Received: 23 May 2023 | Revised: 7 July 2023 | Accepted: 31 July 2023

DOI: 10.1111/liv.15692

Liver INTERNATIONAL WILEY

ORIGINAL ARTICLE

Binge drinking episode causes acute, specific alterations in systemic and hepatic inflammation-related markers

Evelina Stankevic[1] | Mads Israelsen[2,3] | Helene Bæk Juel[1] | Anne Lundager Madsen[1] | Lars Ängquist[1] | Peter Stuart Jacob Aldiss[1] | Nikolaj Torp[2,3] | Stine Johansen[2,3] | Camilla Dalby Hansen[2,3] | Johanne Kragh Hansen[2,3] | Katrine Holtz Thorhauge[2,3] | Katrine Prier Lindvig[2,3] | Bjørn Stæhr Madsen[2,3] | Karolina Sulek[4] | Cristina Legido-Quigley[4] | Maja Sofie Thiele[2,3] | Aleksander Krag[2,3] | Torben Hansen[1]

[1]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

[2]Department of Gastroenterology and Hepatology, Odense University Hospital, Odense C, Denmark

[3]Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense C, Denmark

[4]Steno Diabetes Center Copenhagen, Herlev, Denmark

Correspondence
Torben Hansen, The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark.
Email: torben.hansen@sund.ku.dk

Aleksander Krag, Department of Gastroenterology and Hepatology, Odense University Hospital, J.B. Winsløws Vej 4, Entrance 126, 2nd floor, 5000 Odense C, Denmark.
Email: aleksander.krag@rsyd.dk

Funding information
Novo Nordisk Foundation, Grant/Award Number: NNF15OC0016692; European Union's Horizon 2020 Research and Innovation Program, Grant/Award Number: 668031

Handling Editor: Luca Valenti

Abstract

Background: Frequent binge drinking is a known contributor to alcohol-related harm, but its impact on systemic and hepatic inflammation is not fully understood. We hypothesize that changes in immune markers play a central role in adverse effects of acute alcohol intake, especially in patients with early liver disease.

Aim: To investigate the effects of acute alcohol intoxication on inflammation-related markers in hepatic and systemic venous plasma in people with alcohol-related liver disease (ArLD), non-alcoholic fatty liver disease (NAFLD) and healthy controls.

Methods: Thirty-eight participants (13 with ArLD, 15 with NAFLD and 10 healthy controls) received 2.5 mL of 40% ethanol per kg body weight via a nasogastric tube. Seventy-two inflammation-related markers were quantified in plasma from hepatic and systemic venous blood, at baseline, 60 and 180 min after intervention.

Results: Alcohol intervention altered the levels of 31 of 72 and 14 of 72 markers in the systemic and hepatic circulation. All changes observed in the hepatic circulation were also identified in the systemic circulation after 180 min. Only FGF21 and IL6 were increased after alcohol intervention, while the remaining 29 markers decreased. Differences in response to acute alcohol between the groups were observed for 8 markers, and FGF21 response was blunted in individuals with steatosis.

Conclusion: Acute alcohol intoxication induced changes in multiple inflammation-related markers, implicated in alcohol metabolism and hepatocellular damage. Differences identified between marker response to binge drinking in ArLD, NAFLD and healthy controls may provide important clues to disease mechanisms and potential targets for treatment.

Abbreviations: ALT, alanine aminotransferase; ArLD, alcohol-related liver disease; AST, aspartate aminotransferase; BMI, body mass index; CAP1, controlled attenuation parameter; FDR, false discovery rate; GGT, gamma-glutamyl transferase; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; INR, international normalized ratio; IQR, interquartile range; kPa, kilopascal; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NF-kB, nuclear factor–kappa B; NPX, normalized protein expression; SD, standard deviation; TE, transient elastography.

Clinical trial number: NCT03018990

KEYWORDS
alcohol, inflammation, liver disease, proteomics

1 | INTRODUCTION

Liver diseases represent a large global healthcare burden. Multiple factors contribute to the progression from healthy to diseased liver with alcohol consumption being the most common cause for the development of chronic liver disease.[1] More than half of all liver-related deaths are attributed to alcohol-related liver disease (ArLD).[2] Alcohol intake in various stages of ArLD increases the risk of disease progression from asymptomatic steatosis via inflammation and fibrosis to irreversible decompensated liver cirrhosis.[3] The effect of moderate alcohol intake in non-alcoholic fatty liver disease (NAFLD) is debated[4] with more recent findings suggesting that even low amounts of alcohol associated with increased risks of alcohol-related liver harm.[5] Generally, interaction between multiple risk factors and disease mechanisms, often, a combination of alcohol overuse and obesity, leads to an even greater risk of developing liver-related complications.[5] However, liver-related complications and mortality are higher among the ArLD patients.[6]

Mechanistically, alcohol overuse leads to gut mucosal alterations and suppressed expression of intestinal tight junction proteins, increasing gut permeability.[7] Once this barrier is disrupted, the translocation of bacteria and their metabolites from the gut to the portal circulation increases, enabling pathogens to interact with the liver cells and circulating immune cells.[8] When these patterns are recognized by intrahepatic cells via toll-like receptors, a sequence of inflammatory responses is initiated. This leads to the generation of pro-inflammatory markers, and infiltration of monocytes and neutrophils, ultimately resulting in hepatocellular damage.[9,10] The effect of alcohol on circulating inflammation-related markers depends on the alcohol consumption pattern. Acute alcohol intoxication is shown to suppress the release of pro-inflammatory immune mediators in mice and humans.[11,12] In contrast, chronic alcohol consumption is linked to increased systemic inflammation and upregulation of pro-inflammatory response.[13–16] However, the impact of acute alcohol consumption in people with early chronic liver disease remains largely unexplored.

With this study, we aimed to understand how acute alcohol consumption affects a broad range of immune mediators and whether the differences between acute alcohol effect across healthy, ArLD and NAFLD groups could point to disease-specific mechanisms. Knowledge generated from this study contributes to advances in the development of potential biomarkers and hepatic pharmacological treatments of alcohol overuse.

Key points

Our study shows that a binge of alcohol (drinking 4–5 alcoholic beverages in a short period) causes disruption of the normal inflammatory processes in the body: Most inflammation products went down, while interleukine-6 and the liver hormone FGF-21 went up. This disruption was different in healthy individuals compared to people with non-alcoholic fatty liver disease and people with alcohol-related liver disease. Our results may help to understand how alcohol harms the liver and how binge drinking can be even more harmful for people with existing liver disease.

2 | MATERIALS AND METHODS

2.1 | Study overview

To investigate the effects of acute alcohol intoxication on inflammatory markers we quantified the hepatic and systemic venous plasma levels of 72 inflammation-related markers in 38 individuals from three distinct groups – ArLD ($N=13$), NAFLD ($N=15$) and healthy controls ($N=10$), before and after (60, 180 min) an alcohol intervention (Figure 1).

2.2 | Study population

All recruited participants provided a informed written consent under the study protocol, approved by the Ethical Committee of Southern Denmark (S-20160083). The study was registered at ClinicalTrials.gov (NCT03018990) and was conducted in compliance with the Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

Three groups of age-matched individuals were recruited: healthy controls, individuals with ArLD, and NAFLD, as described earlier.[17] In brief, participants were recruited through Odense Liver Research Centre, seeking individuals aged 18–75 years, with body weight >50 kg and capable to abstain from alcohol for 48 h before the investigations. Inclusion criteria for individuals with ArLD were prior and/or current heavy alcohol intake (defined by >36 and >24 g alcohol/day for males and females, respectively), liver fibrosis, proven on a prior liver biopsy in keeping with histological features of ArLD and no intention of becoming abstinent from

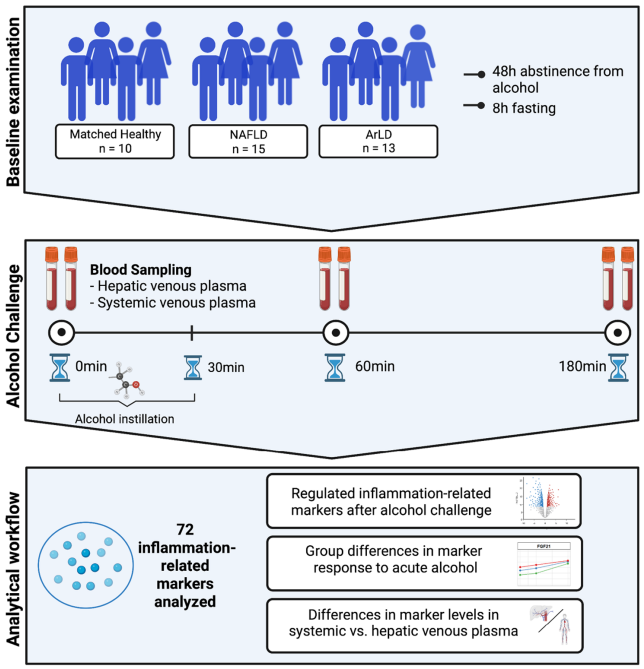

FIGURE 1 Study overview. Systemic and hepatic venous plasma levels of 72 inflammation-related markers were quantified in 13 individuals with ArLD, 15 individuals with NAFLD and 10 healthy controls before (0 min) and after (60; 180 min) alcohol intervention. Figure was created with BioRender.com.

alcohol. There were no signs of malnutrition in the ArLD group. Inclusion criteria for individuals with NAFLD were liver fibrosis, proven on a prior liver biopsy in keeping with histological features of NAFLD and no history of heavy alcohol intake. Lastly, inclusion criteria for healthy controls were normal liver stiffness (defined by <6 kPa as measured by transient elastography), body mass index (BMI) <30 kg/m^2, normal broad blood biochemistry at baseline and no history of heavy alcohol intake. Participants were not included if they were diagnosed with liver cirrhosis at a prior liver biopsy, were suffering from liver disease of another aetiology, insulin-dependent diabetes mellitus or any type of cancer, were pregnant, breastfeeding or were using antibiotics within 4 weeks prior to the investigations.

One participant with ArLD was excluded due to lack of adherence to baseline requirements (48 h of abstinence from alcohol), and one participant with ArLD was excluded due to all measured inflammation-related markers failing internal quality control, equalling to $N = 38$ study participants.

All participants filled out food frequency questionnaire daily, 7 days before the intervention day, and a structured self-reported alcohol intake questionnaire. The participants were informed not to change their dietary habits and alcohol intake during this period. It was a requirement for all study participants to be abstinent from alcohol for 48 h before the intervention day, confirmed by quantifying blood ethanol levels on the intervention day.[18]

2.3 | Clinical examination

Clinical examinations were performed after 8 h of fasting. A catheter was placed under local anaesthesia in the hepatic vein via the right jugular vein and inferior vena cava for sampling hepatic venous plasma.[19] An additional catheter was placed in the jugular vein for sampling systemic venous plasma. Radiographic X-ray evaluation ensured that the catheters were placed correctly before sampling.

Hepatic and systemic venous plasma was simultaneously sampled at baseline, and 60 and 180 min after alcohol intervention. Routine biochemistry was performed according to standard operating procedures at the Department of Biochemistry and Pharmacology at Odense University Hospital. After processing, blood samples for targeted plasma proteomics were collected in lithium heparin tubes and stored at −80°C.

2.4 | Alcohol intervention

Alcohol intervention was performed by instilling hospital pharmacy-produced 40% pure ethanol in 9 mg/mL NaCl through a nasogastric tube. A dose of 2.5 mL of 40% ethanol per kg body weight was instilled over 30 min by infusion pump. The dose was decreased from 2.5 to 0.5 mL for each kg body weight above the BMI of 25 kg/m^2 to avoid severe intoxication in participants with high body fat percentage.

2.5 | Targeted plasma proteomics

Ninety-two inflammation-related proteins were quantified from hepatic and systemic venous plasma using the Target-96 Inflammation panel from Olink Proteomics. The proteomics Target-96 assay is based on the proximity extension assay technology, quantifying arbitrary Normalized Protein eXpression (NPX) values on $\log_2$ scale.[20] The samples were randomized across 96 well plates, normalized using internal and inter-plate controls and analysed in one batch. Twenty proteins were excluded from the analysis due to >75% of measurements being below the limit of detection. The samples are flagged during QC if internal control samples deviate from the median of all samples on the plate by a pre-determined value of ±0.3. The assumption of normal distribution of the outcome has been evaluated and described in more detail in Appendix S1, along with an abbreviation list for marker names.

2.6 | Statistical analyses

Statistical analyses and data visualization were performed in R version 4.1.2 with Rstudio (IDE version 2022.02.3). Data are reported as mean and standard deviation (SD) for normally distributed variables,

as median and interquartile range (IQR) for non-normally distributed variables and frequencies and percentages for categorical variables.

Linear mixed models using generalized least squares (*gls* function from the R package *nlme*[21]) were used to investigate changes in circulating inflammation-related proteins after alcohol intake. Corresponding interaction models were fitted as follows: (1) (time × site) to investigate the effect of alcohol intervention on the difference between protein levels in hepatic and systemic venous plasma and (2) (time × group) to investigate if the liver disease status had an effect on the changes in protein levels due to alcohol intervention.

The results were reported as effect size estimates on $\log_2$ scale and 95% CI and False Discovery Rate (FDR)-adjusted *p*-values.[22] *p*-values were considered significant below .05 after FDR adjustment.

As a post hoc analysis, we compared the effect of alcohol on FGF21 between healthy controls and individuals with chronic fatty liver disease by pooling ArLD and NAFLD into one group ($N = 28$). Additionally, we stratified the study participants ($N = 38$) based on the degree of steatosis and hepatic inflammation. Lastly, we stratified the liver disease group with the chronic fatty liver disease according to its severity: mild fibrosis (F0–F1, $N = 13$) vs significant fibrosis (≥F2, $N = 15$). *p*-values presented were derived from an unpaired *t* test.

A detailed description of statistical modelling within main analyses, stratification and testing in post hoc analyses is provided in Appendix S1 section.

3 | RESULTS

3.1 | Study participants

Thirty-eight participants were included in our study. The baseline characteristics of ArLD ($N = 13$), NAFLD ($N = 15$) and healthy controls ($N = 10$) are summarized in Table 1. Participants with ArLD had higher daily alcohol consumption compared to NAFLD and healthy controls. Significant fibrosis (F ≥ 2) was seen in 46.2% of participants in the ArLD group and in 60.1% of participants in the NAFLD group. All healthy controls had normal liver stiffness measurements (transient elastography <6 kPa).

3.2 | Acute alcohol consumption alters the levels of multiple circulating inflammation-related markers

In the systemic circulation, the alcohol intervention significantly altered the levels of 31 out of 72 (43%) inflammation-related markers of which 29 decreased and 2 (FGF21 and IL6) increased. The levels of 6 markers were significantly changed 60 min after acute alcohol intervention, while a more pronounced effect was seen after 180 min, where the levels of all 31 affected markers were significantly altered (Figure 2A and Table S1).

Similarly, in hepatic circulation, alcohol intervention significantly affected the levels of 14 out of 72 (19%) inflammation-related markers of which 11 decreased and 3 (FGF21, IL6 and ADA) increased. The levels of 3 markers were significantly altered after 60 min while the levels of 13 markers were significantly changed after 180 min (Figure 2B and Table S2).

The variance explained by the differences between the three groups accounted for a median of 9.0% (IQR [0.3–16.8]) of the variation across the dataset, while intervention and sample site accounted for <5% of the variation across the dataset each (Figure S2).

The effect of alcohol on all 72 markers in systemic and hepatic venous plasma is summarized in Appendix S1, including the uncorrected *p*-values, and the results of the same models applied on inverse-normal rank transformed protein levels (Figures S3 and S4, Tables S1 and S2).

3.3 | Differences between marker profile in systemic and hepatic circulation

All 13 markers that changed in the hepatic circulation, were also altered in the systemic venous plasma 180 min after alcohol intervention (Figure 2C). The levels of 12 markers were higher in the systemic compared to the hepatic venous plasma at baseline (Figure 2D). No significant interaction between the sample site and alcohol intervention was observed (Table S3). Differences between hepatic and systemic venous plasma levels of quantified inflammation-related markers 60 and 180 min after alcohol intervention are provided in Figure S7 and Table S3.

3.4 | The response to alcohol intervention is different between ArLD, NAFLD and healthy controls

In systemic venous plasma, 7 inflammation-related markers displayed different response to alcohol intervention between the groups (Figure 3A). Primarily, the ArLD and NAFLD groups had a lower increase in FGF21 compared to healthy controls. The ArLD group had a larger decrease in HFG and OSM, compared to the healthy control group. Furthermore, FGF23 decreased in response to alcohol intervention only in the ArLD group.

In hepatic venous plasma, 2 inflammation-related markers displayed differential response to alcohol intervention between the groups (Figure 3B). After 60 min OSM and CXCL1 increased in the NAFLD group while both inflammation-related markers decreased in the ArLD and healthy controls (Figure 3B).

The results from all analysed inflammation-related markers are summarized in Tables S1 and S2.

3.5 | Impact of binge alcohol on FGF21, implicated in alcohol craving

Between-individual variation, liver group, alcohol intervention and sample site could explain 91% of the total variation in FGF21 levels

TABLE 1 Descriptive characteristics of study participants at baseline.

	Overall	Healthy	NAFLD	ArLD
Participants, *n*	38	10	15	13
Age, years	53.2 (10.8)	53.4 (10.1)	52.9 (12.1)	53.3 (10.4)
Sex, female *n* (%)	14 (36.8)	5 (50.0)	8 (53.3)	1 (7.7)
Daily alcohol consumption, g	6.0 [0.0–48.0]	12.0 [0.0–12.0]	0.0 [0.0–0.0]	60.0 [48.0–108.0]
Anthropometrics				
Weight, kg	88.8 [73.7–103.6]	72.8 [67.9–83.0]	99.1 [89.1–106.0]	86.6 [73.5–104.0]
BMI, kg/m^2	28.8 [25.3–32.2]	25.3 [23.3–27.0]	32.2 [30.7–40.3]	27.6 [23.3–30.9]
Waist circumference, cm	105.0 [91.0–118.0]	88.0 [87.0–102.0]	113.0 [106.0–118.0]	105.0 [91.0–120.0]
Handgrip strength, kg	80.93 (30.13)	74.47 (31.87)	84.41 (34.39)	82.15 (25.09)
Liver parameters				
Fibrosis stage (0/1/2/3/4), *n*	3/10/11/2/2	[a]	1/5/7/1/1	2/5/4/1/1
Steatosis grade (0/1/2/3), *n*	3/11/7/7	[a]	0/7/5/3	3/4/2/4
CAP1, dB/m	303.7 (72.9)	229.6 (49.2)	340.5 (35.2)	318.3 (81.2)
Liver stiffness by TE, kPa	8.8 [4.9–10.5]	4.5 [3.9–5.0]	10.4 [9.6–11.4]	8.9 [5.9–10.1]
ALT, U/L	37.0 [25.2–57.8]	26.0 [17.8–34.5]	44.0 [27.5–61.5]	45.0 [28.0–71.0]
AST, U/L	29.5 [24.8–58.8]	23.0 [22.0–28.0]	29.0 [24.5–54.5]	53.5 [30.5–82.8]
AST/ALT ratio	1.1 (0.5)	1.0 (0.3)	0.9 (0.3)	1.4 (0.8)
Bilirubin, μmol/L	10.0 [7.2–13.0]	10.0 [9.0–12.2]	10.0 [7.0–12.5]	12.0 [7.0–19.0]
INR	1.0 [0.9–1.1]	1.1 [1.0–1.1]	1.0 [0.9–1.0]	1.0 [0.9–1.1]
GGT, U/L	63.5 [26.0–131.5]	21.5 [15.8–25.2]	65.0 [47.0–83.5]	255.0 [103.0–634.0]
Blood biochemistry				
Albumin, g/L	45.0 [42.0–47.0]	46.5 [41.0–47.8]	45.0 [43.5–48.0]	44.0 [41.0–46.0]
Fasting plasma glucose, mmol/L	6.2 [5.5–6.8]	5.7 [5.5–6.0]	6.7 [6.2–7.5]	6.5 [5.6–6.8]
HOMA-IR	4.9 [1.9–8.7]	1.9 [1.3–2.0]	8.1 [6.7–10.4]	3.0 [1.9–8.8]
Insulin, pmol/L	92.5 [44.8–178.6]	43.2 [34.0–47.3]	170.5 [130.3–185.9]	63.5 [46.0–182.9]
HbA1C, mmol/L	38.0 [34.2–45.0]	35.0 [31.5–36.8]	46.0 [41.0–52.5]	36.0 [31.0–40.0]
HDL cholesterol, mmol/L	1.2 [1.1–1.6]	1.5 [1.1–1.9]	1.1 [0.9–1.2]	1.5 [1.2–1.6]
LDL cholesterol, mmol/L	2.6 [2.2–3.6]	3.1 [2.6–4.2]	2.6 [1.3–3.4]	2.3 [2.2–3.1]
Total cholesterol, mmol/L	4.9 [4.2–6.0]	5.3 [4.6–6.0]	4.4 [3.9–5.3]	4.9 [4.4–6.2]
Triglycerides, mmol/L	1.3 [0.8–2.1]	0.8 [0.7–1.1]	1.5 [0.8–3.3]	1.5 [0.9–2.5]
Platelet count, 10^9/L	223.5 [191.5–265.5]	209.5 [174.2–227.5]	252.0 [202.5–297.5]	221.0 [206.0–244.0]

Note: Data are shown as means and standard deviations (SD), medians and interquartile ranges [IQR] and frequencies (%). Daily alcohol consumption is shown as grams of ethanol per day.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP1, controlled attenuation parameter 1; GGT, gamma-glutamyl transferase; HbA_{1c}, haemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; INR, international normalized ratio; LDL, low-density lipoprotein; TE, transient elastography.

[a]Fibrosis stage and steatosis grade were not evaluated in the healthy control group.

(Figure S5), of which 43% of the variation was explained by the alcohol intervention alone (Figure S6). The individual differences between participants in the levels of FGF21 could explain 30% and differences across groups could explain 18% of the variation in FGF21 levels.

We found that the increase in systemic FGF21 was significantly higher in the healthy control group compared to liver disease group, with pooled ArLD and NAFLD groups (Figure 4A). The late FGF21 response (60–180 min after alcohol intervention) in participants with steatosis was lower compared to individuals without steatosis (Figure 4B). We show that the change in FGF21 after 60 and 180 min negatively associates with baseline cholesterol and LDL-cholesterol levels (Figure S8). There were no differences in FGF21 response to the alcohol intervention between participants with and without hepatic inflammation (Figure 4C) and groups with mild and significant fibrosis (Figure 4D).

4 | DISCUSSION

We demonstrate that acute alcohol intervention affects a wide range of circulating inflammation-related markers involved in several

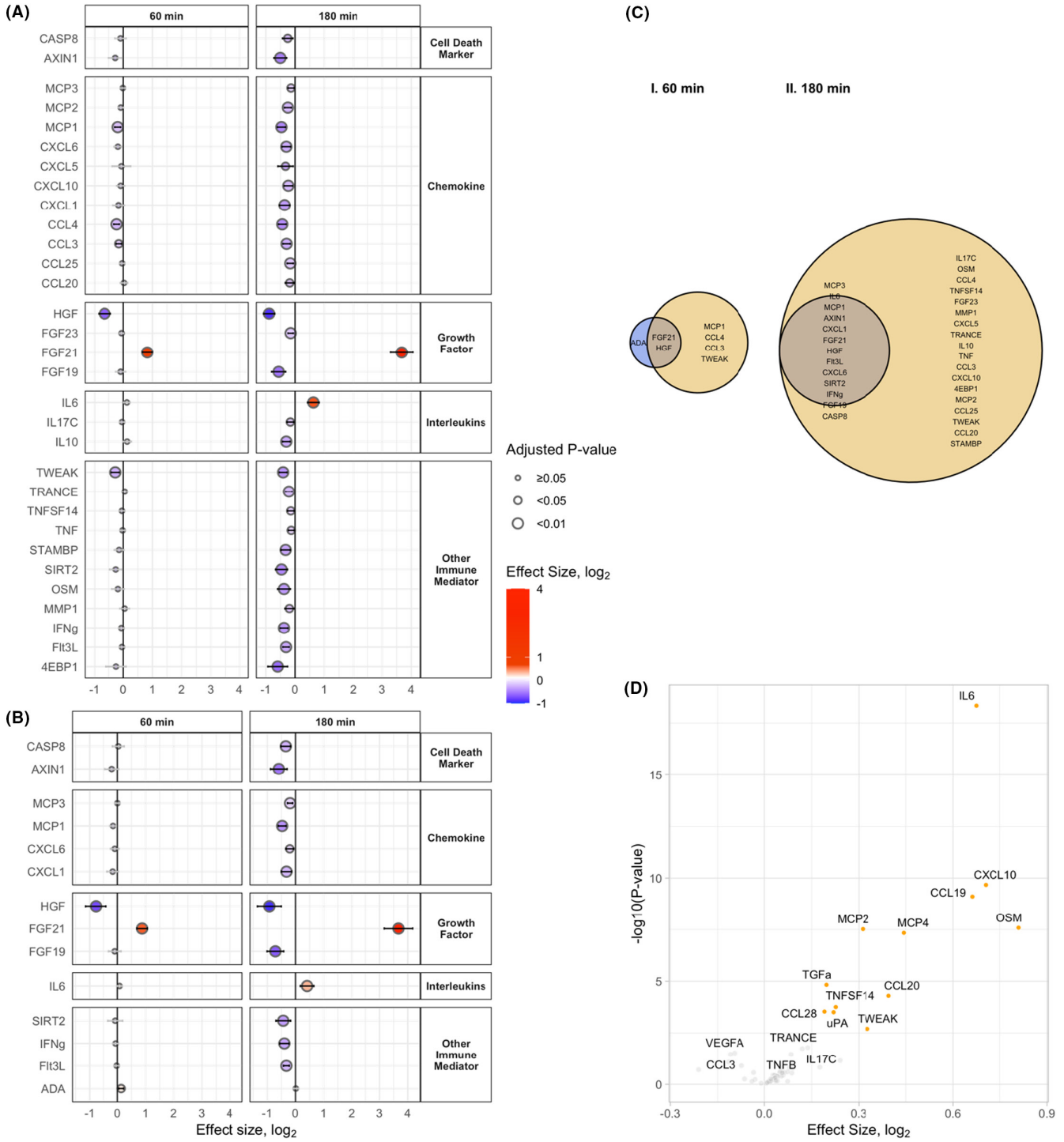

FIGURE 2 (A–D) Marker levels in systemic and hepatic venous plasma after alcohol intervention. (A, B) Forest plot showing upregulated (red) and downregulated (blue) inflammation-related markers in (A) systemic and (B) hepatic venous plasma 60 and 180 min after alcohol instillation. Only significant changes in marker levels are shown. (C) Venn diagram visualizing the overlap of significant changes in marker levels between hepatic (blue) and systemic (golden) venous plasma after 60 min (I.) and 180 min (II.). (D) Volcano plot visualizing marker levels in systemic versus hepatic venous plasma at baseline. Inflammation-related markers coloured in orange were significantly higher and in blue were lower in systemic versus hepatic venous plasma after FDR correction. Figure 2C was created with BioRender.com.

functional pathways (Figure 5). Despite few inflammation-related markers (FGF21 and IL6), the general trend was that alcohol reduced marker levels and this effect was more pronounced in the systemic circulation. Notably, the FGF21 response was significantly reduced in individuals with hepatic steatosis.

Acute alcohol intervention alters the levels of circulating systemic and hepatic members of the 'FGF19 superfamily': FGF19, FGF21 and FGF23, which play an important role in alcohol metabolism.[23] Circulating FGF21 is primarily produced by the liver[24] and regulates nutrient and energy homeostasis through pleiotropic

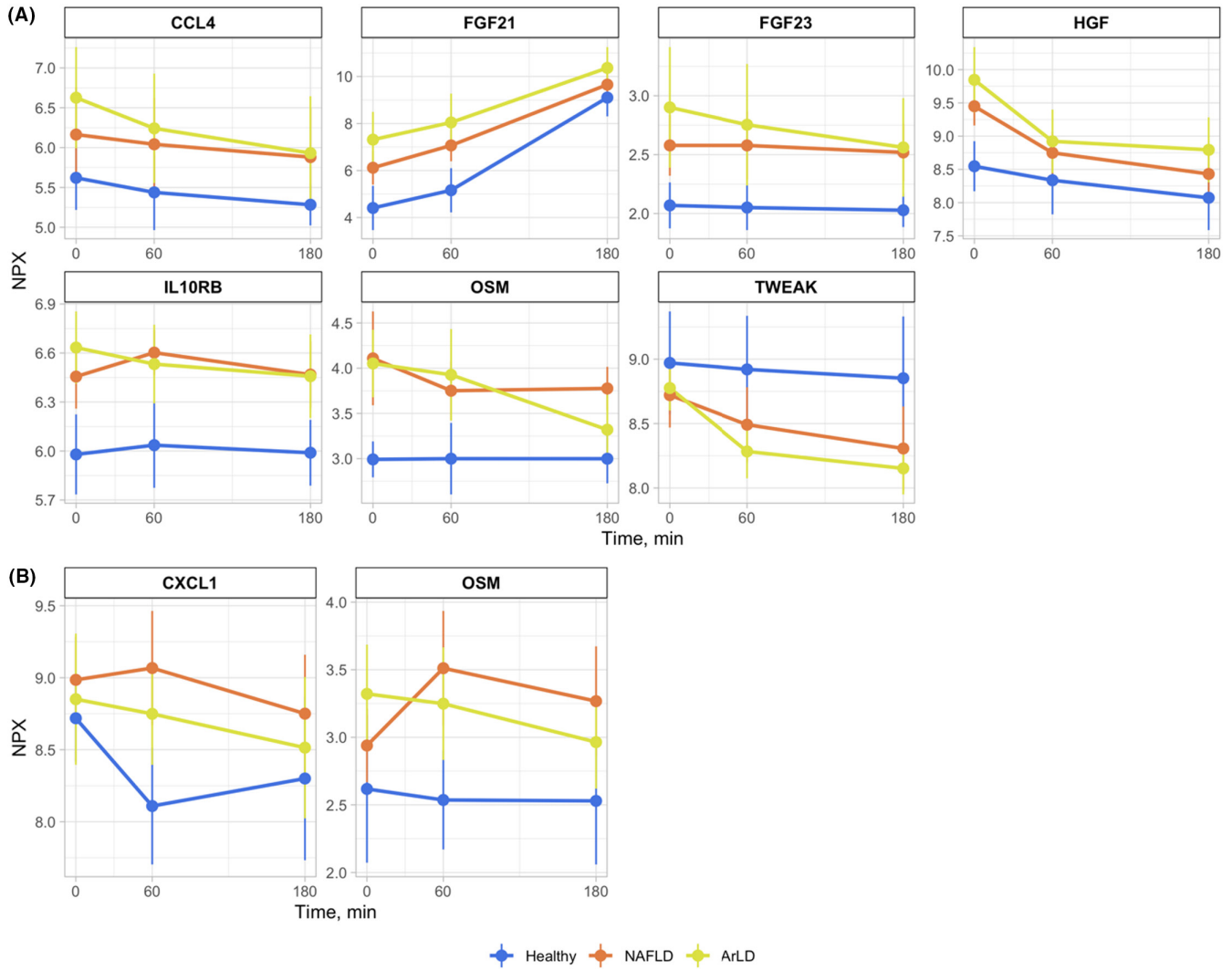

FIGURE 3 (A, B) Group-specific changes in inflammatory marker levels after alcohol intervention. Line plots displaying significantly different temporal trajectories between healthy, NAFLD and ArLD individuals in (A) systemic and (B) hepatic venous plasma. The line plots are coloured by group. The dot represents the mean level of marker in the respective group at baseline, 60 and 180 min after alcohol intervention. The error bars represent the 95% confidence intervals.

actions on multiple tissues.[25] Previous work has shown that alcohol induces a rapid increase of FGF21 in young healthy men.[26–28] Here we expand the knowledge by showing that the increase of FGF21 is driven by the hepatic release as indicated by the higher levels of FGF21 in hepatic venous plasma compared to systemic venous plasma 60 min after alcohol intake (Figure S7, Table S3A).

Genome-wide association studies have identified single nucleotide polymorphisms in *FGF21* and *KLB*, encoding FGF19 and FGF21 co-receptor, β-klotho, to associate with increased alcohol consumption.[29] Furthermore, in their recent work in non-human primates Flippo et al[30] identify a liver-to-brain pathway where FGF21 signalling enhances excitability of KLB-expressing neurons in basolateral amygdala, which, in turn, suppresses alcohol consumption suggesting a potential pharmacological target to reduce alcohol intake. Also, most recently, FGF21 has been shown to protect mice against effects of ethanol toxicity.[31] In line with this, we show that alcohol induces a lower increase of systemic FGF21 in individuals with ArLD compared to healthy. Interestingly, individuals with NAFLD also had a lower FGF21 response compared to healthy. Earlier studies show that fasting serum FGF21 is positively correlated with lipid features, including triglycerides and LDL-cholesterol, as well as insulin and HOMA-IR.[32] Our data show that stimulated FGF21 negatively associates with LDL-cholesterol and total cholesterol, indicating a blunted acute FGF21 response to acute alcohol intake in connection with hypercholesterolemia. Stimulated FGF21 did not associate with HOMA-IR or fasting insulin (Figure S8). Importantly, we also show that individuals with steatosis have a blunted increase in FGF21, compared to those without steatosis. We found no significant differences in FGF21 response to the alcohol intervention between varying degrees of hepatic inflammation, as well as those with mild and significant fibrosis. This indicates that the lower response in FGF21 is not a result of reduced liver function in relation to hepatic inflammation or fibrosis. Thus, instead, we speculate, that the factors influencing FGF21 response in ArLD and NAFLD (excessive

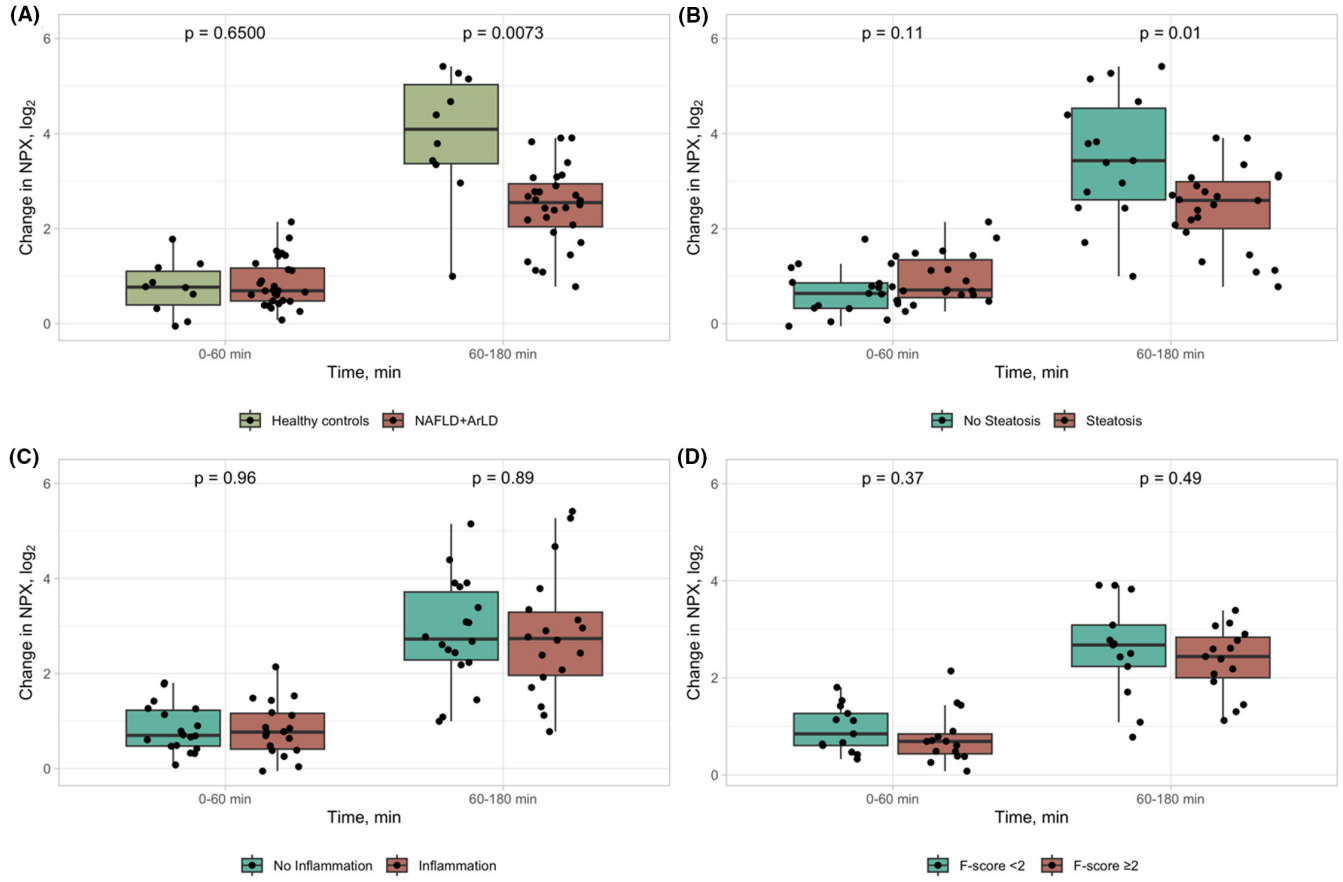

FIGURE 4 (A–D) Change in systemic FGF21 after alcohol intervention. Box plots displaying temporal trajectories of change of FGF21 levels in systemic venous plasma between participants (A) from healthy control group and with liver disease (ArLD and NAFLD combined), (B) with and without steatosis, (C) with and without hepatic inflammation and (D) with fatty liver disease, stratified by liver fibrosis score. Plots are coloured by respective strata. The boxes represent the two middle quartiles (50%) of the change in FGF21 in the respective group at 60 and 180 min after alcohol intervention. Whiskers represent lower and upper quartiles, and the dots represent each observation. *p*-values were estimated by a *t* test.

alcohol intake, obesity and insulin resistance) may all be linked to liver steatosis, and the same imbalance in the liver-to-brain feedback loop that controls the intake of alcohol and sugar.

Evidence from observational studies support that FGF21 plays a role in the development and pathogenesis of liver diseases. Elevated FGF21 levels have found to be independent predictors of the presence and development of acute-on-chronic liver failure.[33] In a different disease group, FGF21 was identified as an independent predictor of liver steatosis.[34] In two separate randomized clinical trials, modified human FGF21analogs *Efruxifermin* and *Pegbelfermin* have shown beneficial effects, namely reduction of hepatic fat fraction, in the treatment of non-alcoholic steatohepatitis.[35,36] In a phase 2b randomized controlled trial, treatment with the FGF21 analogue *Pegozafermin* led to significant improvements in liver fibrosis, and secondary endpoints, including reduction in liver fat, markers reflecting liver injury and liver inflammation. The results from the trial also indicate that treatment with an FGF21 analogue may positively affect some of the classical traits of metabolic derangements, including adiponectin, serum triglyceride and HDL cholesterol levels.[37] The results from the outlined trials support the idea that treatments targeting the FGF21 pathway may have dual beneficial effect for the many people with ArLD who also carry metabolic risk factors.[38]

Our results demonstrate a decrease in systemic levels of FGF23 in the ArLD group, but not in healthy controls or the NAFLD group. FGF23 has previously been suggested as a cardiovascular risk factor among alcohol abusers,[39] strongly tied to liver function, but functional studies of FGF23 in liver disease remain to be explored.[40] Our study replicated a previous finding of decrease in FGF19 levels[27] in the systemic circulation and demonstrated that the decrease in FGF19 is also evident in hepatic circulation. FGF19 is secreted from the enterocytes and regulates bile acid homeostasis and metabolism,[29,41] and its inhibition after alcohol consumption partly contributes to rise in plasma triglyceride concentrations.[27] In a previous work, we have also demonstrated a rise in triglyceride concentrations after acute alcohol intervention, in healthy control individuals and ArLD patients, but not in the NAFLD group.[17]

Many of the immune mediators, contributing to liver damage via sustained hepatic inflammation either activate or are activated by nuclear factor-κB (NF-κB) – a key signalling cascade playing an important role in hepatic inflammation.[42] In contrast to the increased activation of NF-κB and its targets as a result of chronic alcohol

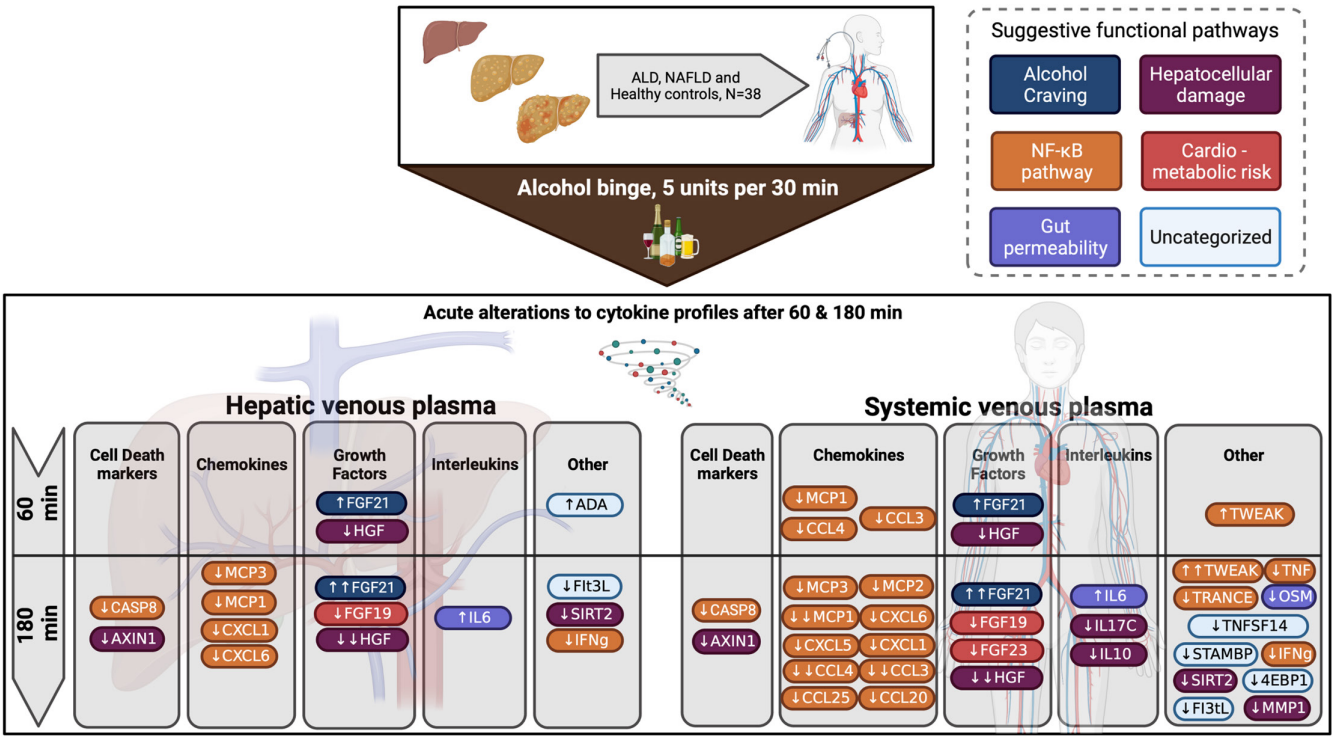

FIGURE 5 Overview of acute alcohol effect on inflammation-related markers and suggestive functional pathways. Acute alcohol intervention in ArLD, NAFLD and healthy controls leads to acute alterations to marker profiles in hepatic and systemic circulation. The affected markers include markers of cell death, chemokines, growth factors, interleukins and other inflammation-related markers. Based on their known functions identified in literature by previous studies, inflammatory markers are coloured by the suggestive functional pathways. Figure was created with BioRender.com.

intake, acute alcohol treatment inhibits NF-κB activation.[43] Reduced NF-kB activation consequently results in decreased downstream activation of its targets,[44] including chemokines, which play an important role in the activation of immune cells during inflammation in the liver.[45] Our study indeed demonstrates a decrease in several inflammation-related markers, known to activate NF-κB pathway, including TNF, IFN-gamma, HGF and subsequent depression of NF-κB targets, including a number of chemokines, compromising key immunoregulatory functions.[46–48]

Previous research shows that HGF can activate the expression of MMP-1 in hepatic stellate cells, contributing to repair and regeneration of the liver,[49] therefore, acute alcohol-induced inhibition of MMP1, evident in our study, may be a downstream effect of decreased levels of HGF. Moreover, participants with NAFLD and those with ArLD had a steeper decrease in the systemic HGF during the first 60 min after alcohol binge, compared to healthy controls. In a previous work from the same intervention study, Torp et al[18] show a transient inhibition of hepatic fibrogenesis marker PRO-C3 during the first hours of acute alcohol intervention, followed by activation of fibrogenesis from 180 min after the binge episode in participants with significant liver fibrosis (F ≥ 2). Taken together, these results indicate that binge alcohol inhibits the turnover of hepatic extracellular matrix, in the first hours after alcohol intoxication.

We replicate the previous findings of plasma concentrations of IL6 rise as a consequence of acute alcohol intake in heavy-drinking individuals,[50] as well as after binge drinking.[51] OSM, a member of IL6 family, did not fluctuate with alcohol intake in healthy individuals in the systemic circulation, but the trajectories of OSM response to alcohol intervention were different in both ArLD and NAFLD groups. One of the explanations for the differential response to alcohol intervention between the three groups might be due to the development of leaky gut, as OSM downregulates the expression of tight junction proteins[52] and is consistently increased in inflamed intestinal tissue.[53] In a recent study, acute alcohol intervention induced transient increase in circulating microbial DNA quantity in ArLD patients, but not those with NAFLD or healthy controls, supporting that an acute alcohol intervention induces leaky gut in patients with ArLD.[54] Targeting gut-liver axis is already showing promising effects in ArLD, with results from a randomized, double-blinded phase 2 trial demonstrating, that locally acting gut-specific antibiotic *Rifaximin-alpha* halts the progression of hepatic injury in ArLD.[55]

Study limitations should be taken into consideration. The sample size was fairly small; therefore, the study might have limited power to capture all fluctuations in marker responses to alcohol and differences between the study groups. Moreover, ArLD patients often exhibit metabolic comorbidities. Components of metabolic syndrome and overweight may lead to worsened liver disease progression in individuals with ArLD.[56] Due to small sample size, sensitivity analyses stratifying the participants by weight have not been performed. The quantified circulating systemic and hepatic inflammation-related markers might not reflect hepatic

protein expression profiles, and we did not investigate the direct effect of these proteins on the liver tissue; therefore, further mechanistic studies are warranted. In this study, we aimed to recruit balanced number of individuals from both genders; however, it was more challenging to reach balanced distribution of both genders in the ArLD group. Higher proportion of men drink heavily compared to women, however, alcohol consumption in women is increasing at alarming rates,[57] and even with lesser exposure, women are at higher risk of development of liver-related complications.[58] Further studies investigating the acute alcohol effects in women with ArLD are highly warranted.

However, our study expands current knowledge on how circulating inflammation-related markers respond to acute alcohol intervention in ArLD, NAFLD and in health. Many of these inflammation-related markers are novel with respect to the effect of binge drinking, and their response to alcohol intervention has not been analysed in liver diseases. We provide evidence for liver disease status-specific responses of several circulating markers to acute alcohol intervention. These are of particular interest for the development of potential biomarkers for liver disease, as fluctuations with alcohol intake might hinder the use of these inflammation-related markers as diagnostic and/or prognostic biomarkers. On the other hand, it might also suggest potential mechanisms of immune response changes with respect to chronic alcohol intake and metabolic disturbances.

In conclusion, we show that acute alcohol intervention alters the levels of circulating systemic and hepatic inflammatory markers. The identified markers are implicated in pathways that are involved in alcohol metabolism and hepatocellular damage (Figure 5). Results from our study may be informative of mechanisms of early liver disease and potential targets for treatment.

AUTHOR CONTRIBUTIONS

Evelina Stankevic, Mads Israelsen, Helene Bæk Juel, Bjørn Stæhr Madsen, Aleksander Krag and Torben Hansen involved in conceptualization. Evelina Stankevic, Mads Israelsen, Helene Bæk Juel, Nikolaj Torp, Stine Johansen, Camilla Dalby Hansen, Johanne Kragh Hansen, Katrine Holtz Thorhauge, Katrine Prier Lindvig and Bjørn Stæhr Madsen involved in data collection and data curation. Evelina Stankevic involved in formal analysis and visualization and wrote the original draft. Evelina Stankevic, Mads Israelsen, Helene Bæk Juel, Anne Lundager Madsen, Lars Ängquist, Karolina Sulek, Maja Sofie Thiele, Aleksander Krag and Torben Hansen involved in methodology and interpretation. Evelina Stankevic, Mads Israelsen, Helene Bæk Juel, Anne Lundager Madsen, Lars Ängquist, Peter Stuart Jacob Aldiss, Nikolaj Torp, Stine Johansen, Camilla Dalby Hansen, Johanne Kragh Hansen, Katrine Holtz Thorhauge, Katrine Prier Lindvig, Bjørn Stæhr Madsen, Karolina Sulek, Cristina Legido-Quigley, Maja Sofie Thiele, Aleksander Krag and Torben Hansen reviewed and edited the manuscript.

ACKNOWLEDGEMENTS

The authors thank Mie Balle Hugger, Annette Fialla, Annette Nielsen, Lotte Kappen and Jane Brudvig-Lauridsen and the entire staff at Odense Liver Research Centre; Louise Skovborg Just and Lise Ryborg for the managing of GALAXY and MicrobLiver consortia; Annemette Forman, Tina Hvidtfeldt Lorentzen from University of Copenhagen, Center for Basic Metabolic Research; Mette Andreasen, Anette Tyrsted and Lea Grip from Open Patient data Explorative Network; Professor Peter Rossing at Steno Diabetes Centre Copenhagen; the MicrobLiver consortium partners.

FUNDING INFORMATION

Challenge grant 'MicrobLiver', NNF15OC0016692, Novo Nordisk Foundation. The European Union's Horizon 2020 Research and Innovation Program (GALAXY, grant number 668031).

CONFLICT OF INTEREST STATEMENT

JKH has received speaking fees from Norgine. KPL has received speaking fees and support for travels from Siemens. MT has received speaking fees from Siemens Healthcare, Norgine, Echosens and Tillotts Pharma; consulting fees from GE Healthcare, Boehringer Ingelheim and GSK. AK has served as a speaker for Norgine, Siemens and Nordic Bioscience and participated in advisory boards for Norgine and Siemens, all outside the submitted work. AK receives royalties from Gyldendal and has received equipment, drugs or other services from Norgine, Siemens and Echosense. All other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Evelina Stankevic https://orcid.org/0000-0002-6433-6975
Peter Stuart Jacob Aldiss https://orcid.org/0000-0002-1809-2092
Stine Johansen https://orcid.org/0000-0002-5031-2294
Katrine Holtz Thorhauge https://orcid.org/0000-0003-0411-0373
Bjørn Stæhr Madsen https://orcid.org/0000-0002-2846-3598

REFERENCES

1. Blachier M, Leleu H, Peck-Radosavljevic M, Valla D-C, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol*. 2013;58:593-608.
2. Sheron N. Alcohol and liver disease in Europe – simple measures have the potential to prevent tens of thousands of premature deaths. *J Hepatol*. 2016;64:957-967.
3. Teli MR, Day CP, James OFW, Burt AD, Bennett MK. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. *Lancet*. 1995;346:987-990.
4. Ajmera VH, Terrault NA, Harrison SA. Is moderate alcohol use in nonalcoholic fatty liver disease good or bad? A critical review. *Hepatology*. 2017;65:2090-2099.
5. Åberg F, Puukka P, Salomaa V, et al. Combined effects of alcohol and metabolic disorders in patients with chronic liver disease. *Clin Gastroenterol Hepatol*. 2020;18:995-997.e2.
6. Mallet V, Parlati L, Martinino A, et al. Burden of liver disease progression in hospitalized patients with type 2 diabetes mellitus. *J Hepatol*. 2022;76:265-274.

7. Calleja-Conde J, Echeverry-Alzate V, Bühler KM, et al. The immune system through the lens of alcohol intake and gut microbiota. *Int J Mol Sci*. 2021;22:7485.
8. Tripathi A, Debelius J, Brenner DA, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol*. 2018;15:397-411.
9. Seki E, Schnabl B. Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. *J Physiol*. 2012;590:447-458.
10. Anand G, Zarrinpar A, Loomba R. Targeting dysbiosis for the treatment of liver disease. *Semin Liver Dis*. 2016;36:37-47.
11. Afshar M, Richards S, Mann D, et al. Acute immunomodulatory effects of binge alcohol ingestion. *Alcohol*. 2015;49:57-64.
12. Monnig MA, Lamb PS, Parra JM, et al. Immune response to an acute moderate dose of alcohol in healthy young adults. *Alcohol Alcohol*. 2020;55:616-623.
13. Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. *Biol Psychiatry*. 2014;76:725-733.
14. Khoruts A, Stahnke L, McClain CJ, Logan G, Allen JI. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. *Hepatology*. 1991;13:267-276.
15. Adams C, Conigrave JH, Lewohl J, Haber P, Morley KC. Alcohol use disorder and circulating cytokines: a systematic review and meta-analysis. *Brain Behav Immun*. 2020;89:501-512.
16. Bjørkhaug ST, Neupane SP, Bramness JG, et al. Plasma cytokine levels in patients with chronic alcohol overconsumption: relations to gut microbiota markers and clinical correlates. *Alcohol*. 2020;85:35-40.
17. Israelsen M, Kim M, Suvitaival T, et al. Comprehensive lipidomics reveals phenotypic differences in hepatic lipid turnover in ALD and NAFLD during alcohol intoxication. *JHEP Rep*. 2021;3:100325.
18. Torp N, Israelsen M, Nielsen MJ, et al. Binge drinking induces an acute burst of markers of hepatic fibrogenesis (PRO-C3). *Liver Int*. 2022;42:92-101.
19. Bosch J, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol*. 2009;6:573-582.
20. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9:e95192.
21. Pinheiro JC, Bates DB. *Mixed-Effects Models in S and S-PLUS. Statistics and Computing*. Springer. doi: 10.1007/b98882
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57:289-300.
23. Kharitonenkov A, DiMarchi R. Fibroblast growth factor 21 night watch: advances and uncertainties in the field. *J Intern Med*. 2017;281:233-246.
24. Hansen JS, Clemmesen JO, Secher NH, et al. Glucagon-to-insulin ratio is pivotal for splanchnic regulation of FGF-21 in humans. *Mol Metab*. 2015;4:551-560.
25. Flippo KH, Potthoff MJ. Metabolic messengers: FGF21. *Nat Metab*. 2021;3:309-317.
26. Desai BN, Singhal G, Watanabe M, et al. Fibroblast growth factor 21 (FGF21) is robustly induced by ethanol and has a protective role in ethanol associated liver injury. *Mol. Metab*. 2017;6:1395-1406.
27. Lanng AR, Gasbjerg LS, Bergmann NC, et al. The effect of acute intragastric vs. intravenous alcohol administration on inflammation markers, blood lipids and gallbladder motility in healthy men. *Alcohol*. 2020;87:29-37.
28. Søberg S, Andersen ES, Dalsgaard NB, et al. FGF21, a liver hormone that inhibits alcohol intake in mice, increases in human circulation after acute alcohol ingestion and sustained binge drinking at Oktoberfest. *Mol Metab*. 2018;11:96-103.
29. Schumann G, Liu C, O'Reilly P, et al. KLB is associated with alcohol drinking, and its gene product β-Klotho is necessary for FGF21 regulation of alcohol preference. *Proc Natl Acad Sci USA*. 2016;113:14372-14377.
30. Flippo KH, Trammell SAJ, Gillum MP, et al. FGF21 suppresses alcohol consumption through an amygdalo-striatal circuit. *Cell Metab*. 2022;34:317-328.e6.
31. Choi M, Schneeberger M, Fan W, et al. FGF21 counteracts alcohol intoxication by activating the noradrenergic nervous system. *Cell Metab*. 2023;35:429-437.e5.
32. Lee Y, Lim S, Hong ES, et al. Serum FGF21 concentration is associated with hypertriglyceridaemia, hyperinsulinaemia and pericardial fat accumulation, independently of obesity, but not with current coronary artery status. *Clin Endocrinol (Oxf)*. 2014;80:57-64.
33. Ruiz-Margáin A, Pohlmann A, Ryan P, et al. Fibroblast growth factor 21 is an early predictor of acute-on-chronic liver failure in critically ill patients with cirrhosis. *Liver Transpl*. 2018;24:595-605.
34. Praktiknjo M, Djayadi N, Mohr R, et al. Fibroblast growth factor 21 is independently associated with severe hepatic steatosis in non-obese HIV-infected patients. *Liver Int*. 2019;39:1514-1520.
35. Harrison SA, Ruane PJ, Freilich BL, et al. Efruxifermin in non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled, phase 2a trial. *Nat Med*. 2021;27:1262-1271.
36. Sanyal A, Charles ED, Neuschwander-Tetri BA, et al. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet*. 2019;392:2705-2717.
37. Loomba R, Sanyal AJ, Kowdley KV, et al. Randomized, controlled trial of the FGF21 analogue pegozafermin in NASH. *N Engl J Med*. 2023. doi: 10.1056/NEJMoa2304286
38. Israelsen M, Juel HB, Detlefsen S, et al. Metabolic and genetic risk factors are the strongest predictors of severity of alcohol-related liver fibrosis. *Clin Gastroenterol Hepatol*. 2022;20:1784-1794.e9.
39. Quintero-Platt G, González-Reimers E, Rodríguez-Gaspar M, et al. Alpha Klotho and fibroblast growth factor-23 among alcoholics. *Alcohol Alcohol*. 2017;52:542-549.
40. Seitz T, Hellerbrand C. Role of fibroblast growth factor signalling in hepatic fibrosis. *Liver Int*. 2021;41:1201-1215.
41. Owen BM, Mangelsdorf DJ, Kliewer SA. Tissue-specific actions of the metabolic hormones FGF15/19 and FGF21. *Trends Endocrinol Metab*. 2015;26:22-29.
42. Xiao C, Ghosh S. NF-kappaB, an evolutionarily conserved mediator of immune and inflammatory responses. *Adv Exp Med Biol*. 2005;560:41-45.
43. Mandrekar P, Catalano D, Szabo G. Inhibition of lipopolysaccharide-mediated NFκB activation by ethanol in human monocytes. *Int Immunol*. 1999;11:1781-1790.
44. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:1-9.
45. Cao S, Liu M, Sehrawat TS, Shah VH. Regulation and functional roles of chemokines in liver diseases. *Nat Rev Gastroenterol Hepatol*. 2021;18:630-647.
46. Jahnke A, Johnson JP. Synergistic activation of intercellular adhesion molecule 1 (ICAM-1) by TNF-alpha and IFN-gamma is mediated by p65/p50 and p65/c-Rel and interferon-responsive factor Stat1 alpha (p91) that can be activated by both IFN-gamma and IFN-alpha. *FEBS Lett*. 1994;354:220-226.
47. Cheshire JL, Baldwin AS. Synergistic activation of NF-kappaB by tumor necrosis factor alpha and gamma interferon via enhanced I kappaB alpha degradation and de novo I kappaBbeta degradation. *Mol Cell Biol*. 1997;17:6746-6754.
48. Müller M, Morotti A, Ponzetto C. Activation of NF-κB is essential for hepatocyte growth factor-mediated proliferation and tubulogenesis. *Mol Cell Biol*. 2002;22:1060-1072.

49. Ozaki I, Zhao G, Mizuta T, et al. Hepatocyte growth factor induces collagenase (matrix metalloproteinase-1) via the transcription factor Ets-1 in human hepatic stellate cell line. *J Hepatol*. 2002;36:169-178.
50. Lee MR, Abshire KM, Farokhnia M, Akhlaghi F, Leggio L. Effect of oral alcohol administration on plasma cytokine concentrations in heavy drinking individuals. *Drug Alcohol Depend*. 2021;225:108771.
51. Bala S, Marcos M, Gattu A, Catalano D, Szabo G. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLoS One*. 2014;9:e96864.
52. Tan B, Luo W, Shen Z, et al. Roseburia intestinalis inhibits oncostatin M and maintains tight junction integrity in a murine model of acute experimental colitis. *Scand J Gastroenterol*. 2019;54:432-440.
53. West NR, Hegazy AN, Owens BM, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor–neutralizing therapy in patients with inflammatory bowel disease. *Nat Med*. 2017;23:579-589.
54. Israelsen M, Alvarez-Silva C, Madsen BS, et al. Impact of acute alcohol consumption on circulating microbiome in asymptomatic alcohol-related liver disease. *Gut*. 2023;gutjnl-2023-330360. doi:10.1136/gutjnl-2023-330360
55. Israelsen M, Madsen BS, Torp N, et al. Rifaximin-α for liver fibrosis in patients with alcohol-related liver disease (GALA-RIF): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Gastroenterol Hepatol*. 2023;8:523-532. doi:10.1016/S2468-1253(23)00010-9
56. Idalsoaga F, Kulkarni AV, Mousa OY, Arrese M, Arab JP. Nonalcoholic fatty liver disease and alcohol-related liver disease: two intertwined entities. *Front Med*. 2020;7:448.
57. Mellinger JL. Epidemiology of alcohol use and alcoholic liver disease. *Clin Liver Dis*. 2019;13:136-139.
58. Roerecke M, Vafaei A, Hasan OSM, et al. Alcohol consumption and risk of liver cirrhosis: a systematic review and meta-analysis. *Am J Gastroenterol*. 2019;114:1574-1586.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Stankevic E, Israelsen M, Juel HB, et al. Binge drinking episode causes acute, specific alterations in systemic and hepatic inflammation-related markers. *Liver Int*. 2023;00:1-12. doi:10.1111/liv.15692