Serum lipopolysaccharides predict advanced liver disease in the general population
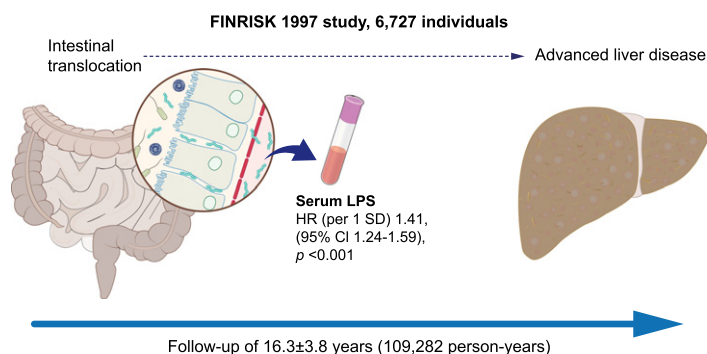
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Graphical abstract

FINRISK 1997 study, 6,727 individuals

Intestinal translocation --→ Advanced liver disease

Serum LPS
HR (per 1 SD) 1.41,
(95% CI 1.24-1.59),
p <0.001

Follow-up of 16.3±3.8 years (109,282 person-years)

Highlights

- Serum LPS was associated with incident advanced liver disease in the general population.

- LPS was associated with liver-related risk of hospitalization, cancer or death.

- The highest LPS tertile may account for up to 30% of the risk of incident liver disease.

- The risk of liver disease was accentuated among carriers of the *PNPLA3* I143M.

- Serum LPS was not associated with all-cause mortality.

Lay summary
Lipopolysaccharide, a gut-derived bacterial endotoxin, has been implicated in the development of chronic liver disease, but its relevance at the population level remains unclear. We found that serum lipopolysaccharide levels were associated with incident advanced liver disease in the general population, with the highest tertile accounting for up to 30% of the risk of hospitalization, cancer or death related to liver disease.

Serum lipopolysaccharides predict advanced liver disease in the general population

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Background & Aims: Gut-derived endotoxemia has been implicated in the development of chronic liver disease, but its relevance at the population level remains unclear. We analyzed whether endotoxemia is associated with incident advanced liver disease in the general population.

Methods: Serum lipopolysaccharide (LPS) was measured in 6,727 (3,455 male and 3,272 female, mean age 53.4 ± 10.9 years, mean body mass index 27.2 ± 4.5) individuals participating in the Finnish population-based health examination survey FINRISK 1997. Data were linked with electronic health registers for incident advanced liver disease (hospitalization, cancer or death related to liver disease). During a mean follow-up of 16.3 ± 3.8 years (109,282 person-years), 86 liver events occurred. Univariate and multivariate Cox regression, and Kaplan-Meier analyses were performed.

Results: Serum LPS predicted incident advanced liver disease with a hazard ratio per 1 SD of 1.41 (95% CI 1.24–1.59; p <0.001) when adjusted for age, sex, gamma-glutamyltransferase, metabolic syndrome, alcohol use, patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) I148M, waist-hip ratio and type 2 diabetes. This association remained robustly significant in additional multivariate analyses with various levels of adjustment. The association was accentuated among carriers of the *PNPLA3* risk variant. The population attributable fraction of the highest LPS tertile for liver events was 29.7%. However, LPS was not associated with all-cause mortality.

Conclusion: Serum LPS is associated with hospitalization, cancer or death related to liver disease in the general population, with the highest tertile potentially accounting for 30% of the risk of liver disease.

Introduction

Chronic liver disease is a major health problem and an increasing cause for morbidity and mortality.[1,2] Finland has one of the highest liver disease mortality rates in Europe.[2] The 2 most common chronic liver diseases in Western populations are non-alcoholic fatty liver disease (NAFLD) and alcohol-related liver disease (ALD).[3] The global prevalence of NAFLD is 25%, and with continuously rising obesity rates, the incidence of NAFLD is rising too, explaining the majority of the increasing incidence of chronic liver disease.[2,4] Alcohol-related liver disease is another major cause of liver cirrhosis, and it is the leading cause of liver mortality in the Western world.[2] Together obesity and alcohol have a synergistic effect on the risk of liver disease.[5] Genetic factors also modify the risk of liver disease. The 2 most important identified genetic risk variants are patatin-like phospholipase domain-containing 3 (*PNPLA3*) at rs738409 C>G and transmembrane 6

superfamily member 2 (*TM6SF2*) at rs58542926 C>T. In Western populations, 35–40% of individuals are heterozygous and 5% homozygous for the *PNPLA3* variant,[6] whereas about 7% are heterozygous for *TM6SF2*.[7]

The role of gut microbiota in the pathogenesis of various liver diseases has garnered interest in recent years. Alterations in gut microbiota have been associated with liver cirrhosis, ALD (including alcoholic hepatitis) and NAFLD. Interestingly, the degree of gut microbiota dysbiosis associates with the stage of liver injury.[8,9] However, the pathogenetic mechanisms behind these associations are still largely unknown. The relevance of gut dysbiosis for development of liver disease on a population level is also unknown.

Lipopolysaccharide (LPS) is a bacterial endotoxin, derived from intestinal gram-negative bacteria. In healthy individuals, only small amounts of LPS translocate through the gut and reach the liver, where LPS is taken up by Kupffer cells and removed.[10] This is an important process with the aim of preventing activation of toll-like receptor 4 (TLR4), which would lead to the release of inflammatory cytokines, induction of liver fibrosis development, and progression to cirrhosis.[11] As a marker of gut dysbiosis and increased intestinal translocation, increased serum LPS activity has been associated with ALD, NAFLD and non-alcoholic steatohepatitis (NASH), and with liver fibrosis and hepatocellular carcinoma (HCC).[11,12]

We aimed to investigate if the level of circulating LPS predicts liver-related outcomes at the population level in a cohort of 6,272 individuals from the general population.

Materials and methods

Study population

The National FINRISK 1997 Study is a population-based health survey that originally consisted of 8,446 individuals (from 11,500 invited). It was conducted in 5 geographical areas in Finland, and the age range of study participants was 25 to 74 years.[13] The survey methods follow the WHO MONICA protocol.[14] The study included a self-administered questionnaire and clinical examination of weight, height, and blood pressure measurements, as well as blood samples. The study was approved by the ethics committee of the National Public Health Institute, and it was conducted according to the Declaration of Helsinki. All individuals gave informed consent for the study and for future registry linkage.

Baseline variables

Of the original sample of 8,446 adults, 6,727 individuals who had an available serum LPS measurement were included in this study. At baseline, study participants were asked to report how often they consumed alcoholic beverages during the previous year and the average amount they consumed per week during the previous month. Average alcohol consumption (grams of 100% alcohol per day) was calculated based on these data. The respondents were also questioned how often they drank alcohol to the level that they felt intoxicated (weekly, monthly or less often). In addition, smoking status (never, former, current smoker), amount of smoking (number of cigarettes per day) and exercise habits (frequency of moderate-intensity or high-intensity physical exercise for at least half an hour) were asked. Diabetes was defined either by taking diabetes medication or a known diabetes diagnosis. Metabolic syndrome was defined according to the Joint Interim Statement criteria.[15]

Laboratory analysis

Participants were asked to fast 4 h and to avoid heavier meals prior to blood sampling. The median fasting time was 5 (interquartile range 3–7) h. Lipids and gamma-glutamyltransferase (GGT) measurements were done from fresh serum samples. The rest of the serum and plasma biomarkers were determined from samples stored at -70°C. Ultrasensitive C-reactive protein (CRP) was determined with Architect c8000 analyzer (Abbott Laboratories, Abbott Park, IL). Serum endotoxin activities were determined by a Limulus amebocyte lysate assay coupled with a chromogenic substrate (HyCult Biotechnology B.V., Uden, the Netherlands), and the interassay coefficient of variation was 9.2% (n = 75). The laboratory analyses have been described in detail previously.[16] Non-high-density lipoprotein (HDL) cholesterol was calculated by subtracting HDL from total cholesterol.

PNPLA3 at rs738409, TM6SF2 at rs58542926, membrane-bound O-acyltransferase domain-containing protein 7 (MBOAT7) at rs641738 and hydroxysteroid 17-beta dehydrogenase (HSD17B13) at rs72613567 were genotyped using Illumina's 610K, Omniexpress and HumanCoreExpress chips.

Sources of outcome data

Follow-up data for hospitalizations were obtained from the National Hospital Discharge Register, which covers all hospitalizations in Finland beginning in 1969. Data for cancers were obtained from the Finnish Cancer Registry, and vital status and cause of death data were obtained from Statistics Finland, which systematically collects data about the deaths of all Finnish citizens. In Finland, each person who dies is by law assigned a cause of death (in accordance with the International Classification of Diseases) to the official death certificate, issued by the treating physician based on medical or autopsy evidence, or forensic evidence when necessary; the death codes are then verified by medical experts at the register and recorded according to systematic coding principles. One or several International Statistical Classification of Diseases and Related Health Problems (ICD) diagnoses are assigned to each hospitalization at discharge; these diagnosis codes are systematically recorded in the National Hospital Discharge Register. Data collection to all these registries is obligatory and general quality is consistent and complete. Linkage was performed using the unique personal identifiers assigned to all Finnish residents. Follow-up for death and hospitalizations was until December 2013, and for cancers until December 2012.

Definition of outcome events

The primary study endpoint was the first hospitalization due to advanced liver disease or liver-related death or a diagnosis of primary liver cancer, whichever came first. Regarding liver death, outcome events comprised ICD8/9 codes 155.0, 570-573 and 155.0, and ICD10 codes K70-K77 and C22.0, in line with previous studies.[17,18] Regarding hospitalization, we included events with the following ICD codes reflective of advanced liver disease, cirrhosis or equivalent: ICD8: 571.0, 571.8, 571.9, 573.0, 573.9; ICD-9: 571.1, 571.2, 571.3, 571.5, 571.8 and ICD10: K70.1-K70.4, K70.9, K72.0, K72.1, K72.9, K74.0-K74.2, K74.6. Liver cancer was defined by ICD8/9 code 155.0 and ICD10 code C22.0. We excluded participants with records showing that any liver event had occurred before the study baseline.

Statistics

For comparing groups, we used Chi-squared, Mann-Whitney U, or Kruskal-Wallis tests as appropriate. Correlations between continuous variables were calculated by Spearman correlation. The effect of LPS on the risk of liver events was analyzed by univariate and multivariate Cox regression with time to first liver event as the outcome variable. LPS was tested both as a continuous variable and divided into tertiles. In the multivariate Cox regression analysis, we adjusted for variables associated with liver events with a p value <0.1 and/or variables with known association with liver disease from previous studies. To prevent overfitting from the inclusion of too many variables, we performed multiple multivariate models with various levels of adjustment. The main multivariate model was adjusted for well-acknowledged risk factors of liver disease, also taking into account the number of liver events to prevent overfitting: age, sex, waist-hip ratio, diabetes, average alcohol use, metabolic syndrome, GGT, and PNPLA3 I148M variant. Additional models were adjusted for age and sex, significant (p <0.1 on univariate analysis) metabolic factors, alcohol factors, other lifestyle factors, genetics, GGT and CRP.

Two-way interaction effects for incident liver events were tested between LPS and PNPLA3, TM6SF2 and MBOAT7 risk variants by including LPS and the genetic risk factors as well as an interaction term in the Cox regression. In case of a significant interaction effect, we performed subgroup analyses to evaluate how the risk estimate of LPS differed in the subgroups.

The population attributable fraction for the highest LPS tertile was calculated, following Miettinen,[19] as $p_c(1-1/HR)$, where p_c is

the prevalence of the highest LPS tertile among individuals who developed the liver outcome and HR is the hazard ratio for liver outcomes in the highest LPS tertile. We used the Kaplan-Meier method and Log-Rank test to analyze the cumulative incidence of liver events and all-cause mortality by LPS tertiles. $p < 0.05$ was considered statistically significant. A possible non-linear relationship between LPS and the liver outcome was examined using the penalized spline smoothing method.

Data were analyzed with SPSS version 25 (IBM Inc., Armonk, NY and R software version 3.6.0).

Results

Clinical characteristics

The study cohort consisted of 6,727 individuals, 3,455 men and 3,272 women, mean age 53.4 ± 10.9 and mean body mass index (BMI) 27.2 ± 4.5 (Table 1). Mean follow-up was 16.3 ± 3.8 years (range 0–17.8, 109,282 person-years of follow-up). During follow-up, 86 individuals experienced a severe liver event (first hospitalization due to advanced liver disease or liver disease-related death or a diagnosis of primary liver cancer). The first liver event occurred a mean of 10.6 ± 3.7 years (range 1–18 years) after baseline. Of the liver events, 57 (66%) occurred among men and 29 (34%) among women, with a mean time to first event of 10.4 and 11.0 years, respectively ($p < 0.001$). The first recorded liver event was hospitalization in 31 cases, liver cancer in 18 cases, and liver disease-related death in 37 cases.

The mean LPS activity in serum was 63.0 ± 37.4 pg/ml. We divided the study cohort into tertiles based on the LPS level, those with LPS <43.4 pg/ml (n = 2,258, mean 31.6 ± 33 pg/ml), those with 43.4–68 pg/ml (n = 2,228, mean 54.7 ± 54.3 pg/ml) and those with >68 pg/ml (n = 2,241, mean 102.8 ± 91.1 pg/ml).

There were statistically significant differences among the LPS tertiles in all baseline variables except sex, alcohol use (grams per week and status of alcohol use), frequency of intoxication episodes, amount of exercise, *TM6SF2* rs58542926 genotype and *MBOAT7* rs641738 genotype (Table 1). We found a trend towards a higher burden of metabolic risk factors in the higher LPS tertiles, but less smoking and non-significant differences in alcohol exposure (Table 1). Individuals in the highest LPS tertile were slightly less often *PNPLA3* I148M carriers than those in the lowest or middle tertiles (38% *vs.* 41%, $p = 0.03$).

The correlation coefficient between LPS and BMI was 0.232, ($p < 0.001$). There was no meaningful correlation between LPS and weekly alcohol intake (r = -0.004, $p = 0.776$), carbohydrate-deficient transferrin (r = -0.098, $p < 0.001$), GGT (r = 0.186, $p < 0.001$) or waist-hip ratio (r = 0.187, $p < 0.001$).

As thrombocytopenia might reflect underlying asymptomatic/ undetected advanced liver disease, we analyzed whether there was a correlation between platelet level and LPS in a subgroup of 536 individuals with platelet measurements available. However, we found no such correlation between LPS and platelet level (r = -0.004, $p = 0.92$, Fig. S1). In addition, we tested the correlation between LPS and CRP, but found only a weak correlation (r = 0.15, $p < 0.001$, Fig. S2).

Serum LPS predicts liver-related outcome

Univariate Cox regression results are shown in Table S1. Serum LPS level at baseline predicted liver-related outcomes on univariate analysis (HR per 1 SD 1.41; 95% CI 1.24–1.59; $p < 0.001$). In the main multivariate model shown in Table 2, the HR for LPS in predicting incident advanced liver disease was 1.31 (95% CI

1.11–1.54; $p < 0.001$). The HR for LPS remained fairly stable (1.3–1.5) in additional models with various level of adjustment (Table 2).

When adjusted for age and sex, individuals in the highest LPS tertile had a 2-fold increased risk of incident severe liver disease compared to those in the lowest tertile (Fig. 1).

PNPLA3 I143M modifies the association between LPS and liver-related outcomes

We found a significant interaction effect for liver events between LPS and *PNPLA3* I143M ($p = 0.015$ for the interaction term), but not with the other genetic risk variants. When we stratified the cohort by *PNPLA3* I143M carrier status (heterozygote/homozygote *vs.* no risk variant), we found that HRs for liver events of the highest LPS tertile were higher among individuals with the G variant of *PNPLA3* (HR 3.7; 95% CI 1.604–8.646; $p = 0.002$) than among those without the *PNPLA3* risk variant (HR 2.6; 95% CI 1.103–6.250; $p = 0.029$) (Fig. 2).

Population attributable fraction

The population attributable fraction of the highest LPS tertile for incident advanced liver disease was 26.8% when adjusting for age and sex, and 29.7% when additionally adjusting for metabolic syndrome, diabetes, waist-hip ratio, weekly alcohol use, GGT and *PNPLA3* I143M variant.

Serum LPS had no association with all-cause mortality

During follow-up, 1,417 individuals died. We found no difference in all-cause mortality according to the LPS tertile (Fig. 3); compared to the lowest LPS tertile, HR for the middle tertile was 1.04 (95% CI 0.91–1.18; $p = 0.58$) and HR for the highest tertile was 1.08 (95% CI 0.95–1.23; $p = 0.22$). This indicates that the competing risk of death does not explain the association between LPS and liver events.

Discussion

Our main finding was that serum LPS is independently associated with incident advanced liver disease in the general population. This association remained significant after adjusting for relevant confounders in multiple multivariate models. As circulating LPS (endotoxemia) is likely derived from the gut, our findings suggest that the gut is an important player in the development of liver disease on a population level. In fact, we estimated that up to 27–30% of cases of advanced liver disease in the population are attributable to a high LPS level (highest tertile). Therefore, targeting the factors that lead to increased mucosal permeability and endotoxemia may be a key in preventing advanced liver disease. To our knowledge, this is the first study linking serum LPS with incident advanced liver disease at a population level.

An increase in serum LPS levels likely reflects endotoxemia resulting from increased mucosal permeability, impaired intestinal mucosal integrity and alterations in gut microbiota, because gram-negative bacteria are known to produce LPS.[10] Alterations in gut microbiota are linked with many types of chronic liver disease including NAFLD, ALD, viral hepatitis, liver cirrhosis and HCC.[8] These changes, together with altered intestinal capacity to neutralize LPS toxicity, decreased liver degradation of LPS, and disrupted mucosal integrity, predispose to endotoxemia and progression of liver disease.[8,20]

Previous clinical studies reporting associations between endotoxemia and advanced liver diseases have been performed in

Table 1. Baseline characteristics of the whole study population.

	All subjects (mean ± SD)	LPS lowest tertile (mean ± SD)	LPS middle tertile (mean ± SD)	LPS highest tertile (mean ± SD)	*p* value
	n = 6,272	n = 2,258	n = 2,228	n = 2,241	
Sex male/female (%)	3,455/3,272 (49)	1,123/1,125 (50)	1,162/1,066 (48)	1,170/1,071 (48)	0.165
Age (years)	53.4 ± 10.9	52.5 ± 11.3	53.5 ± 10.9	54.1 ± 10.3	<0.001
BMI (kg/m^2)	27.2 ± 4.5	26.1 ± 4.3	27.2 ± 4.3	28.3 ± 4.6	<0.001
Waist circumference (cm)	90.3 ± 13.4	87.1 ± 12.9	90.1 ± 13.1	93.6 ± 13.4	<0.001
Waist-hip ratio	0.88 ± 0.1	0.86 ± 0.1	0.88 ± 0.1	0.90 ± 0.1	<0.001
Metabolic syndrome (%)	1,355 (20)	283 (13)	406 (18)	666 (30)	<0.001
Type 2 diabetes (%)	478 (7)	111 (5)	170 (8)	197 (9)	<0.001
Alcohol use (g/week)	72 ± 139	69 ± 130	71 ± 129	77 ± 156	0.760
Alcohol use					0.488
Life-time abstainer	600 (9)	185 (8)	203 (9)	212 (10)	
Current abstainer	349 (5)	120 (5)	108 (5)	121 (6)	
Alcohol user	5,680 (84)	1,927 (86)	1,886 (86)	1,867 (85)	
Frequency of intoxication episodes					0.567
Less often	4,219 (63)	1,440 (77)	1,407 (77)	1,372 (75)	
Monthly	944 (14)	305 (16)	313 (17)	326 (18)	
Weekly or more often	374 (6)	124 (7)	117 (6)	133 (7)	
Systolic blood pressure (mmHg)	139.0 ± 20.4	136.4 ± 20.2	139.2 ± 20.7	141.4 ± 20.1	<0.001
Diastolic blood pressure (mmHg)	84.0 ± 10.9	82.3 ± 10.8	83.9 ± 10.7	85.8 ± 11.0	<0.001
Total cholesterol (mmol/L)	5.7 ± 1.0	5.4 ± 1.0	5.7 ± 1.0	6.0 ± 1.1	<0.001
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.4	1.3 ± 0.4	<0.001
Non-HDL cholesterol (mmol/L)	4.2 ± 1.0	3.9 ± 0.9	4.3 ± 0.9	4.7 ± 1.0	<0.001
GGT (U/L)	38.0 ± 62.5	32.6 ± 36.0	35.1 ± 37.1	46.4 ± 94.6	<0.001
CDT (%)	0.61 ± 0.26	0.64 ± 0.27	0.60 ± 0.24	0.59 ± 0.26	<0.001
CRP (mg/L)	2.5 ± 5.8	2.5 ± 7.0	2.3 ± 4.6	4.7 ± 1.1	<0.001
Smoking					0.003
Current	1,459 (22)	534 (25)	459 (21)	466 (21)	
Former	1,642 (24)	505 (23)	539 (25)	598 (27)	
Never	3,416 (51)	1,144 (52)	1,157 (54)	1,115 (51)	
Exercise 20-30 mins per week					0.277
At least 2 times a week	3,784 (56)	1,296 (58)	1,252 (58)	1,236 (57)	
2-4 times a month	1,740 (26)	597 (27)	552 (26)	591 (27)	
Less often	1,042 (16)	325 (15)	361 (17)	356 (16)	
PNPLA3 rs738409					0.033
CC	3,520 (52)	1,176 (59)	1,153 (60)	1,191 (61)	
CG	2,042 (30)	721 (36)	655 (34)	666 (34)	
GG	320 (5)	103 (5)	128 (7)	89 (4)	
TM6SF2 rs58542926					0.129
CC	5,218 (78)	1,750 (87)	1,725 (89)	1,743 (89)	
CT	664 (10)	249 (12)	214 (11)	201 (10)	
TT	19 (0.3)	8 (0.4)	3 (0.2)	8 (0.4)	
MBOAT7 rs641738					0.873
CC	1,962 (29)	670 (39)	655 (39)	637 (38)	
CT	2,345 (35)	781 (45)	774 (46)	790 (47)	
TT	780 (11)	269 (16)	249 (15)	262 (16)	
HSD17B13 rs72613567					0.941
T/T	3,657 (62)	1,253 (63)	1,196 (62)	1,208 (62)	
T/TA	1,968 (33)	656 (33)	655 (34)	657 (34)	
TA/TA	265 (5)	94 (4)	86 (4)	85 (4)	

Table 2. Hazard ratios for incident severe liver events (hospitalization, liver cancer or liver deaths) according to serum LPS based on Cox regression analyses.

	Adjusted HR (/1 SD) of LPS	95% CI	*p* value
Main model	1.31	1.11-1.54	0.001
Sensitivity analyses			
Model 1	1.41	1.24-1.61	<0.001
Model 2	1.35	1.15-1.58	<0.001
Model 3	1.37	1.16-1.62	<0.001
Model 4	1.40	1.20-1.64	<0.001
Model 5	1.49	1.30-1.71	<0.001
Model 6	1.39	1.19-1.63	<0.001
Model 7	1.29	1.10-1.51	0.001

BMI, body mass index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HR, hazard ratio; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; LPS, lipopolysaccharide; MBOAT7, membrane-bound O-acyltransferase domain-containing protein 7; PNPLA3, patatin-like phospholipase domain-containing protein 3; TM6SF2, transmembrane 6 superfamily member 2.
Adjusted for: Main model: Age, sex, GGT, metabolic syndrome, alcohol usage, *PNPLA3* at rs738409, waist-hip ratio and type 2 diabetes. Model 1: Age and sex. Model 2: Age, sex, BMI, waist-hip ratio, elevated blood pressure, cholesterol, non-HDL cholesterol. Model 3: Age, sex and alcohol (alcohol usage, intoxication episodes). Model 4: Age, sex and alcohol (carbohydrate-deficient transferrin and intoxication episodes). Model 5: Age, sex, genetics (*PNPLA3* rs738409, *TM6SF2* rs58542926, *MBOAT7* rs641738, *HSD17B13* rs72613567). Model 6: Age, sex and lifestyle factors (smoking, alcohol use, amount of exercise, intoxication episodes). Model 7: GGT and C-reactive protein.

quite small populations. For example, endotoxemia was associated with NAFLD in a general population cohort of 920 individuals, but not with liver fibrosis measured by elastography.[21] However, in a smaller study of 237 individuals by the same authors, endotoxemia was associated with NASH and liver fibrosis.[22] Endotoxemia has also been linked with ALD.[8] Interestingly, it seems that the effect of alcohol on intestinal permeability is transient in the absence of pre-existing liver cirrhosis.[23] However, that indicates that also binge drinking, even without liver disease, can lead to increased LPS activity and possibly activation of TLR4 in the liver.[24] Epidemiological data strongly suggest that binge drinking is responsible for an increased incidence of cirrhosis.[25] There is also evidence linking endotoxemia with other chronic liver diseases including viral hepatitis, primary biliary cholangitis and primary sclerosing cholangitis.[11] Interestingly, endotoxemia has been linked with the development of portal hypertension in mice,[26] and also with complications from portal hypertension in those with liver cirrhosis. For example, higher levels of endotoxemia have been reported in those with liver cirrhosis and hepatic encephalopathy[27] and it has been hypothesized to be a trigger of coagulopathy in cirrhosis.[28] These findings support our results of serum LPS as a predictor or indicator of advanced liver disease.

It is important to keep in mind that although LPS can activate inflammatory pathways and induce progression of NAFLD[29] and ALD,[30] there may also be other gut-derived metabolites (for example bile acids), which are important in the pathogenesis of liver diseases.[29] Taken together, our study

suggests that gut microbiota-derived endotoxemia (as a marker of LPS in the circulation) is associated with advanced liver disease, highlighting the importance of the gut-liver axis. Thus, more research is needed on the role of gut microbiota in different liver diseases, and to identify gut microbiota-derived molecules that could act as novel biomarkers.

Our study raises the question of whether LPS could be used as a biomarker for predicting advanced liver disease. We found a rather small but significant association of LPS with liver-related outcomes (Fig. 1, Table 2). However, when we analyzed study individuals in LPS tertiles, those in the highest LPS tertile had a higher cumulative risk of liver-related outcomes compared to others (Fig. 1). Whether LPS measurements could be used as an independent marker (or as part of a non-invasive score) of advanced liver disease, for example in a selected population with known liver disease, should be tested in future studies.

We found that those in highest LPS tertile had significantly higher cumulative incidence for liver events, but there was no difference in all-cause mortality (Fig. 3). This suggests that the association between LPS and liver events are not confounded by competing risk issues. Previously, serum LPS activity has been associated with metabolic syndrome and it has been suggested that LPS-mediated processes could be key factors in metabolic disturbances associated with obesity.[31] However, also in that case, circulating LPS could originate from the gut and reflect underlying NAFLD, which is a cause of many metabolic disturbances.[32] This is supported by the fact that in our cohort LPS was an independent predictor of liver-related outcomes after adjusting for metabolic factors. We acknowledge that not all LPS in the circulation is derived from the gut, as it can also originate from the oral cavity, respiratory, and genitourinary tracts, or from food; it can also be modulated by antibiotics.[33] However, the gut is the major reservoir of LPS, because the gut microbiota is the largest source of gram-negative bacteria in the body.[34]

To our knowledge, this is the first population study to analyze interactions between LPS and liver disease-associated genetic variants. Interestingly, circulating LPS was a stronger risk factor for liver-related outcomes in those with *PNPLA3* I148M G risk allele compared to those without the risk allele (Fig. 2). However, this finding should be interpreted with caution, because the confidence intervals were very wide, which implies that statistical power is suboptimal for this subgroup analysis. It is known that *PNPLA3* I148M variant as such causes more aggressive liver disease and it is suggested to contribute directly to fibrogenesis and carcinogenesis via retinol availability in stellate cells.[35] The explanation of our results is possibly that *PNPLA3* I148M could be a surrogate marker of NAFLD, because those with risk variants have markedly higher liver fat content.[36] Alternatively, variants could confer an additive risk of liver disease and at this point it is unclear whether the *PNPLA3* genotype could have a direct effect on gut microbiota (the expression level of *PNPLA3* in the gut is minimal[37]), thus increasing the probability of liver disease.

BMI, body mass index; CDT, carbohydrate-deficient transferrin; CRP, C-reactive protein; GGT, g-glutamyltransferase; HDL, high-density lipoprotein; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; LPS, lipopolysaccharide; MBOAT7, membrane-bound O-acyltransferase domain-containing protein 7; PNPLA3, patatin-like phospholipase domain-containing protein 3; TM6SF2, transmembrane 6 superfamily member 2. Missing individuals in different categories: Smoking n = 210, alcohol use n = 98, frequency of intoxication episodes n = 1,190, exercise n = 161, *PNPLA3* rs738409 n = 845, *TM6SF2* rs58542926 n = 826, *MBOAT7* rs641738 n = 1,640, *HSD17B13* rs72613567, n = 837. The difference between LPS tertiles was tested with Kruskal-Wallis test and Pearson Chi-Square test (between categorial variables).
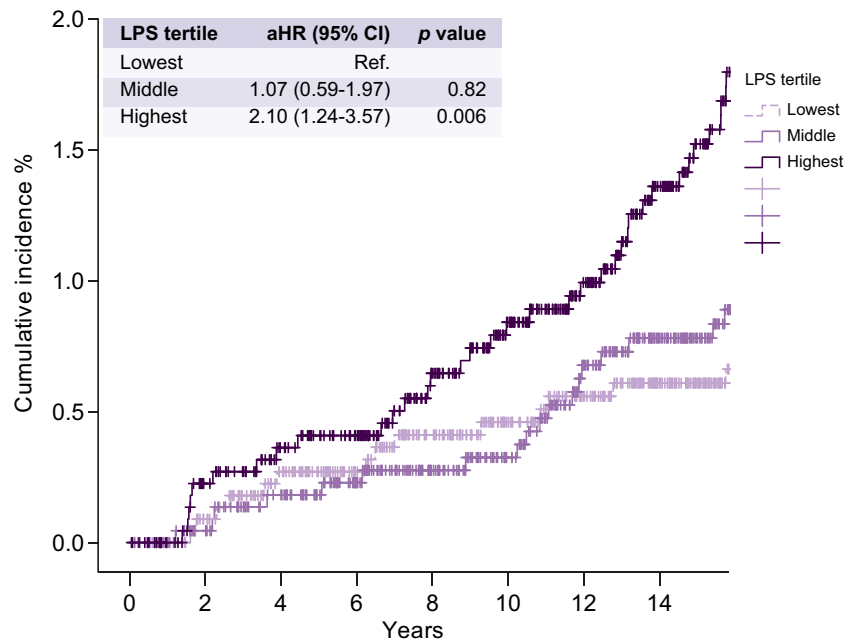
Fig. 1. Cumulative incidence by Kaplan-Meier analysis and age- and sex-adjusted hazards ratios by Cox regression analysis of LPS tertiles for incident advanced liver disease. LPS, lipopolysaccharide.

A strength of this study is the large and well-characterized population-based cohort of men and women. Another strength is the longitudinal, complete follow-up and use of hard endpoints (hospital admission, cancer, death) derived from reliable and mandatory national registries. Furthermore, we were able to adjust our analyses for multiple known confounders. We also excluded the cases with chronic viral hepatitis at baseline or during follow-up to avoid confounding by these factors.

Our population-based study has some limitations. First, because of the study design, we cannot conclude with certainty that LPS is a causative player in liver disease. Secondly, alcohol consumption was measured only once at baseline, but we have previously reported that, in general, 82% of individuals in the FINRISK and Health 2000 surveys show stable alcohol consumption over time.[38] Furthermore, we did not have liver biopsy or other direct measures of the possible presence of liver disease at baseline. However, we did have GGT measurements, and GGT is the most sensitive liver enzyme for liver injury and disease.[39] Importantly, we also excluded individuals with known clinical liver disease at baseline. Low platelet levels are a common finding in advanced liver disease,[40] and could be an additional marker of underlying liver
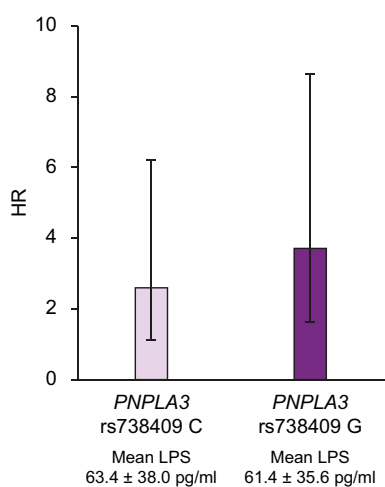

Fig. 2. Hazard ratios for incident liver disease stratified by the *PNPLA3* I143M carrier status (heterozygote/homozygote *vs.* no risk variant). Mean ± SD of LPS level is shown according to groups. LPS, lipopolysaccharide; PNPLA3, patatin-like phospholipase domain-containing protein 3.
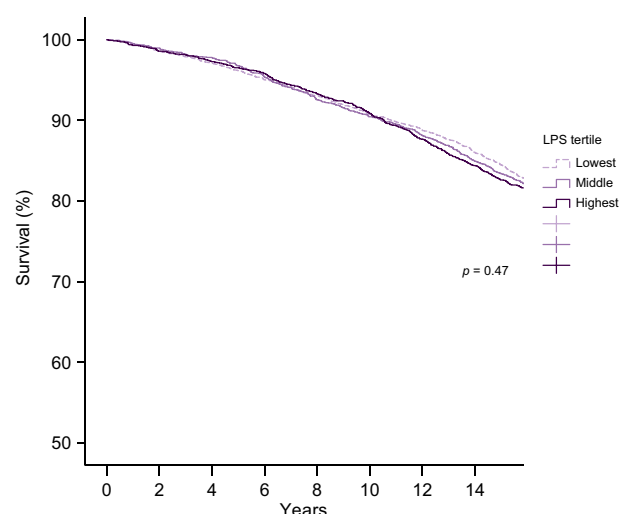

Fig. 3. Cumulative incidence by Kaplan-Meier analysis for overall survival by LPS tertile. LPS, lipopolysaccharide.

disease at baseline in this cohort. However, we found no correlation between LPS level and platelet count. All of this supports that an association between gut endotoxemia and the risk for liver disease exists beyond that explained by possible undetected underlying advanced liver disease.

Conclusion

Serum LPS is associated with incident advanced liver disease in the general population. We found that up to 30% of such liver disease may be attributed to high circulating LPS levels.

Abbreviations

ALD, alcoholic liver disease; BMI, body mass index; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HR, hazard ratio; HSD17B13, Hydroxysteroid 17-beta dehydrogenase 13; LPS, lipopolysaccharide; MBOAT7, membrane-bound O-acyltransferase domain-containing protein 7; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein 3; TLR4, toll-like receptor 4; TM6SF2, transmembrane 6 superfamily member 2.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

VM: writing the article; MF: critical revision of the article; PP, AJ, SM, AL, LV, VS and MP: design and data collection for the FINRISK 1997 study, critical revision of manuscript; FÅ: study design, statistical analyses, writing the article.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2019.09.001.

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