by HBsAg and anti-HCV statuses, unconditional logistic regression method was used with the inclusion of all matching factors (i.e., age, sex, dialect group, and year of enrollment) since the matched pairs were broken.

All statistical analyses were conducted using the computing software SAS version 9.4 (SAS Institute Inc., Cary, NC). All *P* values were two-sided and 0.05 was used as a threshold of statistical significance.

RESULTS

The mean (±SD) age at the cancer diagnosis of the 561 HCC cases included in this study was 70.8 (±8.4) years. The median time interval from the date of baseline interview to the diagnosis of HCC was 11.4 years (range 0.03–22.2 years).

We observed lower proportions of secondary school or higher education and aMED score in quartile 4 among cases than non-cases. However, there was no significant difference in sleep duration between HCC cases and non-cases. Compared to those in the lowest composite score, those in the highest score were on average approximately 3 years younger at

recruitment, and more likely to be women, to attain higher level of education or to engage physical exercise, but less likely to be Hokken ethnic group and to have a history of diabetes (all $P<0.0001$) (Table 1).Amongst HCC cases, there were higher proportions of men, Hokkien, BMI≥23.0 Kg/m^2, cigarette smokers, alcohol drinkers with ≥7 drinks/week, aMED score in quartile 1, or those with a history of type 2 diabetes compared with non-HCC individuals (Supplementary Table 1).

Significantly lower HR of HCC was associated with BMI<23.0 kg/m^2, never smoking, non-heavy drinking, or highest 25 percentile of aMED score. We combined light drinkers (≤14 drinks/week for men and ≤7 drinks/week for women) with non-drinkers due to their similar risk for HCC. Compared with heavy drinkers, HRs (95% CIs) for HCC in light drinkers and non-drinkers were 0.37 (0.24–0.57) and 0.42 (0.28–0.63), respectively. A daily sleep of 6–8 hours was associated with a statistically nonsignificant 10% decrease in risk of HCC 10% (Table 2).

Higher composite score of the five healthy lifestyle factors was significantly associated with lower risk of HCC in a dose-dependent manner ($P_{trend}<0.0001$). Compared with the score of 0–4, the HRs (95% CIs) of HCC for those with the scores of 5, 6, 7, and 8 were 0.67 (0.52–0.85), 0.61 (0.48–0.77), 0.49 (0.37–0.65), and 0.13 (0.06–0.30), respectively, after adjustment for age, gender, history of diabetes, daily energy intake, and other potential confounders. The increment per composite score was associated with a statistically significant 22% reduction in risk of HCC. This inverse association was present consistently and comparable in both men and women (Table 3).

We determined HBsAg status on 197 HCC cases and 465 matched controls. In addition, 80 HCC cases and 222 controls also were tested for anti-HCV status. Among tested HCC cases, 41.3% were positive for HBsAg and 5.0% positive for anti-HCV. The corresponding figures in controls were 3.2% and 1.4%.

In the case-control analysis with additional adjustment for chronic infection with HBV and/or HCV, we found a similar inverse association between the composite score of healthy lifestyle factors and HCC risk overall (Table 4). Among HBsAg-negative and anti-HCV negative/unknown subjects, HR of HCC decreased by more than 60% for those with the highest composite score compared with the lowest score category of healthy lifestyle factors (OR=0.38, 95% CI: 0.19–0.79, $P_{trend}=0.001$). Given the small sample size for HBsAg or anti-HCV positive individuals, the risk estimates for HCC associated with composite score of healthy lifestyle varied greatly and were not informative (Table 4).

We conducted several sensitivity analyses. First, we excluded HCC cases and person-years within the first 2 years of observation and found that the results were similar as those based on the entire cohort. Compared with the composite score of 0–4, the multivariable-adjusted HRs (95% CIs) of HCC for the composite scores of 5, 6, 7, and 8 were 0.66 (0.51–0.85), 0.62 (0.48–0.79), 0.50 (0.37–0.62), and 0.14 (0.06–0.32), respectively ($P_{trend}< 0.0001$) (Supplementary Table 2). Then, we excluded sleep duration from the composite score because it alone was not statistically significantly associated with HCC risk (Table 2). The association between the composite score without sleep duration and HCC risk weakened, but

remained statistically significant; HRs (95% CIs) for new scores 5, 6, and 7 were 0.65 (0.53–0.80), 0.57 (0.45–0.71) and 0.28 (0.17–0.43), respectively, compared with new score 0–4 (P_{trend} < 0.0001). We also conducted similar analysis by removing aMED from the composite score and yielded similar inverse, but weakened association; HRs (95% CIs) for scores 5 and 6 were 0.62 (0.51–0.76) and 0.46 (0.36–0.58), respectively, compared with score 0–4 (P_{trend} <0.0001).

Stratified analyses were performed to assess the heterogeneity in the composite score-HCC risk association by selected factors including diabetes status and BMI. The association between the composite score and HCC was more apparent in participants without history of diabetes (P_{trend}<0.0001) than in those with history of diabetes (P_{trend}=0.0005); however the difference between the two was not statistically significant ($P_{heterogeneity}$=0.278). The association was more apparent in participants with BMI<23.0 kg/m^2 (P_{trend}<0.0001) than in those with BMI≥23.0 kg/m^2 (P_{trend}=0.06), with a statistical significance ($P_{heterogeneity}$=0.004) in heterogeneity between the two subgroups (Supplementary Table 3).

DISCUSSION

In the present analysis in a population-based prospective cohort study of more than 60,000 individuals with an average follow-up of 17.7 years, we found that higher composite score of healthy lifestyle factors including BMI, smoking, alcohol consumption, Mediterranean-style diet and sleep duration was associated with decreased risk of HCC in a dose-dependent manner. This inverse relationship was robust and present in both men and women and in those with at least two or more years of follow-up. The observed inverse association was confirmed among individuals with negative HBsAg and negative anti-HCV, suggesting that the effect of these lifestyle factors on HCC risk was independent of chronic infection with HBV and HCV, major risk factors for HCC.

The current study showed lower risk of HCC for individuals who adhered to a healthier lifestyle including adhering heathier diet based on Mediterranean dietary habits (i.e., eating more vegetables, fruit and nuts/legumes, and fish and fatty foods), maintaining a normal range of BMI, moderate or no alcohol consumption, never smoking cigarettes, and regular sleep duration of 6–8 hours a day. Compared with the lowest score, individuals with the highest composite score of healthy lifestyle factors had reduced by 85% in the risk of developing HCC overall, and by 90% in those without chronic infection with HBV.

Previously, we reported an inverse association between a similarly constructed composite score of healthy lifestyle factors and risk of colon and gastric cancers[30,31]. Accordingly, Odegaard et al.[30] found that this same composite score was significantly associated with lower risk of colon cancer, but not with risk of rectal cancer. A similar inverse association was observed for gastric cancer in our study population[31].

Several prior studies provide supportive evidence for an inverse association between the individual healthy lifestyle factors and HCC risk. A meta-analysis including 26 prospective cohort studies with a total of 9 million study participants, including 16 Asian studies, four US studies and six European studies, showed that both overweight and obesity were

associated with higher risk of primary liver cancer[32]. Recently, an analysis on registry data of more than 1.2 million Swedish men enlisted for conscription between 1969 and 1996 reported that overweight and obese men were at higher risk of severe liver disease (i.e., end-stage liver disease, HCC, or death from any liver disease) or gallbladder or bile-duct carcinoma[33]. Obesity is an underlying cause for the development and progression of NAFLD, which includes nonalcoholic steatohepatitis (NASH) and cirrhosis, a pre-condition for HCC[4].

Alcohol abuse is an important risk factor for HCC. A recent meta-analysis of 19 prospective cohort studies from Asia (15 cohorts), Europe (2 cohorts) and the U.S. (2 cohorts) showed that the pooled relative risks were 0.91 (95% CI: 0.81–1.02) for moderate drinking (1–2 drinks/day) and 1.16 (95% CI: 1.01–1.34) for heavy drinking (≥3 drinks/day) compared with non-drinking[34]. Excessive alcohol consumption, potentially via carcinogenicity of acetaldehyde, the first metabolite of alcohol[35], is associated with alcoholic cirrhosis, which increases risk of HCC[36]. However, the association between moderate drinking and HCC risk is inconsistent. Our study showed that non- or moderate-drinkers (≤14 drinks per week for male or ≤7 drinks per week for female) had a statistically significant 59% lower risk of HCC than heavy drinkers. Among non-Asians in Los Angeles, we previously reported that there was no increased risk of HCC for consumption of 1–4 drinks per day[37]. There may be different alcohol threshold for risk of HCC among different race/ethnic groups, especially at the lower end of the alcohol consumption level with the risk of HCC in Asian compared with non-Asian populations. More studies are warranted to clarify this observation.

Cigarette smoking has also been reported as an independent risk factor for HCC. In a meta-analysis of 38 cohort studies and 58 case-control studies, Lee at al.[38] reported that the adjusted pooled relative risk estimates were 1.51 (95% CI: 1.37–1.67) for current smokers and 1.12 (95% CI: 0.78–1.60) for former smokers compared with never smokers. Our results were consistent with these previous studies[38]. Tobacco smoke contains a myriad of chemical compounds that are carcinogenic or toxic to the liver[39]. For example, N-Nitrosodimethylamine, which is abundant in cigarette smoke, can induce liver tumor in rodents[40].

aMED was found to be associated with lower risk of HCC across different populations. In the U.S., four prospective cohort studies[41–43], all reported an inverse association between aMED and risk of HCC, with a 25–39% lower risk of HCC with highest adherence to aMED. These results were consistent with our findings (i.e., 22% risk reduction of HCC for the highest quartile compared with the lowest quartile), as shown in Table 2.

Recent studies have demonstrated an association between sleep duration and the risk of liver cancer[10] or NAFLD[11,12]. Mechanistically, the circadian clock is believed to play a role in mitochondrial dysfunction[44] and in the regulation of hepatic triglyceride accumulation, oxidative stress, and inflammation[45]; all of which may contribute to the development and progression of NAFLD. In wild-type mice, chronic circadian misalignment led to the disruption of circadian clock and induced spontaneous NAFLD-related HCC independent of dietary exposure, exogenous genotoxic stress and/or germline gene mutation[46].

Strengths of the current study include: prospective study design, a comprehensive questionnaire collecting multiple lifestyle factors and detailed dietary information before the HCC occurrence, large sample size allowing for robust estimates of HCC risk, use of a composite score of healthy lifestyle factors that comprehensively characterized an individual person's profile, long-term and nearly complete follow-up that reduced potential bias due to loss of follow-up or the reverse impact of disease on the exposure-HCC risk association, and known HBsAg and anti-HCV serology on a sizable subset of HCC cases and the controls presenting the entire cohort. This robust study design allowed us to examine these lifestyle factors on HCC risk among HBsAg-negative and anti-HCV positive individuals only, which ruled out the potential confounding effect of both HBV and HCV infections on the observed association.

One potential limitation of our study was the misclassification of lifestyle factors since their assessment at baseline might not reflect the level of long-term exposure due to the change of lifestyle after baseline assessment. As in any observational studies, such misclassification is usually non-differential to both HCC cases and non-HCC participants. Non-differential misclassification usually results in the underestimation of hazard ratios toward null. Thus, the significant association between composite score of healthy lifestyle factors and HCC risk observed in the present study may be underestimated, but the conclusion is valid.

In summary, the current study shows a strong, dose-dependent association between the composite score of healthy lifestyle factors and lower risk of HCC development. The overall results, especially among HBsAg-negative and anti-HCV negative individuals, support the development of a prevention strategy including multiple lifestyle factors that would result in significant reduction in HCC incidence in general populations. This finding highlights the importance in promoting healthy living for primary prevention of HCC, even in a population with a relatively high prevalence of HBV infection and high background risk of HCC. Our findings are pertinent to the populations in the U.S. and other populations where non-viral factors are projected to have increasingly greater impact on HCC incidence and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424. [PubMed: 30207593]

2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–76. [PubMed: 17570226]
3. Yu MC, Yuan J-M. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127:S72–78. [PubMed: 15508106]
4. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019;
5. American Cancer Society. Cancer Facts & Figures 2020 Atlanta, GA: American Cancer Society, 2020.
6. Noone A, Howlader N, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis D, Chen H, Feuer E, et al. SEER Cancer Statistics Review, 1975–2015, National Cancer Institute Bethesda, MD, https://seer.cancer.gov/csr/1975_2015/, based on November 2017 SEER data submission, posted to the SEER web site, April 2018. 2018;
7. Petrick JL, McGlynn KA. The changing epidemiology of primary liver cancer. Curr Epidemiol Rep 2019;6:104–11. [PubMed: 31259140]
8. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol 2018;68:526–49. [PubMed: 28989095]
9. Koh W-P, Robien K, Wang R, Govindarajan S, Yuan J-M, Yu MC. Smoking as an independent risk factor for hepatocellular carcinoma: the Singapore Chinese Health Study. Br J Cancer 2011;105:1430–5. [PubMed: 21915129]
10. Royse KE, El-Serag HB, Chen L, White DL, Hale L, Sangi-Haghpeykar H, Jiao L. Sleep Duration and Risk of Liver Cancer in Postmenopausal Women: The Women's Health Initiative Study. J Womens Health 2002 2017;26:1270–7.
11. Kim D, Kim HJ, Kushida CA, Heo N-Y, Ahmed A, Kim WR. Short Sleep Duration Is Associated With Abnormal Serum Aminotransferase Activities and Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol 2018;16:588–90. [PubMed: 28882688]
12. Kim C-W, Yun KE, Jung H-S, Chang Y, Choi E-S, Kwon M-J, Lee E-H, Woo EJ, Kim NH, Shin H, Ryu S. Sleep duration and quality in relation to non-alcoholic fatty liver disease in middle-aged workers and their spouses. J Hepatol 2013;59:351–7. [PubMed: 23578884]
13. Yuan J-M, Stram DO, Arakawa K, Lee H-P, Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomark Prev 2003;12:890–8.
14. Koh W-P, Yuan J-M, Sun C-L, Lee H-P, Yu MC. Middle-aged and older Chinese men and women in Singapore who smoke have less healthy diets and lifestyles than nonsmokers. J Nutr 2005;135:2473–7. [PubMed: 16177215]
15. Eaglehouse YL, Koh W-P, Wang R, Aizhen J, Yuan J-M, Butler LM. Physical activity, sedentary time, and risk of colorectal cancer: the Singapore Chinese Health Study. Eur J Cancer Prev 2017;26:469–75. [PubMed: 28542077]
16. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, Yu MC. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer 2001;39:187–95. [PubMed: 11759279]
17. Seow A, Shi CY, Franke AA, Hankin JH, Lee HP, Yu MC. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. Cancer Epidemiol Biomark Prev 1998;7:135–40.
18. Seow A, Shi CY, Chung FL, Jiao D, Hankin JH, Lee HP, Coetzee GA, Yu MC. Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. Cancer Epidemiol Biomark Prev 1998;7:775–81.
19. Koh W-P, Dan YY, Goh GB-B, Jin A, Wang R, Yuan J-M. Dietary fatty acids and risk of hepatocellular carcinoma in the Singapore Chinese health study. Liver Int 2016;36:893–901. [PubMed: 26443688]
20. Johnson S, Koh W-P, Wang R, Govindarajan S, Yu MC, Yuan J-M. Coffee consumption and reduced risk of hepatocellular carcinoma: findings from the Singapore Chinese Health Study. Cancer Causes Control CCC 2011;22:503–10. [PubMed: 21258859]

21. US Department of Agriculture (USDA). Agriculture Handbook. US Department of Agriculture (USDA),
22. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L, Trichopoulos D. Diet and overall survival in elderly people. BMJ 1995;311:1457–60. [PubMed: 8520331]
23. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599–608. [PubMed: 12826634]
24. Fung TT, Hu FB, McCullough ML, Newby PK, Willett WC, Holmes MD. Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. J Nutr 2006;136:466–72. [PubMed: 16424129]
25. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157–63. [PubMed: 14726171]
26. Pan W-H, Yeh W-T. How to define obesity? Evidence-based multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. Asia Pac J Clin Nutr 2008;17:370–4. [PubMed: 18818155]
27. Tsong WH, Koh W-P, Yuan J-M, Wang R, Sun C-L, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. Br J Cancer 2007;96:821–7. [PubMed: 17311023]
28. Parkin DM, Whelan S, Ferlay J, Teppo L, Thomas D, eds. Cancer incidence in five continents. Vol. 8: Cancer incidence in five continents ; Vol. 8. Lyon: IARC Press, 2002 781p
29. Nieters A, Yuan J-M, Sun C-L, Zhang Z-Q, Stoehlmacher J, Govindarajan S, Yu MC. Effect of cytokine genotypes on the hepatitis B virus-hepatocellular carcinoma association. Cancer 2005;103:740–8. [PubMed: 15643599]
30. Odegaard AO, Koh W-P, Yuan J-M. Combined lifestyle factors and risk of incident colorectal cancer in a Chinese population. Cancer Prev Res Phila Pa 2013;6:360–7.
31. Wang Z, Koh W-P, Jin A, Wang R, Yuan J-M. Composite protective lifestyle factors and risk of developing gastric adenocarcinoma: the Singapore Chinese Health Study. Br J Cancer 2017;116:679–87. [PubMed: 28125822]
32. Chen Y, Wang X, Wang J, Yan Z, Luo J. Excess body weight and the risk of primary liver cancer: an updated meta-analysis of prospective studies. Eur J Cancer Oxf Engl 1990 2012;48:2137–45.
33. Hagström H, Tynelius P, Rasmussen F. High BMI in late adolescence predicts future severe liver disease and hepatocellular carcinoma: a national, population-based cohort study in 1.2 million men. Gut 2018;67:1536–42. [PubMed: 28320770]
34. Turati F, Galeone C, Rota M, Pelucchi C, Negri E, Bagnardi V, Corrao G, Boffetta P, La Vecchia C. Alcohol and liver cancer: a systematic review and meta-analysis of prospective studies. Ann Oncol 2014;25:1526–35. [PubMed: 24631946]
35. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. Vol. 96 Alcohol Consumption and Ethyl Carbamate. Lyon, France: International Agency for Research on Cancer, WHO, 2010.
36. La Vecchia C, Negri E, Cavalieri d'Oro L, Franceschi S. Liver cirrhosis and the risk of primary liver cancer. Eur J Cancer Prev 1998;7:315–20. [PubMed: 9806120]
37. Yuan J-M, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer 2004;101:1009–17. [PubMed: 15329910]
38. Lee Y-CA, Cohet C, Yang Y-C, Stayner L, Hashibe M, Straif K. Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. Int J Epidemiol 2009;38:1497–511. [PubMed: 19720726]
39. International Agency for Research on Cancer. Tobacco smoke and involuntary smoking. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans Lyon, France: International Agency for Research on Cancer, 2004 36–1187p
40. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. Some N-notroso compounds Lyon, France: International Agency for Research on Cancer, 1978.

41. Ma Y, Yang W, Simon TG, Smith-Warner SA, Fung TT, Sui J, Chong D, VoPham T, Meyerhardt JA, Wen D, Giovannucci EL, Chan AT, et al. Dietary Patterns and Risk of Hepatocellular Carcinoma Among U.S. Men and Women. Hepatol Baltim Md 2018;

42. Li W-Q, Park Y, McGlynn KA, Hollenbeck AR, Taylor PR, Goldstein AM, Freedman ND. Index-based dietary patterns and risk of incident hepatocellular carcinoma and mortality from chronic liver disease in a prospective study. Hepatol Baltim Md 2014;60:588–97.

43. Bogumil D, Park S-Y, Le Marchand L, Haiman CA, Wilkens LR, Boushey CJ, Setiawan VW. High-Quality Diets Are Associated With Reduced Risk of Hepatocellular Carcinoma and Chronic Liver Disease: The Multiethnic Cohort. Hepatol Commun 2019;3:437–47. [PubMed: 30859154]

44. Jacobi D, Liu S, Burkewitz K, Kory N, Knudsen NH, Alexander RK, Unluturk U, Li X, Kong X, Hyde AL, Gangl MR, Mair WB, et al. Hepatic Bmal1 Regulates Rhythmic Mitochondrial Dynamics and Promotes Metabolic Fitness. Cell Metab 2015;22:709–20. [PubMed: 26365180]

45. Adamovich Y, Rousso-Noori L, Zwighaft Z, Neufeld-Cohen A, Golik M, Kraut-Cohen J, Wang M, Han X, Asher G. Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. Cell Metab 2014;19:319–30. [PubMed: 24506873]

46. Kettner NM, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, Katchy CA, Lee C, Moore DD, Fu L. Circadian Homeostasis of Liver Metabolism Suppresses Hepatocarcinogenesis. Cancer Cell 2016;30:909–24. [PubMed: 27889186]

Table 1.

Distributions of Baseline Characteristics among Study Participants by Composite Score of Healthy Lifestyle Factors, the Singapore Chinese Health Study

Characteristics	Total # subjects	Composite Score						*P-value*
		0–3	4-	5	6	7	8	
Number of subjects	61,321	922	2960	10218	20427	20694	6100	
Mean age (±SD), years	56.4±8.0	56.9±7.7	58.7±8.1	57.9±8.1	56.9±8.0	56.4±7.8	54.1±7.3	<0.0001
Gender (%)								
Male	27,293 (44.5)	837 (90.8)	2,237 (75.6)	6,411 (62.7)	3,443 (46.2)	6,567 (31.7)	1,798 (29.5)	<0.0001
Female	34,028 (55.5)	85 (9.2)	723 (24.4)	3,807 (37.3)	10,984 (53.8)	14,127 (68.3)	4,302 (70.5)	
Dialect								
Cantonese	28,325 (46.2)	320 (34.7)	1,151 (38.9)	4,196 (41.1)	9,034 (44.2)	10,191 (49.2)	3,433 (56.3)	<0.0001
Hokkien	32,996 (53.8)	602 (65.3)	1,809 (61.1)	6,022 (58.9)	11393 (55.8)	10,503 (50.7)	2,667 (43.7)	
Highest level of education (%)								
No formal education	16,661 (27.2)	197 (21.4)	857 (28.9)	2,988 (29.2)	6,051 (29.6)	5,495 (26.5)	1,073 (17.6)	<0.0001
Primary school	27,224 (44.4)	566 (60.3)	1,549 (52.3)	5,038 (49.3)	9,116 (44.6)	8,594 (41.5)	2,371 (38.9)	
Secondary school or higher	17,436 (28.4)	168 (18.3)	554 (18.7)	2,192 (21.4)	5,260 (25.7)	6,605 (31.9)	2,656 (43.5)	
Diabetes (%)								
No	55,852 (91.1)	833 (90.4)	2,643 (89.3)	9,136 (89.4)	18,500 (90.6)	19,087 (92.2)	5,053 (92.7)	<0.0001
Yes	5,469 (8.9)	89 (9.6)	317 (10.7)	1,082 (10.6)	1,927 (9.4)	1,607 (7.8)	447 (7.3)	
Any weekly physical activity (%)								
No	41,083 (67.0)	785 (85.1)	2,659 (89.8)	9,198 (90.0)	18,593 (91.0)	19,079 (92.2)	5,498 (90.1)	<0.0001
Yes	20,238 (33.0)	137 (14.9)	301 (10.2)	1,020 (10.0)	1,834 (9.0)	1,615 (7.8)	602 (9.9)	
Mean total energy intake (±SD), Kcal	1,556.6± 566.2	1,861.4± 730.0	1,530.7± 609.1	1,481.7± 573.7	1,505.9± 556.0	1,563.3± 537.3	1,796.1± 553.7	<0.0001

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Table 2.

Association Between Individual Lifestyle Factors and Risk of Hepatocellular Carcinoma in the Singapore Chinese Health Study

Lifestyle Factor (index score)	%	HCC incidence rate per 100,000 person-years[a]	HR[b] (95% CI)
BMI Kg/m^2			
≥23.0 (0)	52.1	61.59	1.00
<23.0 (1)	47.9	40.84	0.68 (0.58–0.81)
Smoking status[c]			
Heavy smoker (0)	3.8	71.54	1.00
Light smoker (1)	26.8	68.01	0.74 (0.54–1.01)
Never smoker (2)	69.4	46.03	0.60 (0.43–0.83)
Alcohol consumption[d]			
Heavy drinker (0)	1.6	86.61	1.00
Non-heavy drinker (2)	98.4	50.21	0.41 (0.28–0.61)
Alternative Mediterranean diet score			
Quartile 1 (0)	21.1	61.85	1.00
Quartiles 2 & 3 (1)	59.4	52.10	0.88 (0.72–1.08)
Quartile 4 (2)	19.5	39.08	0.71 (0.53–0.96)
Sleep duration (hours/day)			
<6 or >8 (0)	16,5	60.41	1.00
6–8 (1)	83.5	50.25	0.90 (0.72–1.11)

[a]Adjusted for age and sex.

[b]Hazard ratio (HR) were derived from Cox proportional regression models that included all factors in the table simultaneously in addition to age, sex, dialect, year of enrollment, education level, diabetes status, and daily energy intake. CI, confidence interval

[c]Heavy smokers were those who started to smoke before 15 years of age and smoked 13 or more cigarettes per day, and light smokers were all remaining ever smokers.

[d]Heavy drinker were defined as those who consumed ≥15 drinks/week for men and ≥8 drinks/week for women, following definitions from the US Center for Disease Control and Prevention (https://www.cdc.gov/alcohol/pdfs/excessive_alcohol_use.pdf)

Table 3.

Association Between Composite Score of Healthy Lifestyle Factors and Risk of Hepatocellular Carcinoma in the Singapore Chinese Health Study

Composite Score	Persons	Person-year	Cases	HR[a] (95% CI)
Total subjects				
0 to 4	5,981	92,721	111	1.00
5	14,018	235,792	155	**0.67 (0.52–0.85)**
6	22,177	396,833	194	**0.61 (0.48–0.77)**
7	15,325	285,141	95	**0.49 (0.37–0.65)**
8	3,820	73,003	6	**0.13 (0.06–0.30)**
P_{trend}				**<0.0001**
Continuous scale				**0.78 (0.72–0.83)**
Men				
0 to 4	4,335	66,286	95	1.00
5	7,559	122,345	117	**0.68 (0.52–0.89)**
6	9,062	154,999	129	**0.64 (0.49–0.83)**
7	5,177	93,726	60	**0.53 (0.38–0.74)**
8	1,160	21,661	4	**0.16 (0.06–0.43)**
P_{trend}				**<0.0001**
Continuous scale				**0.79 (0.73–0.86)**
Women				
0 to 4	1,646	26,436	16	1.00
5	6,459	113,447	38	0.60 (0.33–1.07)
6	13,115	241,834	65	**0.52 (0.30–0.90)**
7	10,148	191,415	35	**0.38 (0.20–0.70)**
8	2,660	51,342	2	**0.09 (0.02–0.38)**
P_{trend}				**0.0003**
Continuous scale				**0.72 (0.61–0.84)**
$P_{heterogeneity}$				0.51

[a]Hazard ratio (HR) were derived from Cox proportional regression models that also included age, sex, dialect, year of enrollment, education level, diabetes status, and daily energy intake. CI, confidence interval

Table 4.

Association Between Composite Score of Healthy Lifestyle Factors and Risk of Hepatocellular Carcinoma Stratified by Hepatitis B and/or Hepatitis C Viral Serology in the Singapore Chinese Health Study

Composite Score	Case	Control	OR (95% CI)
All subjects[a]			
0 to 4	32	54	1.00
5	44	119	0.58 (0.30–1.12)
6	78	163	0.70 (0.37–1.30)
7	41	101	**0.46 (0.22–0.94)**
8	2	28	**0.04 (0.01–0.23)**
P_{trend}			**0.003**
Continuous scale			**0.75 (0.62–0.90)**
HBsAg-negative and anti-HCV negative subjects[b]			
0 to 4	24	51	1.00
5	32	114	**0.53 (0.28–1.00)**
6	47	157	**0.53 (0.28–0.98)**
7	20	97	**0.38 (0.19–0.79)**
8	0	24	-
P_{trend}			**0.001**
Continuous scale			**0.73 (0.60–0.88)**
HBsAg-positive or anti-HCV positive subjects[c]			
0 to 4	8	3	1.00
5	12	5	1.10 (0.19–6.42)
6	31	6	2.22 (0.41–11.89)
7	21	4	2.46 (0.37–16.49)
8	2	4	0.22 (0.02–2.19)
P_{trend}			0.77
Continuous scale			0.93 (0.60–1.46)
$P_{heterogeneity}$			0.884

[a]Odds ratios (ORs) were derived from conditional logistic regression models that also included level of education, diabetes status, dietary energy intake, and seropositivity of hepatitis B surface antigen (HBsAg) and/or antibodies to hepatitis C virus (anti-HCV). A separate indicator variable for 117 HCC cases and 243 controls with unknown anti-HCV status was included in the logistic model.

[a]Odds ratios (ORs) were derived from conditional logistic regression models that also included age, gender, dialect group, level of education, year of enrollment, diabetes status, dietary calorie intake. For all subject, the model also included HBsAg serology status.

[¶]Conditional logistic regression model was performed

[¥]Unconditional logistic regression model was performed