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# Genetic and life-style risk factors for advanced liver disease among men and women

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**Abstract**

***Background & Aims:*** Liver disease is traditionally categorized as alcoholic and non-alcoholic. We studied various risk factors predictive of advanced non-viral liver disease in general population and analyzed the interaction between these factors and alcohol consumption. ***Methods:*** Persons without underlying liver disease who participated in the Health2000 or FINRISK studies 1992-2012 comprised a cohort of 41260 individuals. Pattern of alcohol consumption and metabolic, life-style-related and anthropometric parameters were analyzed with Cox regression analysis using severe liver disease hospitalization, cancer or death as end-point. Viral liver diseases were excluded. ***Results:*** 355 liver events occurred during the mean 12.4-year follow-up (511789 person-years). In the multivariate model, age (HR 1.03, p=0.0083 for men, HR 1.04, p=0.0198 for women), waist-hip ratio (WHR) (HR 1.52, p=0.0006 for men, HR 1.58, p=0.0167 for women) PNPLA3 mutations (HR 1.9, p=0.024 for men, HR 2.7, p=0.0109 for women) and weekly binge drinking (HR 2.4, p=0.0024 for men, HR 7.4, p<0.0001 for women) predicted development of severe liver disease. Among men, diabetes (HR 2.7, p=0.0002), average alcohol consumption (HR for 10g/d 1.1, p=0.0022) non-married status (HR 1.9, p=0.0397 for single and HR 2.4, p=0.0002 for widow/separated) and serum HDL (HR 2.2, p=0.0022) and non-HDL cholesterol (HR 1.2, p=0.0237) were additional risk factors. Alcohol intake increased the risk especially among persons with high WHR (p for interaction 0.009). ***Conclusions:*** Age, PNPLA3 haplotype and WHR increase the risk for development of severe liver disease. We found strong synergism between alcohol and central obesity. Binge drinking is an additional risk factor.

***Key words:*** alcohol, liver disease, cirrhosis, risk factor

## Introduction

Liver cirrhosis is an end-result of several chronic liver diseases, e.g. viral hepatitis, autoimmune diseases, metabolic diseases and alcoholic liver disease (ALD). In Europe, ALD, non-alcoholic fatty liver disease (NAFLD) and viral hepatitis (B and C) are the leading causes of severe liver disease and liver-related deaths[1]. In case of viral and autoimmune hepatitis, the etiology is often easily diagnosed. Traditionally, ALD has been distinguished from NAFLD by exclusion of alcohol consumption exceeding certain threshold risk levels[2]. Alcohol is the only essential etiological factor in pure ALD[3]. However, other potential risk factors besides alcohol are poorly known[4]. The susceptibility to alcohol-induced liver damage is related to various factors including genetic determinants[5]. The risk factors for both ALD and NAFLD may coexist in same individuals[6] and obesity and metabolic syndrome (MS) are frequent among patients with alcoholic liver cirrhosis[7]. Since the clinical picture and histological changes are similar in both diseases, it is often challenging to distinguish ALD from NAFLD[6]. Moreover, alcohol may modify other potential risk factors of liver disease.

We aimed to determine the effect of genetic and life-style related risk factors of advanced liver disease in a large population-based cohort. Incidence of ALD and liver-related mortality are high in Finland[8, 9, 10], and the incidence has been increasing recently[9]. Thus, Finland offers a suitable ground for population-based studies on severe liver diseases. Aims of our study were to identify potential risk-factors and their interactions for development of non-viral advanced liver disease.

**Methods**

The study population consisted of the population-based health examination surveys, FINRISK and Health 2000. Detailed descriptions of study protocols have been published previously[11,12]. FINRISK is a Finnish population survey on risk factors of various diseases. The surveys with questionnaires, physiological measurements and blood sampling have been carried out every 5 years since 1972 by the National Institute for Health and Welfare (previously National Public Health Institute) using a random and representative population sample. In the present study, we included FINRISK cohorts from 1992 (n=5972), 1997 (n=8330), 2002 (n=8544), 2007 (n=6112) and 2012 (n=5676). The Health 2000 (n=6626) survey is also a comprehensive health interview and health examination study carried out in 2000-2001. DNA, serum and plasma samples have been collected in both the FINRISK and Health 2000 studies. Genotype data were available for a subset of participants (77%).

Participants were asked to report how often they had consumed alcoholic beverages during the previous year and the average portion they had consumed per week. Average alcohol consumption (grams per day) was calculated as the sum of the number of drinks multiplied by the average alcohol content per type of alcoholic beverage. Average intake was converted into daily doses (10 g of ethanol per dose). Consumption of wine, beer, and spirits were assessed separately as well as frequency of binge drinking (at least 60 grams per day) during the last 12 months (included in total average alcohol intake). Exercise was assessed by frequency of physical exercise for 20-30min until slightly out of breath and sweaty. Smoking was categorized as current, former and never smokers. Detailed description of the questionnaires is available at https://kite.fimm.fi/search#/browse .

Elevated blood pressure was defined as systolic blood pressure exceeding 130 mmHg and/or diastolic pressure 85 mmHg, or by the use of antihypertensive medication. Diabetes mellitus was defined by fasting serum glucose ≥7.0 mmol/L, taking diabetes

medication, or a prior known diabetes diagnosis. The non-HDL cholesterol was calculated by subtracting HDL cholesterol from total serum cholesterol concentration.

All residents of Finland have a unique personal identity code which is used in all national health registers. These codes were used to retrieve data of liver-related hospitalizations for the individuals in FINRISK and Health2000 surveys from the National Hospital Discharge register (HILMO), of liver cancers from the Finnish Cancer Registry, and of liver-related death from the national Causes-of-Death Register of Statistic Finland. These registries are nationwide, and follow-up of individuals living in Finland is virtually 100%. The liver diseases were defined by using the International Classification of diseases (ICD). The 10th revision has been used since year 1996. Persons with a diagnosis of any liver disease (ICD10:K70-K77, C22.0; ICD8/9: 570-573, 155.0) at the baseline were excluded. Additionally, individuals with viral hepatitis (ICD10 codes B18) at baseline or during the-follow-up were excluded. The number of viral hepatitis diagnoses in the cohort was low (n=100). The follow-up was done until December 2013. A diagnosis of liver disease (ICD8: 571.0, 571.8, 571.9, 573.0, 573.9; ICD-9: 571.1, 571.2, 571.3, 571.5, 571.8; ICD10: K70.1, K70.2, K70.3, K70.4, K70.9, K72.0, K72.1, K72.9, K74.0, K74.1, K74.2, K74.6), liver-related death (ICD8/9:570-573, 155.0; ICD10: K70-K77, C22.0) or liver cancer (C22.0) were designated as outcomes.

The individuals included to the study had given signed informed consent for the study and future registry linkage. The study was approved by the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District (previously studies also approved by the institutional review board of the National Public Health Institute).

Cox regression analysis with backward stepwise elimination was performed, separately for men and women, with liver-related death, liver disease and liver cancer as the outcome, and with age, marital status (single, married, divorced/widowed), education (low, average, high), employment (part- or full-time employment, retired, other), diabetes, waist-hip ratio (WHR), physical exercise, smoking status (former, current, never), alcohol consumption status (lifetime abstainer, current abstainer, user), average alcohol consumption (in doses of 10 g of ethanol), frequency of binge drinking (weekly, monthly, less often), serum levels of non-HDL cholesterol, HDL cholesterol and

triglycerides, elevated blood pressure, mutations in patatin-like phospholipase-containing domain 3 (PNPLA3), transmembrane 6 superfamily 2 (TM6SF2), HFE C282Y and H63D as independent variables. To analyze coherence in risk factor selection, we also performed Cox regression with forward stepwise selection. The proportional hazards assumption was tested using supremum test based on methods derived from cumulative sums of martingale residuals, and no violations were detected. Each independent risk factor was tested in age-adjusted subgroup analyses by diabetes status, alcohol intake (< vs > 30g/day for men or 20g/day for women), sex-specific WHR median, body mass index (BMI) (<20, 20-25, 25-30, >=30), marital status, and PNPLA3 carrier status. Separate analyses were performed with alcohol-related liver disease (ICD8: 571.0; ICD-9: 571.1, 571.2, 571.3; ICD10:K70.1, K70.2, K70.3, K70.4, K70.9) or non-alcoholic liver disease (ICD8: 571.8, k71.9, 573.0, 573.9; ICD-9: 571.5, 571.8; ICD10: K72.0, K72.1, K72.9, K74.0, K74.1, K74.2, K74.6) as the outcome by a fixed-model Cox regression with the risk factors from the primary backward elimination model as independent variables. In addition, a similar fixed-model analysis was performed with liver-related death as the outcome. The relationship between average alcohol intake, stratified by sex and WHR tertiles, and the risk for liver disease was examined using the penalized spline smoothing method[13] with adjustment for age. P-values <0.05 were considered statistically significant. Data were analyzed with SAS version 9.4, SPSS version 23 and R software version 3.2.5.

**Results**

After exclusion of persons with a known liver disease at baseline (n=288), and those with viral hepatitis (n=100), the final study cohort consisted of 41260 individuals. During the 511789 person-year follow-up, 355 incident liver events (245 in men and 110 in women) occurred, including 196 liver-related deaths and 51 liver cancer cases. The baseline characteristics of the study cohort are presented in Table 1 and a detailed description including details of alcohol consumption in supplementary files.

The final multivariate model included age, diabetes, WHR, average alcohol consumption, binge drinking frequency, serum non-HDL and HDL cholesterol, PNPLA3 carrier status

and marital status for men and age, WHR, binge drinking frequency and PNPLA3 carrier status for women. The hazard ratios (HR) of each factor are presented in Tables 2 and 3. Forward stepwise selection Cox regression yielded the same independent variables as the backward model.

In subgroup analysis, alcohol intake was a consistent risk factor across subgroups among both men (Figure 1) and women (Figure 2), and we found no signs of effect modification of BMI, diabetes, PNPLA3 or marital status on the association between average alcohol intake and liver disease risk. Detailed results of the subgroup analyses are presented in supplementary table.

In a fixed-model Cox regression analysis considering the significant independent risk factors from the primary analysis using alcohol-related and non-alcoholic liver events as end-points, alcohol intake was not a risk factor for non-alcoholic liver disease (HR for 10 g of ethanol 1.0, 95%CI 0.9-1.17 for male) but weekly binge drinking (HR 3.9, 95%CI 1.3-11.6 for men and HR 8.1, 95%CI 1.6-40.4 for women) was. In analysis with liver death as the outcome (139 liver deaths among men and 57 among women) and significant independent risk factors from primary analysis, all the same risk factors remained significant among men (data not shown). Among women, binge drinking and WHR were non-significant probably due to low number of events.

The interaction between alcohol intake and WHR is presented in Figures 3 and 4 showing hazard ratios of alcohol intake among persons divided into WHR tertiles, that indicate a dose-dependent increase of the risk. One daily dose of alcohol in average increases the risk in men with the highest WHR as much as 4 doses among other men (p for interaction 0.009).

**Discussion**

Our large nationwide population-based study showed that age, high WHR, weekly binge drinking and PNPLA3 predict development of incident severe liver disease.

Furthermore, average alcohol intake is a risk factor among women with BMI >20 and among all men. Diabetes increases the risk of severe liver disease in all men and in women with alcohol consumption <20 g/day. The harmful effect of alcohol intake is especially notable in men with high WHR, among whom even 1 daily dose of alcohol in average increased the risk of severe liver event over 5-fold.

In a large Swedish study, alcohol was an independent risk factor for severe liver disease in a dose-dependent pattern without any threshold[14]. Thus, the safe limit of alcohol intake can be questioned. Such "safe" limits may also vary depending on other factors such as central obesity. In our cohort, alcohol consumption was an independent risk factor only among men. Weekly binge drinking was related to severe liver events among both sexes. This might reflect the pattern of alcohol consumption among women suggesting that high overall consumption among women in the cohort might be largely manifested as binge drinking. However, alcohol intake was a risk factor in several female subgroups, e.g. among women with BMI over 20.

Weekly binge drinking predicted severe liver disease in both sexes. This has been reported previously[15]. In the present study, we found binge drinking as a risk factor for both alcohol-related and non-alcohol-related liver diseases even though total alcohol intake was a risk factor only for alcohol-related diagnosis among men. Binge drinking should be recognized as an important risk factor of severe liver disease irrespective of total alcohol intake and emphasized in general health promotion.

Previous reports show differences between various alcoholic beverages in risk of liver cirrhosis[16]. A recent study from UK reported higher risk of liver cirrhosis among women drinking daily and outside meals regardless of the total alcohol intake[17]. Unfortunately, our data did not include details of drinking habits besides amount consumed and frequency of binge drinking. Furthermore, enough data of fractions of various alcoholic beverages consumed by study participants was not available for analysis.

Age was an independent risk factor in males and females. Age is related to more advanced stage of both ALD[18] and NAFLD[19]. Age as a risk factor of severe liver events is comprehensible, since development of advanced liver disease takes time and older persons presumably have longer previous exposure to risk factors, e.g. lengthier history of alcohol consumption.

WHR was an independent risk factor among men and women. Other parameters reflecting overweight have been recognised as risk factors previously. High BMI is a risk factor for ALD[20] and has even a supra-additive effect on the risk of ALD[21]. Obesity is a known risk factor of NAFLD[22]. Overweight increases the risk of hepatocellular carcinoma (HCC), as well[23]. Various components of MS affect the risk of severe liver disease[24]. Our model recognized WHR as an independent risk factor. WHR seems to be the most appropriate indicator of overweight and predisposition to MS in the context of liver disease, as reported previously[25, 26, 27].

Alcohol may modify the effects of other potential risk factors[28]. While high alcohol consumption increases the risk of MS[29, 30], mild to moderate alcohol consumption is associated with lower MS risk[28]. However, in our cohort, even low alcohol intake increased the risk of severe liver disease among men with higher WHR, who presumably are also more susceptible to MS. Both HDL and non-HDL cholesterol were significant risk factors among men. Although high HDL level is usually considered beneficial, in context of liver disease this is contradictory. Higher HDL values are associated with elevated transaminase levels[31]. Furthermore, ethanol is a unique toxin, since it is capable of deteriorating almost all aspects of hepatic lipid metabolism leading to steatosis and changes in cholesterol synthesis and secretion[32]. The interaction between alcohol, lipid metabolism and the risk of liver disease warrants further studies.

Variation in certain genes, e.g. PNPLA3, TM6SF2 and MBOAT7 are related to the risk of liver cirrhosis[33, 34, 35]. Due to low number of PNPLA3 homozygotes among our cohort, we grouped heterozygote (CG) and homozygote (GG) carriers together. In the present study, PNPLA3 mutation increased the risk of severe liver disease in both sexes. Thus,

the genetic profile together with other factors determines an individual's risk of severe liver disease. Other gene variations did not enter the final model in the multivariate analysis as independent risk factors in this cohort.

Single or divorced status of men as an independent risk factor for liver disease is difficult to explain. It hardly has any biological connection to liver disease. Instead, single and divorced males might simply consume more alcohol or live more sedentary lifestyle. However, marital status is related to mortality and morbidity in the context of other diseases. For example, unmarried men have an increased risk of both cardiovascular and non-cardiovascular mortality[36]. Likewise, single, divorced and widowed men have higher mortality from alcohol-related causes[37]. In our study, marital status of men was a significant risk factor only for alcohol-related but not for non-alcohol-related liver diagnosis.

In clinical practice, ALD diagnosis is often based on the history of patient's alcohol consumption[2]. Contrary to many other countries, great majority of liver diagnoses in Finland are categorized as alcohol-related[10]. Due to co-existence and interaction of various risk factors, the dichotomous distinction between alcoholic and non-alcoholic liver disease seems inappropriate. Our results emphasize the importance of counselling about all potential liver-related risk factors as whole in health promotion. In individual level, discourage of alcohol consumption in abdominally obese persons is particularly important due to supra-additive effect of ethanol intake on their liver disease risk. The genetic risk profile is generally not determined in practice, but all modifiable risk factors should be considered in health promotion. Our results provide insights for creating a liver-specific risk score for estimating the risk of individuals, but this warrants further studies.

The strength of our study is the large number of persons in a representative nationwide cohort. The national health registries used in the study are considered precise[38,39]. Limitation of the study is lack of data of cases not requiring hospitalization. Thus, our results are only applicable to risk factors of severe liver disease. Since alcohol consumption and other habits were recorded only once at the time of the survey, the effects of possible changes in risk factors could not be studied. Persons with most

severe alcohol abuse might not participate in health surveys[40]. Furthermore, details of alcohol drinking patterns besides type of alcoholic beverage consumed and frequency of binge drinking (e.g. drinking outside meals, daily versus episodic drinking) were not recorded. Although questionnaires concerning alcohol consumption were very detailed, the amounts were self-reported and determination of actual intake can be somewhat challenging. This might influence the risk estimates of alcohol intake per dose. Some of the subgroup analyses might be underpowered as reflected in relatively wide confidence intervals.

In conclusion, alcohol consumption, especially binge drinking and factors reflecting metabolic syndrome increase the risk of advanced liver disease among both sexes. Genetic predisposition (PNPLA3) increases the risk, as well. Additionally, marital status is related to the risk among men. The development of severe liver disease seems to result from interplay of various genetic and environmental factors making dichotomous distinction between alcoholic and non-alcoholic liver disease inappropriate.

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Table 1. Baseline characteristics (mean and standard deviation) of the participants in the study.

| | All | Men | Women |
|---|---|---|---|
| Number of persons | 41260 | 19472 | 21788 |
| Number of liver events | 355 | 245 | 110 |
| Age, (years) | 49.6 (13.91) | 50.0 (13.63) | 49.2 (14.14) |
| Marital status: (married/single/divorced,widow) n(%) | 29877 (72.6)/ 5452 (13.2)/ 5841 (14.2) | 14774 (76.0)/ 2849 (14.7)/ 1805 (9.3) | 15103 (69.4)/ 2603 (12.0)/ 4036 (18.6) |
| BMI† | 26.8 (4.71) | 27.1 (4.12) | 26.5 (5.16) |
| WHR‡ | 0.9 (0.09) | 1.0 (0.07) | 0.9 (0.09) |
| Smoking status: current/former/never n(%) | 9791 (24.0)/ 9257 (22.6)/ 21822 (53.4) | 5625 (29.23)/ 5741 (29.84)/ 7875 (40.93) | 4166 (19.26)/ 3516 (16.26)/ 13947 (64.48) |
| Alcohol consumption: lifetime abstainer/current abstainer/user, n(%) | 3672 (9.0)/ 1891 (4.7)/ 35118 (86.3) | 936 (4.9)/ 1034 (5.3)/ 17281 (89.8) | 2736 (12.8)/ 857 (4.0)/ 17837 (83.2) |
| Alcohol intake (grams/week) | 75.5 (137.59) | 114.8 (172.2) | 38.6 (77.12) |
| Binge drinking : less often/monthly/weekly n(%) | 13902 (73.8)/ 2786 (14.8)/ 2149 (11.4) | 5239 (59.2)/ 1889 (21.4)/ 1717 (19.4) | 8663 (86.7)/ 897 (8.9)/ 432 (4.4) |
| Physical exercise: Twice a week/2-4 times per month/less often, n (%) | 19993 (57.4)/ 9246 (26.5)/ 5605 (16.1) | 9111 (55.5)/ 4358 (26.5)/ 2963 (18.0) | 10882 (59.1)/ 4888 (26.6)/ 2642 (14.4) |
| Cholesterol (mmol/l) | 5.5 (1.09) | 5.6 (1.10) | 5.5 (1.08) |
| HDL§ cholesterol (mmol/l) | 1.4 (0.38) | 1.3 (0.34) | 1.5 (0.39) |
| Triglycerides (mmol/l) | 1.5 (1.00) | 1.7 (1.14) | 1.3 (0.83) |
| PNPLA3¶ rs738409 CC/CG/GG | 19640 (60.0)/ 11406 (34.9)/ 1665 (5.1) | 9263 (60.1)/ 5370 (34.9)/ 771 (5.0) | 10377 (60.0)/ 6036 (34.9)/ 894 (5.1) |
| Elevated blood pressure, n(%) | 11536 (28.0)/ 29687 (72.0) | 4291 (22.0)/ 15170 (78.0) | 7245 (33.3)/ 14517 (66.7) |
| Diabetes, n(%) | 38121 (92.4)/ 3139 (7.6) | 17899 (91.9)/ 1573 (8.1) | 20222 (91.8)/ 1566 (7.2) |

†body mass index § high density lipoprotein ‡waist-hip ratio

¶ patatin-like phospholipase-containing domain 3 gene

Table 2. Predictors of advanced liver disease with hazard ratios (HR) and 95% confidence intervals (95%CI) among men by multivariate backward stepwise elimination Cox regression analysis.

| | HR | 95%CI | *p* |
|---|---|---|---|
| age (years) | 1.03 | 1.01-1.05 | 0.0083 |
| marital status (single versus married) | 1.85 | 1.03-3.33 | 0.0397 |
| marital status (divorced/widow versus married) | 2.37 | 1.43-3.91 | 0.0008 |
| diabetes | 2.72 | 1.61-4.61 | 0.0002 |
| WHR/SD† | 1.52 | 1.20-1.92 | 0.0006 |
| alcohol consumption (per 10 g ethanol) | 1.13 | 1.08-1.19 | <0.0001 |
| binge drinking weekly | 2.36 | 1.35-4.11 | 0.0024 |
| serum HDL‡ cholesterol | 2.21 | 1.33-3.67 | 0.0022 |
| serum non-HDL cholesterol | 1.19 | 1.02-1.39 | 0.0237 |
| PNPLA3§ (CG or GG versus CC) | 1.88 | 1.25-2.81 | 0.0024 |

†waist-hip ratio/1 standard deviation

‡high density lipoprotein

§patatin-like phospholipase-containing domain 3 gene (rs738409)

Table 3. Predictors of advanced liver disease with hazard ratios (HR) and 95% confidence intervals (95%CI) among women by multivariate backward stepwise elimination Cox regression analysis.

| | HR | 95%CI | *p* |
|---|---|---|---|
| age (years) | 1.04 | 1.01-1.07 | 0.0198 |
| WHR/SD† | 1.58 | 1.09-2.30 | 0.0167 |
| binge drinking weekly | 7.38 | 2.85-19.12 | <.0001 |
| PNPLA3‡ (CG or GG versus CC) | 2.73 | 1.26-5.92 | 0.0109 |

†waist-hip ratio/1 standard deviation

‡patatin-like phospholipase-containing domain 3 gene (rs738409)

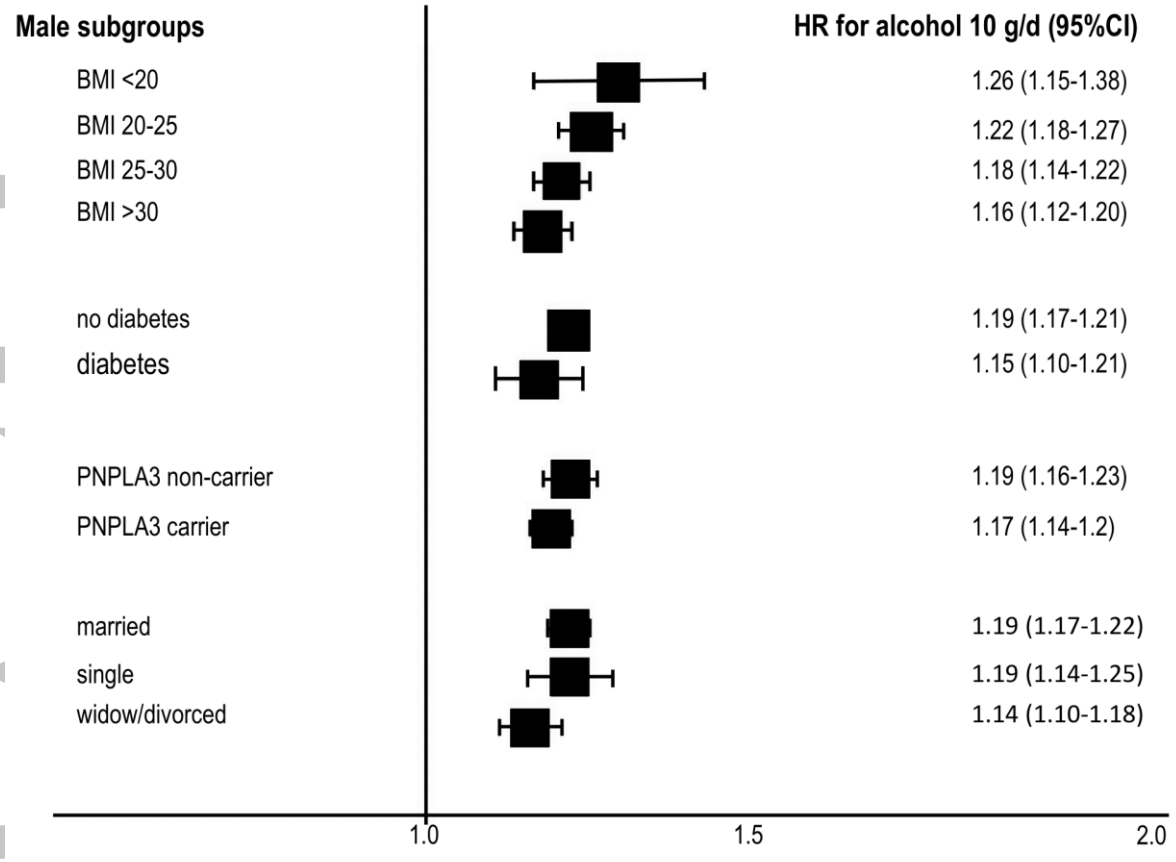


Figure 1. Alcohol intake as a risk factor for advanced liver disease among male subgroups. Hazard ratios (HR) with 95% confidence intervals for 10g alcohol per day.

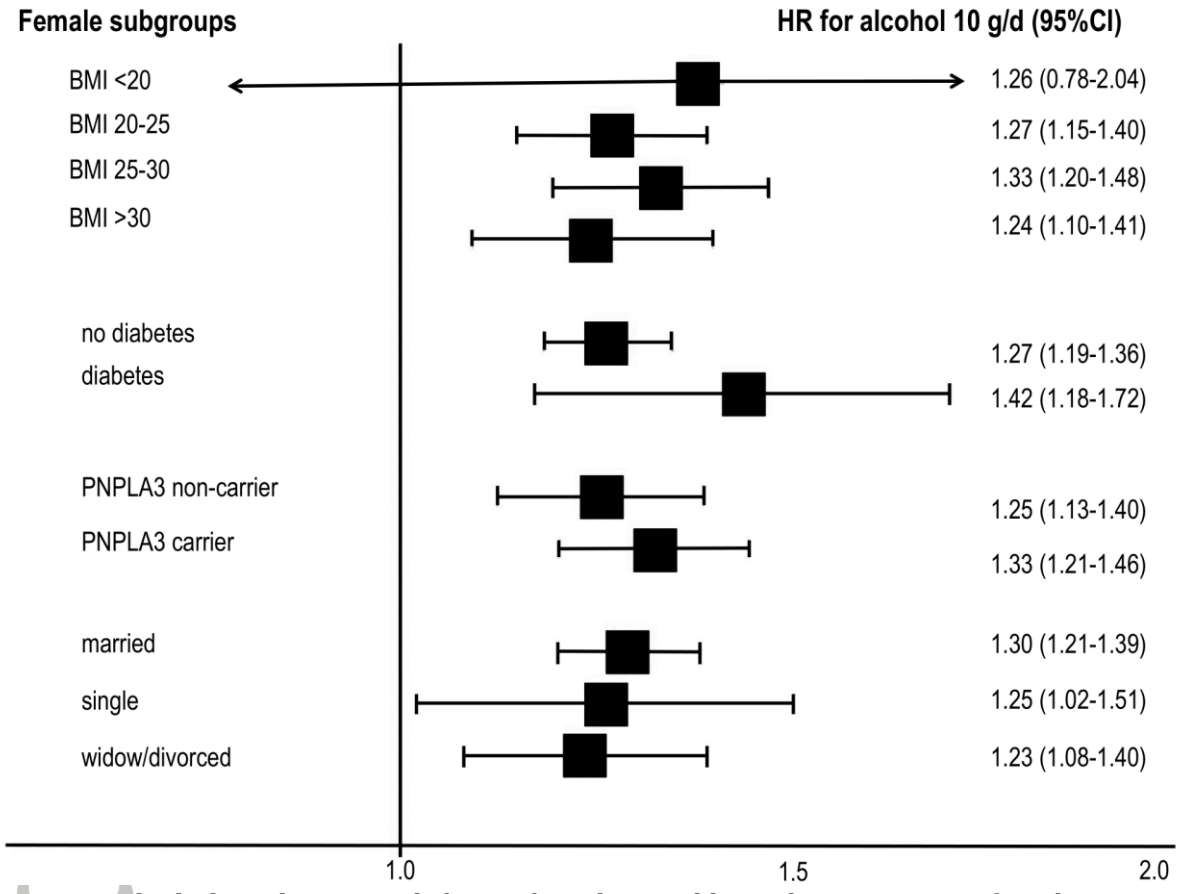


Figure 2. Alcohol intake as a risk factor for advanced liver disease among female subgroups. Hazard ratios (HR) with 95% confidence intervals for 10g alcohol per day.

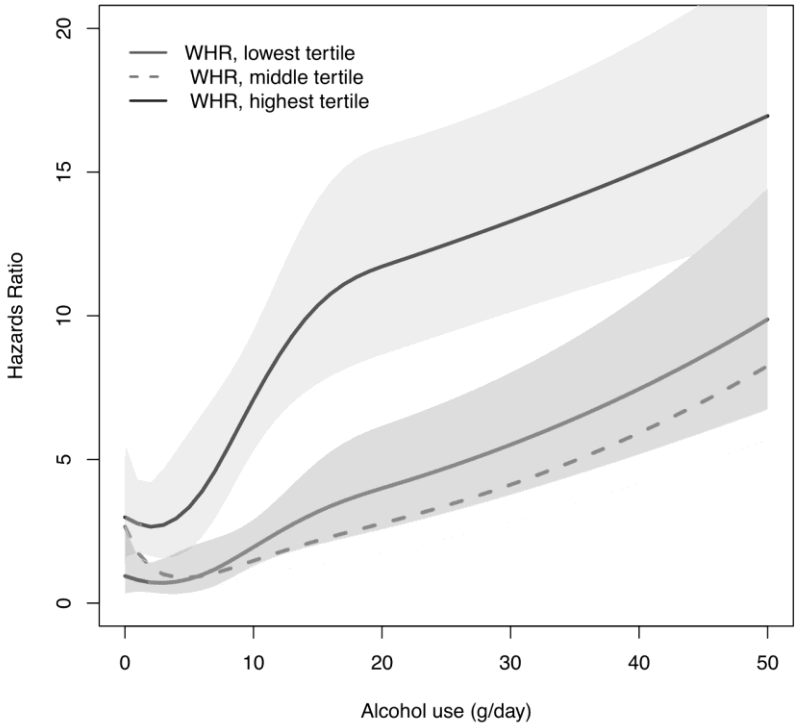


Figure 3. The risk of advanced liver disease based on average alcohol intake in men grouped according to waist-hip ratio (WHR).

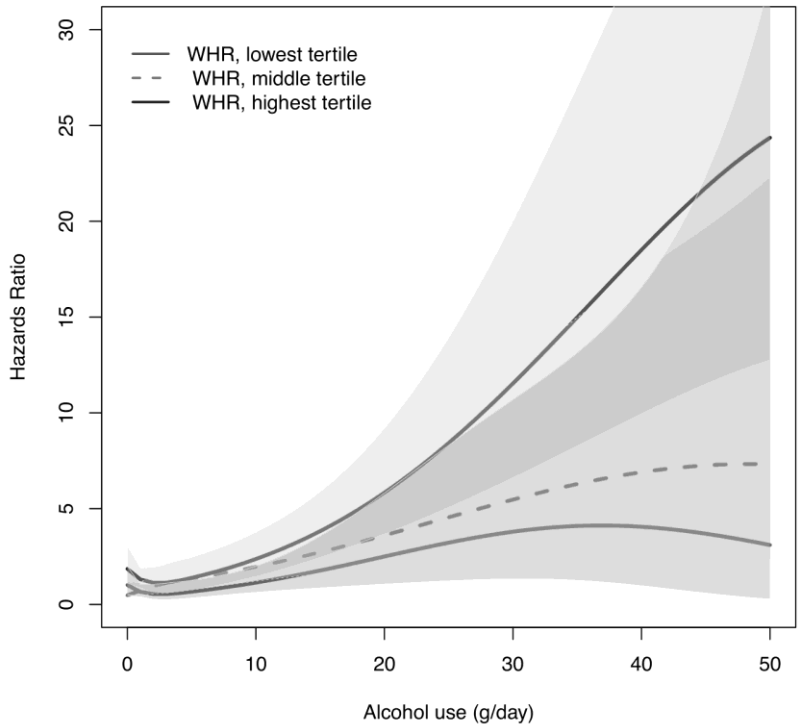


Figure 4. The risk of advanced liver disease based on average alcohol intake in women grouped according to waist-hip ratio (WHR).