Low levels of alcohol consumption, obesity and development of fatty liver with and without evidence of advanced fibrosis

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ABSTRACT

The effects of low level alcohol consumption on fatty liver disease and the potential for effect modification by obesity is uncertain. We investigated associations between low level alcohol consumption, obesity status and the development of incident hepatic steatosis (HS) either with or without, an increase in noninvasive liver fibrosis score category (from low to intermediate or high category). A total of 190,048 adults without HS and a low probability of fibrosis with alcohol consumption <30g/day (men) and <20g/day (women) were followed for up to 15.7 years. Alcohol categories of no, light and moderate consumption were defined as 0, 1-9.9, and 10-29.9 g/day (10-19.9 g/day for women), respectively. HS was diagnosed by ultrasonography, and the probability of fibrosis was estimated using the fibrosis-4 index (FIB-4). Parametric proportional hazards models were used to estimate multivariable-adjusted hazard ratios (aHR) and 95% confidence intervals (CI). 43,466 participants developed HS. 2,983 participants developed HS with an increase in FIB-4 index (to intermediate or high scores). Comparing light-drinkers and moderate-drinkers with non-drinkers, aHR (95% CI) for incident HS were 0.93 (0.90–0.95) and 0.90 (0.87–0.92), respectively; in contrast, comparing light-drinkers and moderate-drinkers with non-drinkers, aHR (95% CI) for developing HS plus intermediate/high FIB-4 were 1.15 (1.04–1.27) and 1.49 (1.33–1.66), respectively. The association between alcohol consumption categories and incident HS plus intermediate/high FIB-4 was observed in both non-obese and obese individuals although the association was stronger in non-obese individuals (p for interaction by obesity=0.017). **Conclusion:** Light/moderate alcohol consumption has differential effects on the development of different stages of fatty liver disease which is modified by the presence of obesity.

Keywords: low level alcohol consumption; obesity; nonalcoholic fatty liver disease; hepatic steatosis; hepatic fibrosis; non-invasive fibrosis marker; cohort study

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver diseases worldwide and comprises a spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) that can progress to fibrosis, cirrhosis, liver failure, or hepatocellular carcinoma (1, 2). Recently, studies have suggested that fibrosis is the most important histologic predictor of liver- and non-liver-related mortality in patients with NAFLD and that steatosis itself is not associated with liver-related outcomes (3-5).

Heavy alcohol consumption is an established risk factor for increased liver morbidity and mortality, but previous studies have suggested that modest alcohol consumption is associated with decreased risk of NAFLD compared to no alcohol consumption (6-8). However, those studies did not consider the effects of alcohol consumption on development of hepatic fibrosis. In contrast, longitudinal studies of patients with existing NAFLD have reported that modest alcohol consumption is associated with unfavorable effects on NAFLD histology (hepatic steatosis (HS) and NASH) and worsening of non-invasive fibrosis markers, compared with no consumption of alcohol (9, 10). Importantly, since HS with fibrosis rather than simple HS alone is associated with adverse hepatic outcomes, inclusion of simple HS as an endpoint (without considering liver fibrosis) may provide misleading evidence for the effect of low levels of alcohol consumption on liver health (3-5).

To date, no large-scale, longitudinal cohort studies have evaluated the effects of modest alcohol consumption on development of HS with fibrosis in the general population and the

effect of low levels alcohol consumption on risk of NAFLD and the potential for effect modification by obesity is uncertain. Therefore, we aimed to investigate the association between low levels of alcohol consumption and the risk of a) incident HS, and b) incident HS with an increase to an intermediate/high probability of liver fibrosis over time, as the two key outcomes. To address these aims, we used data from a large retrospective cohort study of non-obese and obese individuals participating in a health screening examination program with repeated measures of alcohol consumption and other covariates during follow-up.

METHODS

Study populations

The Kangbuk Samsung Health Study is a cohort study of Korean men and women aged 18 years and older who have undergone comprehensive annual or biennial examinations at Kangbuk Samsung Hospital Total Healthcare Center in Seoul or Suwon, South Korea (10, 11). The present analysis was restricted to all study participants who underwent a comprehensive health examination between January 2002 and December 2017 and had at least one follow-up visit through December 31, 2017 (N = 353,609; Figure 1). We excluded 163,561 participants that met the following criteria: missing information on ultrasonography, alcohol consumption, or components of the fibrosis-4 (FIB-4) or NAFLD fibrosis score (NFS); history of malignancy; presence of HS on abdominal ultrasound; history of liver cirrhosis or findings of liver cirrhosis on ultrasound; intermediate or high probability of fibrosis based on FIB-4 or NFS (see below for further details) at baseline; alcohol intake ≥30 g/day for men or ≥20 g/day for women (1); positive serologic markers for hepatitis B or C virus; known liver disease or use of medications for liver disease; and use of steatogenic

medications within the past year such as sodium valproate, amiodarone, methotrexate, tamoxifen, or corticosteroids. Because some individuals met more than one exclusion criterion, a total of 190,048 participants were included in the analysis (see further details in the supporting information and **Supplementary Table 1**).

The Institutional Review Board of Kangbuk Samsung Hospital approved this study (IRB No. 2017-04-027), and informed consent was waived because we used only de-identified retrospective data from a routine health screening process.

Measurements

Data on physical measurements, abdominal ultrasonography, and serum biochemical measurements were collected as part of the basic health check-up program at baseline and follow-up visits. Demographic characteristics, health behaviors, medical history, and medication use were also collected at each visit using standardized, self-administered questionnaires (12). Current alcohol use was assessed as the frequency of alcohol drinking per week and amount of alcohol consumed per drinking day (see further details in the supporting information). In the present study, average alcohol consumption per day was calculated using frequency and amount of alcohol consumed per drinking day. Because there is debate as to the precise dose-response relationships between alcohol intake and major health conditions, non-drinking, light drinking, and moderate drinking were defined as 0 g/day, 1-<10 g/day, and 10–<20 g/day for women and 0 g/day, 1–<10 g/day, and 10–<30 g/day for men, respectively, as previously applied in our and other studies (7, 10, 13). Since 2011, participants were also asked about alcohol flushing, a proxy for aldehyde dehydrogenase 2 (ALDH2) deficiency (see further details in the supporting information).

Participants were categorized into alcohol flushers and non-flushers.

Sitting blood pressure (BP), height, and weight were measured by trained nurses. Obesity was defined as body mass index (BMI) ≥25 kg/m^2, the proposed cutoff for diagnosis of obesity in Asian populations (14). Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current use of antihypertensive medication.

Fasting blood tests included lipid profile, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyltransferase, glucose, insulin, high sensitivity C-reactive protein (hsCRP), albumin, and platelet count. The homeostasis model assessment of insulin resistance (HOMA-IR) for quantifying insulin resistance was calculated as [fasting insulin (uU/mL) × fasting glucose (mmol/L)] / 22.5. Diabetes was defined as fasting serum glucose ≥126 mg/dL, self-reported use of insulin or antidiabetic medication.

The diagnosis of HS was based on abdominal ultrasound performed by experienced radiologists, unaware of the study aims. HS was diagnosed based on known standard criteria, including presence of a diffuse increase in fine echoes in the liver parenchyma compared with kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls (15). Inter-observer and intra-observer reliability values for HS diagnoses were substantial (kappa statistic of 0.74) and excellent (kappa statistic of 0.94), respectively (12).

To assess the risk of development of more severe NAFLD, two non-invasive indices of liver fibrosis were used, the FIB-4 and NFS. The FIB-4 index was calculated as follows: FIB-4 = (age (years) × AST (U/L)) / (platelet count ($\times 10^9$/L) × ALT (U/L)$^{1/2}$). Participants were also categorized into three groups: low (FIB-4 <1.30), intermediate (FIB-4 1.30-2.66), and high (FIB-4 ≥2.67) probability of advanced fibrosis (16). NFS was calculated as follows:

NFS= -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×10^9/L) – 0.66 × albumin (g/dL) (17). Participants were categorized into three groups according to probability of advanced fibrosis: high (NFS >0.676), intermediate (NFS: 0.676 to -1.455), and low (NFS < -1.455) (17).

Statistical analysis

Descriptive statistics were used to summarize the participants' characteristics by alcohol consumption category and those characteristics were compared between groups using χ^2-test and one-way ANOVA.

The primary endpoints were: a) development of incident HS, and b) the development of incident HS plus an increase to intermediate/high probability of liver fibrosis at follow up, based on FIB-4 levels. Incident HS and incident HS combined with worsening of non-invasive fibrosis marker were treated as a separate endpoint in each model. For analysis of the association between alcohol consumption and incident HS, if HS was identified during follow-up, subsequent observations were not incorporated in the analysis. For analysis of the association between alcohol consumption and incident HS combined with worsening of fibrosis markers, if an individual's ultrasonographic finding indicated HS and non-invasive fibrosis markers simultaneously worsened to a higher category (intermediate or high) during the follow-up, the case was assumed to have developed HS combined with fibrosis. Therefore subsequent measurements were excluded from the primary analysis. Person-years were calculated as the sum of the follow-up duration from baseline to the development of a primary endpoint (HS or HS with fibrosis, separately) or until the final examination which

was undertaken before the end of 2017; whichever occurred first. Data for participants who reported alcohol consumption greater than light/moderate (alcohol intake ≥30 g/day for men or ≥20 g/day for women (1)) during follow-up were censored at the visit before the observation of heavy alcohol consumption. Incidence rates were calculated as the number of incident cases divided by person-years of follow-up. Since each primary endpoint would have occurred at an unknown time point between the visit at which primary endpoint was observed and the previous visit, a parametric proportional hazards model was used to account for this type of interval censoring. In these models, the baseline hazard function was parameterized with restricted cubic splines in log time with four degrees of freedom.

Primary analysis of the associations between light and moderate drinking versus non-drinking based on alcohol consumption pattern at baseline and development of incident HS, or incident HS with fibrosis, was based on a parametric proportional hazards model. The hazard ratio (HR) and 95% confidence interval (CI) were calculated for development of a primary endpoint according to alcohol consumption. Models were initially adjusted for age, sex, center (Seoul or Suwon) and year of screening exam (Model 1) and then further adjusted for smoking status (never, past, current, or unknown), regular exercise (<3 times per week, ≥3 times per week, or unknown), education level (below college graduate, college graduate or higher, or unknown), history of hypertension, medication for hypertension, history of diabetes, medication for diabetes, and medication for dyslipidemia (Model 2). To examine whether the relationship between alcohol consumption and development of the primary outcomes was mediated by BMI, model 3 was further adjusted for BMI. The proportional hazards assumption was tested by examining graphs of estimated log (-log (survival)). To test linear trends of incidence, we included the median value of each category (alcohol intake,

gram/day) as a continuous variable in the models. To evaluate the effects of changes in alcohol consumption and covariates during follow-up, we conducted additional analyses using alcohol consumption and other covariates as a time-varying covariate in the models. We also performed a sensitivity analysis using NFS instead of FIB-4.

We evaluated whether or not the associations between alcohol consumption and the risk of HS and fibrosis differed by presence of obesity because the effect of alcohol consumption on liver outcome appears to be increased by the presence of obesity (18, 19). Interactions between alcohol consumption categories and obesity on the risk of HS and intermediate/high probability of advance liver fibrosis were tested using likelihood ratio tests, comparing models with and without multiplicative interaction terms. We also evaluated whether or not the associations between alcohol consumption and the risk of HS and fibrosis differed by presence of alcohol flushing response. Additionally, we performed sensitivity analyses excluding participants who reported binge drinking, which was defined as 60 g or more of alcohol intake on one occasion, because a binge drinking pattern can be another risk factor for liver fibrosis, even if the average alcohol consumption falls into the moderate drinking category (20).

Statistical analyses were carried out using STATA version 15.0 (StataCorp LP, College Station, TX, USA). All p-values less than 0.05 were considered statistically significant.

RESULTS

The mean (SD) values for FIB-4 at baseline were 0.69 (0.20). Compared with non-drinkers (**Table 1**), moderate drinkers were more likely to be younger, male, current smokers, regular exercisers, highly educated, and obese and to have higher values of BMI, BP, triglycerides,

liver enzymes and hsCRP. In contrast, this group had lower values of HOMA-IR.

During the median follow-up period of 4.1 years (interquartile range, 2.1-7.8, maximum 15.7), 43,466 participants developed HS, and 2,983 participants developed HS plus increase in liver fibrosis score category to intermediate/high FIB-4. Compared to subjects who did not develop HS (**Supplementary Table 2**), subjects who developed HS but with a low FIB-4, or HS plus an increase in liver fibrosis score category to intermediate/high FIB-4, were more likely to have diabetes and high levels of BP, triglyceride and HOMA-IR and to have lower HDL-C concentrations with worst profile of metabolic factors seen in those who developed HS plus an intermediate/high FIB-4.

Table 2 shows the cumulative incidence rates and risk of HS, or HS plus intermediate/high FIB-4, according to alcohol consumption category. After adjusting for confounding variables, the multivariable-adjusted HRs (95% CIs) for new-onset HS, (comparing light-drinkers and moderate-drinkers with non-drinkers) were 0.94 (0.92–0.96) and 1.02 (0.99–1.05), respectively (**Table 2, model 2**). After further adjustment for BMI, HRs (95% CIs) for new-onset HS, (comparing light-drinkers and moderate-drinkers with non-drinkers) were 0.93 (0.90-0.95) and 0.90 (0.87-0.92), respectively. Further adjustment for hsCRP and HOMA-IR did not change qualitatively these associations. Conversely, the multivariable-adjusted HRs (95% CIs) for developing HS plus an intermediate/high FIB-4 (comparing light-drinkers and moderate-drinkers with non-drinkers) were 1.16 (1.05–1.28) and 1.65 (1.48–1.84), respectively. After adjustment for BMI, the positive association between alcohol consumption and HS plus an intermediate/high FIB-4 was slightly attenuated but remained significant. When we adjusted for alcohol use and the confounders as time-varying covariates, the association of moderate drinking with HS plus an intermediate/high FIB-4 remained

significant. Similar results were observed when using NFS (**Supplementary Table 3**).

The association between alcohol consumption and risk of HS, and HS plus an intermediate/high FIB-4 differed significantly by the presence of obesity (**Table 3**). In non-obese individuals, moderate alcohol consumption was positively associated with both simple HS and HS plus an intermediate/high FIB-4. In contrast, in obese individuals, moderate alcohol consumption was inversely associated with incident HS, but positively associated with incident HS plus an intermediate/high FIB-4.

When the analyses were restricted to participants without evidence of binge drinking (**Supplementary Tables 4 and 5**), light and moderate drinkers still showed a significantly higher risk of incident HS plus an intermediate/high fibrosis score, compared with non-drinkers. We further divided the light drinkers into those who drink less than once a week (this category indicates 1-3 times a month since drinking less than once a month was not recorded) and those who drink once a week or more (**Supplementary Table 6**). These data showed there was a significant and inverse association between light drinking and incident hepatic steatosis in light drinkers with a frequency of ≥ once a week, but not in light drinkers with a frequency of <once a week. . The positive association between light drinking and HS with intermediate/high fibrosis score was similarly observed in both the light drinking category with a frequency of ≥ once a week and with a frequency of 1-3 times a month.

Since individuals with the *aldehyde dehydrogenase 2 (ALDH2)* gene variant, a very common genotype among East Asians, might be more susceptible to the detrimental effect of alcohol intake and also be at risk for the development of liver fibrosis in the absence of alcohol-related HS, we evaluated whether or not the associations between alcohol consumption and the risk of HS and fibrosis differed by presence of alcohol flushing response

as a marker of the *ALDH2* variant (21-23). Among non-flushers, there was no significant association between alcohol consumption and incident HS; among flushers, alcohol consumption was inversely associated with incident HS. In both non-flushers and flushers, moderate drinking tended to increase the risk of HS with fibrosis. The risk of developing intermediate/high fibrosis score was higher in moderate drinkers than in non-drinkers, with a stronger effect among individuals with flushing. However, there was no evidence of a statistically significant interaction between alcohol consumption and presence of alcohol flushing response on the risk of HS and fibrosis (**Supplementary Table 7**). Increased frequency of drinking alcohol was associated with decreased risk of HS, whereas increased quantity of alcohol consumed per drinking day, tended to be associated with higher risk of HS. Both increased frequency of drinking and alcohol quantity tended to be associated with increased risk of HS with intermediate/high fibrosis FIB-4 (**Supplementary Table 8**).

DISCUSSION

In this large cohort of 190,048 young and middle-aged Korean men and women without NAFLD at baseline, modest alcohol consumption (within the permissible limits to make a diagnosis of NAFLD) was associated with a decreased risk of incident HS (overall). However, in contrast to these findings in the small subgroup of subjects (~6%) who developed more severe NAFLD over time, our data show that the same levels of modest alcohol consumption were associated with an increased risk of developing HS plus an intermediate/high probability of advanced liver fibrosis. Furthermore, the association between moderate drinking and development of incident HS plus an intermediate/high fibrosis score was consistently observed in both non-obese and obese individuals, even though the association

was stronger in non-obese individuals as shown in **Table 3.**

Although some studies have reported that light or moderate drinking has protective effects on NAFLD or liver histology in NAFLD, other studies have reported no association or even harmful effects (6, 7, 24, 25). Meta-analyses have shown that modest alcohol consumption is associated with a lower risk of NAFLD, compared with non-drinkers (6). However, it should be noted that most previous studies have used a cross-sectional study design, making it impossible to comment on the nature of the temporal relationship between alcohol consumption and NAFLD (20). Only a few cohort studies have examined the association between alcohol consumption and the development of NAFLD (7, 8). Studies in the Japanese population have reported that light to excessive amounts of alcohol consumption were associated with a decreased risk of NAFLD (7, 8), but none of those studies reported the effects of alcohol on liver fibrosis. In our study, light to moderate alcohol consumption was found to be significantly associated with a decreased risk for developing HS without considering fibrosis. However, given that NAFLD with advanced fibrosis rather than simple steatosis alone, predicts liver- and non-liver-related mortality (3-5, 11, 26), it is important to evaluate the effects of alcohol intake on measures of liver fibrosis. Moreover, a recent longitudinal study using paired liver biopsies amongst patients with biopsy-proven NAFLD reported that no alcohol consumption was associated with greater improvement in NAFLD histology, including steatosis and NASH over time, compared with moderate alcohol use (9).

In the present study, low levels of alcohol consumption were independently associated with increased risk of developing incident HS plus an intermediate/high probability of advanced fibrosis over a maximum of almost 16 years of follow-up. These results are in line with other previous studies (27-29). A meta-analysis has reported that even alcohol

consumption of 12-24 g per day, which falls within our moderate alcohol consumption category, was associated with an increased risk of cirrhosis-related mortality compared with non-drinking (27, 28). Additionally, other studies have reported an increased risk of developing hepatocellular carcinoma with any alcohol consumption in patients with NASH (28, 29).

The mechanisms underlying the relationship between light to moderate alcohol consumption and HS plus fibrosis remain unclear. The association between alcohol consumption and liver disease may be primarily mediated by metabolic alterations such as insulin resistance (30). However, in our study, the association between moderate alcohol consumption and HS plus an intermediate/high probability of advanced fibrosis was observed, even after adjustment for HOMA-IR (and also hsCRP as a non-specific marker of inflammation). A body of evidence indicates the notion that mitochondria are the primary target of alcohol stress and ethanol-related increases in reactive oxygen species (ROS) in hepatocytes (31). Ethanol consumption promotes the production of pro-inflammatory cytokines such as TNF-α and interleukin-6, which stimulate hepatocytes to generate signals that affect mitochondrial ROS formation, leading to mitochondrial dysfunction. Hepatocyte cell injury resulting from this cascade of events is thought to promote neutrophil infiltration or activate stellate cells to initiate fibrogenesis (31). Studies in rodents suggest that low levels of ingested ethanol can induce hepatic mitochondrial oxidative stress and stimulate hepatic fibrosis (32, 33). Additionally, ethanol reaches the liver via the portal vein, inducing triglyceride accumulation and hepatic oxidative stress, and also increasing gut permeability (34).

Previous epidemiological studies have suggested a synergistic effect of alcohol and

obesity on liver disease morbidity and mortality (18, 19). In our study, there were significant interactions between obesity and alcohol intake categories for incident HS, and between obesity and alcohol intake categories for incident HS plus an intermediate/high probability of advanced fibrosis. The association between moderate alcohol drinking and HS plus an intermediate/high probability of advanced fibrosis was consistently observed in both non-obese and obese individuals, but was stronger in non-obese individuals, even though the absolute incidence of HS with intermediate/high fibrosis score was much higher in obese individuals, than non-obese subjects. Given that other risk factors for HS and fibrosis, such as insulin resistance and type 2 diabetes, can increase with the presence of obesity, the relative effects of alcohol consumption on development of NAFLD with liver fibrosis might be more important in non-obese individuals.

Additionally, several genetic variations, such as *patatin-like phospholipase domain-containing 3*, have been reported to be more commonly represented in non-obese subjects with HS, compared to the general population (35, 36). The occurrence of these genotypes, that are known to be associated with more severe liver disease in NAFLD, may predispose non-obese individuals to increased liver disease susceptibility due to the effects of environmental 'toxins' such as modest alcohol consumption and high dietary fructose intake.

Interestingly, moderate alcohol drinking was inversely associated with incident HS, in obese individuals. The reasons for this inverse association with incident hepatic steatosis are unclear. It is interesting to note that although subjects with moderate alcohol consumption had a higher mean BMI than non-drinkers, moderate drinkers were more insulin sensitive than non-drinkers (**Table 1**). Thus, it is plausible that the more insulin sensitive subjects do not develop HS at follow-up because they more efficiently store excess calories as lipids in

peripheral adipose tissue depots, and not in ectopic sites such as the liver. In keeping with this speculation, the data in **Supplementary Table 1** supports this suggestion, since the mean HOMA-IR in subjects who developed HS was significantly higher than in subjects who did not develop HS. However, there is also a possibility of misclassification of alcohol consumption. Alcohol consumption tends to be underestimated based on self-report, but whether there is differential reporting of alcohol consumption by obesity status is unclear (37, 38). Only a few studies have reported that underreporting of alcohol consumption occurs more frequently in obese than in the non-obese individuals, although other studies have reported there is no differential underreporting of alcohol consumption by obesity status (38, 39). Whilst we acknowledge that there may be misclassification of the etiology of HS (NAFLD versus Alcoholic fatty liver disease) in the non-obese group, we would have anticipated that underreporting of what is very modest alcohol consumption (in our study), would have occurred in both non-obese and obese groups because there is no definite evidence of differential underreporting of alcohol drinking by obesity. Additionally, our outcome measurement and physical examination were carried out after the participants had completed the questionnaires about lifestyle factors including alcohol consumption; thus, we consider that this aspect of the study design would have also minimized the risk of recall bias. We suggest that further studies with objective measures of alcohol consumption as well as genotyping are required to understand this obesity-related difference in the associations between moderate alcohol consumption and different aspects of liver disease severity in NAFLD, specifically differentiating HS with fibrosis from simple steatosis.

The differential effect of alcohol consumption on HS between a) drinking frequency and b) quantity of alcohol consumed (**Supplementary Table 8**) may have important implications

for guidelines and public health. Since heavy alcohol consumption was already excluded at baseline, it is possible that higher drinking frequency can accompany lower absolute quantity of alcohol consumption per drinking day, in keeping with light or moderate overall alcohol consumption. Thus, our study design cannot evaluate the dose-response relationship of both drinking frequency and alcohol quantity with HS and fibrosis. That said, it is noteworthy that higher drinking frequency was inversely associated with HS and increased alcohol quantity consumed per drinking day was positively associated with HS with fibrosis. Whilst our study focused on average alcohol consumption, a myriad of other drinking patterns relating to quantity, frequency, binge pattern consumption, beverage type, and drinking with meals may also affect liver health, and we suggest the effect of these different patterns of alcohol consumption requires further study (40, 41).

We acknowledge the limitations in our study. First, diagnosis of HS with liver fibrosis was based on ultrasound and two validated non-invasive fibrosis scores. Although fatty liver infiltration below a threshold of 10% cannot be detected using ultrasound, ultrasound assessment has acceptable diagnostic accuracy for detecting steatosis and is widely used in both clinical and epidemiological studies (42). FIB-4 and NFS are non-invasive liver fibrosis markers that have been validated as offering good diagnostic performance in identifying advanced liver fibrosis, confirmed by liver biopsy (16, 17). Additionally, previous studies have demonstrated that higher liver fibrosis scores (intermediate and high fibrosis score) are associated with increased liver disease-related mortality both in the United States and in Korean adults (5, 11). Second, the lifestyle variables including alcohol consumption were assessed using self-administered structured questionnaires. An objective marker of alcohol consumption, such as phosphatidylethanol or carbohydrate-deficient transferrin, was not

available in our data. Different types of alcoholic beverages may differently affect health outcomes (40, 43), but detailed information on the different types of alcoholic beverages and lifetime drinking patterns (e.g., prior heavy drinking) were unavailable. Measurement error could introduce some degree of misclassification bias (for example, if former heavy drinkers were classified as non-drinkers) but this would tend to attenuate the strength of the observed associations towards the null. We cannot exclude the possibility of some unmeasured or residual confounding factors. Finally, our study population comprised relatively healthy, educated, young and middle-aged Koreans who were predominantly younger and leaner than the majority of population-based studies on NAFLD. Since NASH and worsening fibrosis increase with age (1, 2) and drinking pattern, and the prevalence of gene variants encoding several of the alcohol-metabolizing enzymes differ among ethnic groups (44, 45), our findings might not be generalizable to other age groups, populations with a higher prevalence of comorbidities, or other race/ethnic groups. On the other hand, our findings from a cohort of asymptomatic and relatively young adults are potentially less likely to be affected by survivor bias and biases related to comorbidities and use of multiple medications than findings from previous cohorts of patients with biopsy proven NAFLD. Because alcohol consumption and heavy drinking tends to decline with age (46), it is likely that the drinking patterns of this relatively young cohort would be fairly stable over time. Individuals with ALDH2 variant, a very common genotype among East Asians including Koreans, might be more susceptible to the detrimental effect of alcohol intake and also be at risk for the development of liver fibrosis in the absence of alcohol-related HS (21, 22). In our study, the associations between alcohol consumption and the risk of HS and fibrosis did not significantly differ by presence of alcohol flushing response, a proxy for ALDH2 genetic

variation. However, given that only one-third of the participants had available information on flushing, and had a much shorter follow-up duration (because this information was only available from 2011), further studies with longer follow-up and *ALDH*2 genotyping are needed, in order to definitively examine the differential effect of alcohol consumption on HS and fibrosis, stratified by ALDH2 polymorphism.

Conclusion

In this large cohort study of young and middle-aged adults at low risk of liver fibrosis without NAFLD at baseline, low levels of alcohol consumption were associated with decreased risk of developing simple HS at follow up. In contrast, low levels of alcohol consumption was associated with an increase in risk of intermediate/high probability of advanced liver fibrosis in subjects with HS at follow up. These data also show the effects of low levels of alcohol consumption on the liver are modified by the presence of co-existing obesity. We suggest that in both obese and non-obese subjects, the thresholds for safe drinking need to be reassessed.

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Figure legends

Figure 1. Selection of the study population.

*Some individuals met more than one criterion for exclusion.

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance.

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Table 1. Baseline characteristics according to alcohol intake category among NAFLD-free participants with a low probability of advanced fibrosis at baseline (n=190,048)

Characteristics	Category of alcohol consumption			*P* value for trend
	Non-drinkers	Light drinkers	Moderate drinkers	
Number	60,443	84,241	45,364	
Age (years) [a]	36.5 (7.2)	34.7 (6.0)	35.5 (6.3)	<0.001
Male (%)	18.4	42.1	82.4	<0.001
Current smoker (%)	6.5	15.3	40.2	<0.001
Regular exercise (%)[c]	13.3	12.4	14.8	<0.001
High education level (%)[d]	73.2	84.6	84.0	<0.001
Hypertension (%)	5.5	5.1	10.7	<0.001
Diabetes (%)	0.8	0.5	0.9	0.122
Medication for dyslipidemia (%)	0.4	0.4	0.5	0.008
Obesity (%)[e]	10.1	11.7	22.1	<0.001
Body mass index (kg/m^2)	21.6 (2.6)	21.8 (2.6)	23.1 (2.6)	<0.001
Systolic BP (mmHg)[a]	107.2 (12.7)	106.9 (12.3)	112.9 (12.4)	<0.001
Diastolic BP (mmHg)[a]	68.6 (9.1)	68.4 (9.1)	72.9 (9.5)	<0.001
Glucose (mg/dl) [a]	90.1 (9.6)	90.6 (9.0)	92.7 (10.8)	<0.001
Total cholesterol (mg/dl) [a]	185.6 (32.6)	185.4 (31.2)	191.6 (32.2)	<0.001
LDL-C (mg/dl) [a]	106.1 (27.8)	108.6 (27.9)	113.6 (28.9)	<0.001
HDL-C (mg/dl) [a]	59.8 (13.3)	60.9 (14.2)	57.7 (13.5)	<0.001
Triglycerides (mg/dl)[b]	77 (58-105)	77 (58-106)	97 (70-138)	<0.001
AST (U/l)[b]	19 (17-22)	19 (16-22)	21 (18-25)	<0.001
ALT (U/l) [b]	15 (12-20)	15 (12-21)	20 (15-27)	<0.001
GGT (U/l) [b]	12 (9-17)	15 (11-21)	23 (16-35)	<0.001
Albumin (g/dL) [a]	4.5 (0.2)	4.6 (0.2)	4.6 (0.2)	<0.001
Platelet ($\times 10^9$/L) [a]	261.5 (54.9)	254.0 (51.2)	252.2 (49.0)	<0.001
hsCRP (mg/l) [b]	0.3 (0.1-0.7)	0.3 (0.2-0.6)	0.4 (0.2-0.8)	<0.001
HOMA-IR[b]	1.53 (1.10-2.03)	1.28 (0.85-1.79)	1.38 (0.94-1.90)	<0.001
Fib-4 [a]	0.71 (0.21)	0.68 (0.20)	0.69 (0.20)	<0.001

Data are expressed as [a]mean (standard deviation), [b]median (interquartile range) or percentage.
[c]≥ 3 times/week;
[d]≥ College graduate;
[e] BMI ≥25kg/m^2.
Abbreviations: ALT, alanine aminotransferase; APRI, aspartate transaminase to platelet ratio index; AST, aspartate aminotransferase; BP, blood pressure; FIB-4, fibrosis-4; GGT, gamma-glutamyl transpeptidase; HDL-C, high-density lipoprotein-cholesterol; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance. LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score

Table 2. Cumulative incidence rates and risk of incident hepatic steatosis or incident hepatic steatosis plus intermediate/high probability of advanced fibrosis (based on FIB-4 levels), according to alcohol consumption category

Categories of alcohol consumption[a]	Person-years (PY)	Incident cases	Incidence (per 10^3 PY)	Cumulative Incidence (per 10^3 person)		Multivariable-adjusted HR[a] (95% CI)			HR (95% CI)[b] in model using time-dependent variables
				2-Year	5-Year	Model 1	Model 2	Model 3	
Hepatic steatosis									
Non-drinkers	389,894.4	11,915	30.6	39.5	131.9	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	408,494.7	16,974	41.6	57.9	185.2	0.95 (0.92-0.97)	0.94 (0.92-0.96)	0.93 (0.90-0.95)	0.87 (0.85-0.90)
Moderate drinkers	222,787.9	14,577	65.4	98.4	284.2	1.05 (1.02-1.08)	1.02 (0.99-1.05)	0.90 (0.87-0.92)	0.87 (0.84-0.89)
P for trend						<0.001	0.079	<0.001	<0.001
Hepatic steatosis plus intermediate/high FIB-4									
Non-drinkers	433,335.3	827	1.9	1.0	5.0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	465,799.1	957	2.1	0.9	4.7	1.17 (1.06-1.30)	1.16 (1.05-1.28)	1.15 (1.04-1.27)	0.99 (0.85-1.15)
Moderate drinkers	274,491.0	1,199	4.4	2.3	10.0	1.71 (1.54-1.91)	1.65 (1.48-1.84)	1.49 (1.33-1.66)	1.28 (1.09-1.51)
P for trend						<0.001	<0.001	<0.001	0.002

[a] Estimated from parametric proportional hazard models. Multivariable model 1 was adjusted for age, sex, center and year of screening exam; model 2: model 1 plus adjustment for smoking status, regular exercise, education level, history of diabetes, medication for diabetes, history of hypertension, medication for diabetes and medication for dyslipidemia; model 3: model 2 plus adjustment for BMI

[b] Estimated from parametric proportional hazard models with alcohol intake, smoking status, regular exercise, diabetes, hypertension and BMI as a time-dependent categorical variables and baseline age, sex, center, year of screening exam, and education level as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.

Table 3. Development of incident simple hepatic steatosis or hepatic steatosis plus intermediate/high probability of advanced fibrosis (based on FIB-4 levels), according to alcohol consumption category and stratified by obesity (defined as BMI ≥25 kg/m^2).

Categories of alcohol consumption	Person-years (PY)	Incident cases	Incidence density (per 10^3 PY)	Cumulative Incidence (per 10^3 person)		Multivariable-adjusted HR[a] (95% CI)		HR (95% CI)[b] in model using time-dependent variables
				2-Year	5-Year	Model 1	Model 2	
Simple hepatic steatosis								
Non-obese								
Non-drinkers	358160.4	8,989	25.1	28.9	103.6	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	365712.5	12,480	34.1	45.0	149.7	0.99 (0.96-1.01)	0.98 (0.95-1.01)	0.88 (0.86-0.91)
Moderate drinkers	178564.9	9,402	52.7	73.1	228.9	1.10 (1.06-1.13)	1.06 (1.03-1.10)	0.90 (0.87-0.94)
P for trend						<0.001	<0.001	<0.001
Obese								
Non-drinkers	31734.0	2,926	92.2	134.5	379.3	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	42782.2	4,494	105.0	153.8	434.4	0.82 (0.78-0.86)	0.81 (0.77-0.85)	0.85 (0.81-0.89)
Moderate drinkers	44223.0	5,175	117.0	186.0	468.3	0.76 (0.73-0.80)	0.74 (0.70-0.77)	0.80 (0.76-0.84)
P for trend						<0.001	<0.001	<0.001
Hepatic steatosis plus intermediate/high FIB-4								
Non-obese								
Non-drinkers	390070.7	583	1.5	0.8	3.7	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	406886.9	703	1.7	0.8	3.9	1.24 (1.11-1.40)	1.23 (1.10-1.39)	1.01 (0.86-1.20)
Moderate drinkers	210641.5	767	3.6	1.8	8.1	1.78 (1.57-2.01)	1.72 (1.51-1.95)	1.31 (1.09-1.58)
P for trend						<0.001	<0.001	0.003
Obese								
Non-drinkers	43264.6	244	5.6	2.8	16.3	1.00 (reference)	1.00 (reference)	1.00 (reference)
Light drinkers	58912.2	254	4.3	2.1	10.7	1.10 (0.83-1.20)	0.99 (0.83-1.19)	0.93 (0.72-1.21)
Moderate drinkers	63849.5	432	6.8	4.0	16.2	1.34 (1.13-1.59)	1.30 (1.10-1.55)	1.21 (0.93-1.57)
P for trend						<0.001	<0.001	0.111

Note: P <0.001 for the overall interaction between obesity and alcohol intake categories for incident hepatic steatosis (model 2) and P =0.017 for the overall interaction between obesity and alcohol intake categories for incident hepatic steatosis plus intermediate/high FIB-4 (model 2)

[a] Estimated from parametric proportional hazard models. Multivariable model 1 was adjusted for age, sex, center and year of screening exam; model 2: model 1 plus adjustment for smoking status, regular exercise, education level, history of diabetes, medication for diabetes, history of hypertension, medication for diabetes and medication for dyslipidemia

[b] Estimated from parametric proportional hazard models with alcohol intake, smoking status, regular exercise, diabetes and hypertension as a time-dependent categorical variables and baseline age, sex, center, year of screening exam, and education level as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.