Divergent Roles of APOAI and APOM in the Identification of Alcohol Use Disorder and their Association with Inflammation and Cognitive Decline: A Pilot Study

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SIGNIFICANCE STATEMENT

Cognitive impairment is a common feature among Alcohol Use Disorder (AUD)-diagnosed individuals, ranging from absent to severe cognitive deterioration. Early identification of vulnerable patients with cognitive impairment attending to an alcohol detoxification program would help the clinicians to provide them with proper assistance, since the diagnostic procedures for cognitive impairment rely on resource-intensive neuropsychological evaluations, often leading to delays in patient categorization and intervention. Our pilot study investigates peripheral biomarkers associated with diagnosis and associated cognitive impairment by understanding the relationship between plasma apolipoproteins, inflammation and cognitive decline in AUD. We showed elevated APOAI and decreased APOM plasma levels associated with inflammation and cognitive deterioration in patients, helping to identify the presence/absence of the disorder and cognitive status. Our study offers a preliminary quantifiable biological approach to support neuropsychological assessment and diagnostic procedures, since we identified plasma APOAI and APOM as potential biomarkers for AUD and related cognitive decline.
ABSTRACT

Background. Alcohol Use Disorder (AUD) courses with inflammation and cognitive decline. Apolipoproteins have emerged as novel target compounds related to inflammatory processes and cognition. Methods. A cross-sectional study was performed on abstinent AUD patients with at least one month of abstinence (n=33; 72.7% men) and healthy controls (n=34; 47.1% men). A battery of plasma apolipoproteins (APOAI, APOAII, APOB, APOCII, APOE, APOJ and APOM), plasma inflammatory markers (LPS, LBP), and their influence on cognition and presence of the disorder were investigated. Results. Higher levels of plasma APOAI, APOB, APOE and APOJ, as well as the proinflammatory LPS, were observed in the AUD group, irrespective of sex, whereas APOM levels were lower versus controls. Hierarchical logistic regression analyses, adjusting for covariates (age, sex, education), associated APOM with the absence of cognitive impairment in AUD, and identified APOAI and APOM as strong predictors of the presence or absence of the disorder, respectively. APOAI and APOM did not correlate with alcohol abuse variables or liver status markers but they showed an opposite profile in their associations with LPS (positive for APOAI; negative for APOM) and cognition (negative for APOAI; positive for APOM) in the entire sample. Conclusions. The HDL constituents APOAI and APOM were differentially regulated in the plasma of AUD subjects compared to controls, playing divergent roles in the disorder identification and associations with inflammation and cognitive decline.

Keywords: Apolipoprotein; ApoE4; Cognition; HDL; LPS.
INTRODUCTION

Alcohol Use Disorder and Cognitive Function

Alcohol Use Disorder (AUD) is associated with health systems burden, with prevalent cognitive impairment affecting memory, attention, executive function or visuospatial abilities (Perry, 2016; Aharonovich et al., 2018; Visontay et al., 2021; Ramos et al., 2022). The degree of severity ranges from absent to severe cognitive deterioration (Hayes et al., 2016). Early identification of cognitively impaired patients in alcohol detoxification programs would help the clinicians provide them with proper assistance. Current diagnostic procedures for cognitive impairment involve neuropsychological evaluations, but they entail a high healthcare burden, with limited availability of professionals, and delay the categorization of patients. Objective diagnostic procedures based on biomarkers related to AUD and cognitive impairment could supplement clinical practices. This approach may aid in timely referral of patients during detoxification programs and potentially lead to the discovery of new therapeutic strategies.

Advances in Biomarker Research

Biological factors related to impaired cognition in AUD are still largely unknown. Alcohol abuse induces neuroinflammation, contributing to cognitive and emotional alterations (Spear, 2018). Exploring the microbiota-gut-brain axis and alcohol-induced inflammation offers new avenues for identifying potential biomarkers of cognitive decline in AUD (Orio et al., 2019; Rodriguez-Gonzalez et al., 2020; Escudero et al., 2023). Our previous studies showed that elevations in proinflammatory molecules in peripheral blood mononuclear cells or plasma of women binge drinkers were associated with worse scores in cognitive flexibility and episodic memory (Orio et al., 2018). Specifically, elevated endotoxin Lipopolysaccharide (LPS) plasma levels were negatively correlated with scores on delayed recall (Orio et al., 2018). Recent studies
highlight the potential of peripheral cytokines and inflammatory-related molecules as biomarkers of alcohol consumption or cognitive impairment in AUD subjects (García-Marchena et al., 2020; Requena-Ocaña et al., 2022; Escudero et al., 2023). However, identifying reliable peripheral biomarkers for detecting cognitive impairment in AUD remains a scientific challenge.

Apolipoproteins have emerged as novel target molecules related to inflammatory processes and cognition. APOE is the most studied in the context of alcohol abuse (Downer et al., 2014; Slayday et al., 2021), since the isoform APOE4 appears to have a role in neuroinflammation (Kloske & Wilcock, 2020; Duro et al., 2022) and cognitive impairment (reviewed in Parhizkar et al., 2022). Recently, we observed that AUD patients carrying the APOE4 isoform exhibited higher levels of plasma Reelin (a protein that shares receptors with APOE4) and more severe cognitive impairment (Escudero et al., 2023). There are other plasma apolipoproteins, such as APOAI, APOB, APOCIII, APOE, APOH, APOM or APOJ that emerge as interesting candidates in this field. Some of them fluctuate in plasma from mid-life and have been related to mild cognitive impairment in old individuals (Song et al., 2012; Muenchhoff et al. 2017) and/or associated with Alzheimer’s disease or dementia (Reynolds et al., 2010; Koch et al., 2018; Chan et al., 2020; Zuin et al., 2021; Xin et al., 2022).

Possible Explaining Mechanisms

Alcohol abuse triggers peripheral inflammation by disrupting the gut barrier (leaky gut), leading to the translocation of bacterial LPS from Gram-negative bacteria to the bloodstream in preclinical models (Antón et al., 2018a, b) and in alcohol-dependent subjects (Keshavarzian et al., 2009; Leclercq et al., 2012; Bala et al., 2014). LPS translocation has been linked to inflammation and activation of the innate immune system, which are closely related to neuroinflammation and behavioral alterations (Dantzer et al., 2008; Alfonso-Loeches et al., 2010;
Pascual et al., 2011, 2014; Crews et al., 2013; Leclercq et al. 2014; Sayd et al., 2015; Antón et al., 2017; Erickson et al., 2019). The alcohol-induced neuroinflammatory effects are enhanced by external administration of LPS in mice (Qin et al., 2008). LPS is transported in blood by the Lipopolysaccharide Binding Protein (LBP) (Opal et al., 1999), which transfers it to its receptor or into lipoproteins (Levels et al., 2005). Apolipoproteins, natural components of lipoproteins, are related to inflammation (Castellani et al., 1997; Berbée et al., 2005; Mousa et al., 2023) and cognition (Lewis et al., 2010; Koch et al., 2018; Shi et al., 2020; Romagnoli et al., 2021), including APOE4 (Kloske & Wilcock, 2020; Duro et al., 2022; Liu et al., 2022).

Increasing evidence suggests that some apolipoproteins may be involved in the detoxification of bacterial LPS, acting as part of the innate immune system (Berbée et al., 2005; Smoak et al., 2010). We observed that APOAI was elevated in plasma and formed aggregates with LPS components within the brain in preclinical models of alcohol abuse, potentially participating in neuroinflammation (Orio et al., 2023; López-Valencia et al., 2024).

**Hypothesis**

This pilot investigation aims to explore possible associations among apolipoproteins, inflammation, and cognitive decline in AUD subjects. Existing literature suggests that alcohol-induced inflammation is negatively associated with cognition, and some apolipoproteins, such as APOE4, may serve as biomarkers for alcohol-induced neuroinflammation and cognitive decline. Emerging evidence suggests that other apolipoproteins may be related to inflammation and modulate cognition in other pathologies, although the direction of influence -if any- is not clear. In the field of alcohol abuse, the relationships among inflammation, apolipoproteins and cognition are highly unexplored.
We hypothesized that: (1) Apolipoproteins (APO: A1, AII, B, CII, E, J and M) and inflammatory markers (LPS, LBP) in plasma are differently regulated in AUD versus controls, while considering sex as a variable; (2) These parameters are associated with the cognitive status of AUD patients or the presence of the disorder. We hypothesized a negative association between inflammation and cognition, but the direction may be different (positive/negative) for each apolipoprotein according to the literature, sometimes controversial (Vuilleumier et al., 2013), and controlling for age, sex and education as covariates; (3) Apolipoproteins related to AUD are associated with alcohol-induced inflammation. We searched for plasma biomarkers of cognitive impairment in AUD subjects undergoing an alcohol detoxification program and, ultimately, those that could potentially identify the disorder.

METHODS

Study Participants

76 participants (Caucasian) were initially recruited: 1) the AUD group [n=39 from an outpatient 'Alcohol Program' at Hospital Universitario 12 de Octubre (Madrid, Spain) (see Supplementary Methods 1.1)]; 2) the control group [n=37 healthy controls]. The final sample (33 AUD and 34 controls) is reported in Supplementary Methods 1.2, Figure S1. The AUD group underwent both pharmacological and psychological interventions as part of the 'Alcohol Program'.

Inclusion criteria. Age ≥ 18 up to 65 years old. In patients, AUD diagnosis was done by expert clinicians, based on DSM-5 criteria (APA, 2014). Abstinence, at least 4 weeks to minimize the potential influence of recent alcohol use (Escudero et al., 2023), was monitored through exhaled breath control. Exclusion criteria. Psychiatric comorbidity, assessed by the
Spanish version of the “Mini International Neuropsychiatric Interview” (MINI) (Ferrando et al., 1998) and DSM-5 (APA, 2014); an history of abuse or dependence to other drugs except tobacco (including alcohol in the control group); chronic medical conditions; infectious diseases (e.g., HIV and/or acute hepatitis); liver diseases (chronic hepatitis, cirrhosis or liver cancer); and chronic use of anti-inflammatory medication. More details in Supplementary Methods 1.2.

This study received approval from the Research Ethics Committee of the Hospital Universitario 12 de Octubre (Madrid, Spain) (Nº CEIm: 19/002) and was conducted in accordance with The Code of Ethics of the World Medical Association Declaration of Helsinki. All participants provided written informed consent and data were kept anonymous and confidential through coding.

Neuropsychological and Clinical Assessment

All participants were assessed by a cognitive screening test specifically validated for AUD: TEDCA “Test of Detection of Cognitive Impairment in Alcoholism” (Jurado-Barba et al., 2017). Cognitive impairment was determined based on the General Cognitive Functioning (GCF) score (cut-off point ≤ 10.5) (Supplementary Methods 1.3.; Table S1 for details).

Patients and controls were assessed for depressive and anxiety symptomatology (in absence of a psychiatric diagnosis), using The Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI), respectively (Supplementary Methods 1.4.).

Sample Collection and Processing

Blood (20 mL) was collected in the morning (8:00-9:00 a.m.) from nurse personnel after a fasting period of 8-12 hours. The blood was drawn by venipuncture into BD vacutainer® tubes containing K2-EDTA anticoagulant (BD, Franklin Lakes, NJ, USA). Plasma was obtained by
centrifugation at 1800 rpm for 10 minutes at 4°C following our previous studies (Escudero et al., 2023). All samples were coded anonymously and stored at -80°C until immunoassay analysis.

**Alcohol Abuse variables and Liver Status parameters**

A semi-structured interview was administered to both groups to obtain data regarding their alcohol consumption history and the use or abuse of other substances. Subjects were asked questions about the type of beverage consumed, quantity, time of abstinence, etc. The liver status of the AUD group was checked by analysis of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase, and bilirubin, which are commonly used in clinical practice. When liver damage was suspected, patients underwent liver ultrasound examinations in the Digestive Department (Supplementary Methods 1.5). No liver analyses were conducted in the control group, assuming a sample of healthy controls.

**Multiplex Immunoassay Technology and Enzyme-Linked Immunosorbent Assay (ELISA)**

Plasma concentrations of apolipoproteins were measured using MAGPIX® multiplexed immunoassay platform (Luminex Corporation). LPS and LBP were determined by ELISA commercial kits, following the manufacturer's instructions (for details, see Supplementary Methods 1.6).

**Statistical Analyses**

Frequency and descriptive statistics were presented as percentages [N (%)] or means with standard deviations [mean (SD)]. Outliers were identified using Grubb’s Test. Sociodemographic differences between the AUD and control groups were examined using Fisher’s exact test, Chi-Square test, or Student's t-test. Two-way ANCOVAs, considering group and sex as factors, were
applied to analyze plasma biological markers and cognition, controlling for age and/or education. Hierarchical logistic regression models, adjusting for significant (age, \( p < 0.05 \)) or marginally significant (sex and education, \( p = 0.050 \) and \( p = 0.053 \), respectively) covariates, assessed the biomarkers' contribution to explain GCF deficit or AUD presence (Supplementary Methods 1.7), alongside Receiving Operating Characteristic (ROC) curve analysis. Pearson correlation analyses with 1% false discovery rate (FDR) (Benjamini & Hochberg, 1995) were conducted to explore associations between apolipoproteins with inflammation, alcohol abuse or liver variables.

Results were analyzed using GraphPad Prism 8.0 and SPSS 25.0, with a significance threshold set at \( p < 0.05 \).

RESULTS

Characteristics of the Sample

Table 1 shows sociodemographic variables for the AUD and control groups, emotional symptomatology and pharmacological treatments. Both groups had similar BMI, but differed significantly in age (\( p = 0.000 \)). Sex approached statistical significance (\( p = 0.05 \)). Although the AUD group showed trends of lower education levels and higher unemployment rates, these differences were not statistically significant (Table 1). Tobacco consumption did not differ between groups. Disulfiram was the most frequently used psychiatric medication, followed by antidepressants, whereas none of the control group received psychiatric medication. Some patients presented psychological symptomatology, including minimal depressive symptoms and mild anxiety, with minimum scores in controls. Statistically significant differences in depressive (\( p = 0.001 \)) and anxious symptoms (\( p = 0.006 \)) were observed between groups (Table 1).
The alcohol abuse outcomes and liver status markers in the AUD group are shown in Table 2. In the control group, the number of Standard Drinking Units (SDU) per month was 7.50 ± 6.02 (mean ± SD). The liver status fell within normal ranges, according to European standards.

**Plasma Apolipoprotein Levels**

Figure 1 shows plasma apolipoproteins (APOAI, APOAII, APOB, APOCII, APOE, APOJ, APOM) in the AUD and control groups disaggregated by sex. The two-way ANCOVAs with group and sex as factors, controlling for age, indicated a group effect in plasma APOAI, APOB, APOE and APOJ [Fig. 1A, log10(APOAI): F(1,61)=48.20, p=0.000, $\eta^2=0.44$ (to be noted a substantial magnitude of effect)] [Fig. 1C, APOB: F(1,62)=6.41, p=0.014, $\eta^2=0.09$] [Fig. 1E, APOE: F(1,62)=4.31, p=0.042, $\eta^2=0.06$] [Fig. 1F, log10(APOJ): F(1,62)=6.75, p=0.012, $\eta^2=0.10$], with higher levels in the AUD group. Plasma APOM showed an inverse profile to other apolipoproteins, showing a significant group effect [F(1,62)=10.06; p=0.002; $\eta^2=0.14$], with lower levels in the AUD subjects compared to the control subjects (Fig. 1G). There was no effect of sex for any of the apolipoproteins (p>0.05). Total apolipoprotein levels for each group are reported in Supplementary Results 2.1.; Table S3. Age did not modulate these results for apolipoproteins (p>0.05), except for APOCII [Fig. 1D; F (1,62)=5.88; p=0.018, $\eta^2=0.09$], but there was no group effect for APOAII (Fig. 1B).

**Plasma Inflammatory Parameter Levels**

Figure 2 displays plasma inflammatory parameters (LPS, LBP) in the AUD and control groups, divided by sex. Two-way ANCOVAs with group and sex as factors and controlling for age, revealed a group effect in plasma LPS [F(1,59)=11.36, p=0.001, $\eta^2=0.16$], disregarding of sex, with higher levels in AUD patients than controls (Fig. 2A). LBP data showed no significant
group differences, and no sex effect was found either (Fig. 2B). Total levels in each group without division by sex is reported in Supplementary Results 2.1; Table S3.

Neuropsychological Data

Figure 3 illustrates cognitive data in AUD and control groups. Two-way ANCOVAs, with age and education as covariates, revealed a group effect in GCF (Fig. 3A; GCF: F(1,61)=15.06, \( p=0.000, \eta^2=0.20 \)). The AUD group showed a GCF deficit in 45.5% of the AUD sample (cut-off point ≤ 10.5). Lower scores were found also in all cognitive subdomains (Fig. 3B; left panel: Visuospatial Cognition: F(1,61)=6.58, \( p=0.013, \eta^2=0.44 \); middle panel: Memory/Learning: F(1,61)=16.57, \( p=0.000, \eta^2=0.21 \); and right panel: EF: F(1,61)=5.62, \( p=0.021, \eta^2=0.08 \)) for the AUD group, being Memory/Learning the most affected subdomain.

Contribution of Apolipoproteins and Inflammation to AUD-induced Cognitive Impairment

Hierarchical Logistic Regression Models were used to determine the biomarkers' contribution to explaining the GCF deficit. The analysis used a two-block approach: block 1) included mandatory covariates: age, sex and education; block 2) added significant biomarkers identified through Pearson correlation analyses.

Pearson Correlation Analyses

Negative correlations with GCF were found in patients for APOE (\( r=-0.61, p=0.000, q=0.00 \)) and LPS (\( r=-0.66, p=0.000, q=0.000 \)), whereas APOM showed a positive association (\( r=0.63, p=0.000, q=0.00 \)). These analyses allowed us to depict the biomarkers to be introduced in block 2, and provided insight into their directional associations (positive or negative). Other parameters did not show significant associations (\( p>0.05 \)) (Pearson correlation analyses, after
We introduced significant biomarkers (APOE, APOM, LPS) found in block 2, after introducing all covariates (age, sex, education) in block 1. Only APOM showed significance up to the final model (Nagelkerke $R^2 = 0.660$; Hosmer and Lemeshow $= 0.889$). The equation results were $[-0.140 \times \text{age}] + [0.907 \times \text{sex}] + [2.768 \times \text{education}] + [-0.782 \times \text{APOM}]$. APOM was significant ($p=0.012$), with an OR=0.458 showing an inverse association. Specifically, the risk of GCF deficit in the AUD group decreased by 54.2% ($\left[e^{0.782 \times 1} - 1\right] 	imes 100$) for each increased unit of APOM, after controlling for covariates. Therefore, higher plasma APOM levels identified the absence of cognitive impairment in the AUD group. Results are shown in Table 3.

**Identifying AUD Presence**

We used Hierarchical Logistic Regression Models to address whether plasma apolipoproteins and/or inflammatory markers could identify the presence of AUD. Block 1 introduced covariates age, sex and education as control factors and block 2 introduced only significant biomarkers that showed an effect of group in the ANCOVAs previously performed (see Figure 1).

Among all significant biomarkers differentially regulated in AUD (APOAI, APOB, APOE, APOJ, APOM, LPS; Fig.1&2), only APOAI and APOM showed significance up to the final model (Nagelkerke $R^2 = 0.899$; Hosmer and Lemeshow $= 0.959$). The resulting equation was $[0.171 \times \text{age}] + [0.743 \times \text{sex}] + [2.629 \times \text{education}] + [0.336 \times \text{APOAI}] + [-0.621 \times \text{APOM}]$. APOAI had an OR=1.399, meaning that for each increased unit of this biomarker, the risk of AUD increased 39.9% $\left[e^{0.399 \times 1} - 1\right] 	imes 100$, after controlling for covariates. For APOM
(OR=0.537), the risk of the disorder decreased by 46.3% \((e^{0.621x1-1}) \times 100\) for each unit of increase after controlling for covariates. The possible contribution of age to the disorder's presence is not ruled out \((p=0.05)\), with a lower risk percentage \((18.6\% \,(e^{0.171x1-1}) \times 100))\). Results are shown in Table 4.

**ROC Analysis of the Association between Biomarkers and Cognition or Identification of the Disorder**

A ROC curve was performed for APOM in the AUD group to elucidate the absence of GCF deficit (Figure 4A). The area under the curve for APOM was 0.86 (95% CI 0.72 - 0.99), suggesting a robust ability of plasma APOM to predict the absence of cognitive impairment. Similarly, ROC curves were performed for APOAI and APOM (Figure 4B) to explain the presence (APOAI) or absence (APOM) of AUD, respectively. The area under the curve for APOAI was 0.92 (95% CI 0.86 - 0.99) (Fig. 4B, left panel) and 0.74 for APOM (95% CI 0.63 - 0.86) (Fig. B, right panel). This suggests that APOAI may have a robust discriminatory power in distinguishing subjects with AUD from controls.

**Exploring Possible Mechanisms**

As previously explained, we found changes in plasma APOAI, APOB, APOE, APOJ and APOM compared to the control group. We found that APOM was associated with impaired cognition in patients and that increased APOAI along with decreased APOM levels in plasma identified the presence of the disorder (with a possible contribution of age). In this section, we conduct a preliminary exploration of the factors that may be associated with the observed changes in APOAI and APOM levels in plasma.
**Alcohol Abuse Variables**

The alcohol abuse outcomes in the AUD group are shown in Table 2. No significant correlations were observed between significant apolipoproteins and these alcohol variables (Pearson correlations; \( p > 0.05 \); data not shown).

**Liver status**

The liver status markers in the AUD group are shown in Table 2. No significant correlations between significant apolipoproteins and liver parameters were found (Pearson correlation \( p > 0.05 \); data not shown).

**Pro-inflammatory State**

We found modest and opposite associations between selected apolipoproteins and LPS (Pearson correlation analysis) and no associations with LBP (\( p > 0.05 \); data not shown). APOAI was not associated with LPS (\( r = 0.159, p = 0.410, \text{n.s.} \)) or cognition (\( r = -0.045, p = 0.808, \text{n.s.} \)) in the AUD group (Figure 5A), and only APOM was negatively associated with LPS \( r = -0.548, p = 0.002 \) and positively with cognition (\( r = 0.631, p = 0.000 \)) in the AUD group (Figure 5B).

We investigated this mechanism also in the entire sample. We found that APOAI correlated positively with LPS (\( r = 0.422, p = 0.001 \)) and negatively with cognition (\( r = -0.426, p = 0.000 \)) in the entire sample (Figure 6A) and opposite associations were found for APOM both with LPS (negative, \( r = -0.426, p = 0.000 \)) and cognition (positive, \( r = 0.566, p = 0.000 \)) (Figure 6B).
DISCUSSION

This study assessed blood apolipoproteins and inflammatory markers in AUD patients, their association with cognitive impairment and their potential for disorder identification. We found higher LPS, APOAI, APOB, APOE and APOJ and lower APOM plasma levels in AUD subjects compared to controls, with no sexual differences. LPS and APOE correlated with impaired cognition and APOM with better performance in the AUD group, with APOM maintaining significance in hierarchical logistic models. APOAI and APOM were indicative of the disorder's presence and absence, respectively (with a possible influence of age), and they diverged also in their associations with LPS and cognition in the entire sample. Whereas APOAI correlated positively with LPS and negatively with GCF, APOM showed the opposite trends.

Apolipoproteins and Inflammatory Markers in AUD versus Controls

We observed changes in components of high-density lipoproteins (HDL: APOAI, APOE and APOM) and low-density lipoproteins (LDL: APOB), together with APOJ, in AUD subjects during abstinence. No sexual differences were found. APOAI levels were remarkably different between the AUD and control groups ($\eta^2=0.44$). APOCII levels were age-influenced in both groups, aligning with previous reports (Sakurabayashi et al., 2001; Muenchhoff et al., 2017). Elevated APOB levels have been described in drinkers (Yin et al., 2011), and we recently found higher APOJ levels in abstinent AUD subjects (Escudero et al., 2023).

We found higher LPS plasma levels in the AUD group, as suggested by animal (Crews et al., 2013; Antón et al., 2017) and human (Bala et al., 2014; Antón et al., 2018a, b; Orio et al., 2018) studies. No changes were observed in plasma LBP, a protein known for transferring or
neutralizing LPS among lipoprotein subclasses (Wurfel et al., 1994; Levels et al., 2005), and no sex differences were found.

**Contribution of Apolipoprotein profile to Cognitive Decline and Identification of the Disorder**

Despite initial associations between cognition and LPS, APOE (negative) and APOM (positive), hierarchical analyses revealed APOM as the only apolipoprotein inversely related with cognitive decline in the AUD group, adjusting for sex, age and education. The role of LPS (reviewed in Brown et al., 2024) and APOE, specifically ApoE-ε4 (Kloske & Wilcock, 2020; Dilliott et al., 2021), in cognition has been documented. Our previous findings highlighted the detrimental impact of APOE4 plasma expression on memory/learning in a similar AUD cohort (Escudero et al., 2023), consistent with reports suggesting that the blood pool of some apolipoproteins (i.e. APOE, APOAI) influence cognitive performance (Lewis et al., 2010; Lane-Donovan et al., 2016; Liu et al., 2022). Interestingly, APOM was the only apolipoprotein reduced in patients, since the rest of apolipoproteins were upregulated in plasma. Although research on APOM remains limited, our results may align with studies showing low APOM levels in AD's patients (Khoonsari et al., 2016; Xin et al., 2022).

Despite the significant plasma APOAI elevations in patients versus controls, with a relevant magnitude of the effect, we did not find associations with cognition in our AUD sample, being this apolipoprotein one of the most robust candidates proposed for cognition association (Kawano et al., 1995; Lewis et al., 2010; Lefterov et al., 2010; Niu et al., 2023).

APOAI and APOM serve as potential AUD biomarkers. Elevated plasma APOAI and decreased APOM levels would explain the presence of AUD disorder, potentially influenced by
age, a covariate approaching significance in the final statistical model. Age-related effects on apolipoprotein expression were observed in very old individuals, with abnormal levels during senescence (Song et al., 2012). However, how these apolipoproteins differ with age is unclear (Muenchhoff et al., 2017), as some exhibit non-linear, inverse trends or weak correlations with age (Song et al., 2012). The divergent roles of APOAI and APOM in AUD identification deserve further investigation.

**Discussion of possible Mechanisms**

The changes in plasma apolipoprotein levels during early AUD abstinence can be discussed from various perspectives.

Firstly, plasma apolipoprotein levels might relate to alcohol consumption variables. Wilkens and colleagues found a link between total APOAI and alcohol consumption in older adults, and associations between HDL subspecies lacking or containing APOC3, APOE or APOJ and alcohol intake (Wilkens et al., 2023). Liappas et al. (2007) found APOE serum levels correlated with alcohol consumption during the previous year of alcohol abuse. However, in our study, plasma apolipoproteins did not correlate with alcohol consumption variables.

Secondly, excessive alcohol intake induces fatty liver, enhancing lipogenesis and lipid hyperaccumulation (Osna et al., 2017). APOB and APOM are linked to fatty liver recovery or fibrosis, although APOM’s role remains controversial (Tahara et al., 1999; Hajny & Christoffersen, 2017; Chen & Hu, 2020). However, our study examines the relationship between plasma apolipoproteins, inflammation and cognition in abstinent AUD subjects without major liver disease. In our sample, AUD patients’ liver parameters were within normal ranges, and we found no associations between apolipoproteins and liver markers, suggesting that
observed alterations likely did not reflect significant liver damage. Accordingly, evidence suggests higher APOAI and II in alcoholic patients without liver damage or mild steatosis compared to controls (Marth et al., 1982; Duhamel et al., 1984).

Thirly, apolipoprotein levels may be related to the inflammatory state in alcohol abuse, contributing to cognitive and emotional alterations (Spear, 2018; Orio et al., 2018). Subjects with high plasma LPS had higher APOAI and lower APOM levels, and all these changes correlate with poorer cognition. These associations were significant in the entire sample, but the effect of APOAI was lost in the AUD group alone, possibly due to the limited sample size. The positive LPS-APOAI association could be related to APOAI's ability to bind LPS and neutralize its inflammatory actions (Li, Dong & Wu, 2008). APOAI has anti-inflammatory properties during LPS-induced endotoxemia (Yan et al. 2006; Vuilleumier et al., 2013). It would be plausible to hypothesize that plasma levels of APOAI are elevated in AUD, where systemic LPS and proinflammatory environment are high, as a compensatory mechanism to mitigate LPS response. However, despite its generally accepted anti-inflammatory properties, APOAI can become dysfunctional or even proinflammatory during chronic systemic inflammation (Vuilleumier et al., 2013). We have recently demonstrated that APOAI may facilitate the transport of LPS to brain (López-Valencia et al., 2024), suggesting deleterious effects. The role of APOAI in cognition remains unclear. APOAI downregulation/deficiency has been associated with cognitive deficits (Kawano et al., 1995; Lefterov et al., 2010) whereas overexpression has been linked to preserved cognitive function (Lewis et al., 2010), contrasting with our data. However, plasma APOAI has recently been identified as a biomarker for AD diagnosis (Niu et al., 2024), so its overexpression in AUD may indicate high inflammation and cognitive deficits.
Instead, APOM maintained a significant negative correlation with LPS both in the entire sample and in the AUD group alone. APOM's anti-inflammatory properties have been described in knock-out mice (Shi et al., 2020), since APOM binds LPS to facilitate its neutralization and clearance (Mousa et al., 2023). APOM is considered a negative acute response protein, with decreased serum levels observed during LPS administration in preclinical models and during infection and inflammation in humans (Feingold et al., 2008), aligning with our findings.

Interestingly, both APOM and APOAI are major components of HDL (Phillips, 2013; Hajny & Christoffersen, 2017). Alterations in HDL-associated proteins have been described in infection and inflammation contexts, suggesting a decreased ability of HDL to protect against lipid peroxidation (reviewed in Khovidhunkit et al., 2000). However, the effects of endotoxin and cytokines on lipoproteins can be both detrimental and beneficial (Hardardóttir et al., 1994).

**Limitations and Conclusions**

These preliminary results are limited by a small sample size, which restricts their generalizability. Confirming and strengthening these findings in a larger sample is crucial. The small sample size affects the inclusion of variables in statistical analysis, making only large differences significant. Nonetheless, adjusting for significant (age, p<0.05) or marginally significant (sex and education, p=0.050 and p=0.053, respectively) covariates, and included significant biomarkers for the hierarchical analyses. Despite the small sample size, we found considerable effects in the expression of some apolipoproteins.

Other limitations of the study include: (1) the high co-occurrence of AUD with other psychiatric disorders, so the cohort of patients in this study may not be fully representative; (2) associations between biological parameters and cognition do not imply causality, so caution may
be taken in the extrapolation of results; (3) more precise liver damage evaluation using MRI-PDFF instead of ultrasound or computed axial tomography (CAT) scans for exclusion criteria.

Despite these limitations, key strengths include the homogeneity of sample regarding the abstinence period of recruitment, the use of comprehensive protocols for diagnosis and evaluation and the rigorous inclusion-exclusion criteria. This study analyzes a wide spectrum of biomarkers that may have contrasting effects (i.e. anti- or pro-inflammatory actions) and controls for important confounded variables, enhancing the translational value of the findings.

Conclusions: (1) several apolipoproteins and inflammatory markers (LPS) are altered in the plasma of AUD patients during early abstinence, independent of sex. (2) key HDL components APOAI and APOM are differentially regulated in AUD subjects compared to controls, playing divergent roles in disorder identification. (3) APOM inversely correlates with inflammation and may serve as a biomarker for AUD-related cognitive impairment. Understanding these peripheral biomarkers in AUD could facilitate early diagnosis and monitorization of disease progression.
FUNDING

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data in the manuscript are freely available upon request to the corresponding author (lorio@psi.ucm.es).
REFERENCES


FIGURE LEGENDS

**Figure 1.** Analysis of plasma apolipoproteins in the AUD and control groups: (A) log10 (APOAI), (B) APOAI, (C) APOB, (D) APOCII, (E) APOE, (F) log10 (APOJ), and (G) APOM. Results are presented as mean (SD). Two-way ANCOVAs (group and sex), controlling for age, were conducted. N= 32-33 (AUD group); N = 34 (control group). Overall effect of group is indicated by * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001. Abbreviations: AUD= Alcohol Use Disorder.

**Figure 2.** Analysis of plasma inflammatory parameters in the AUD and control groups: (A) LPS, (B) LBP. Results are presented as mean (SD). Two-way ANCOVAs (group and sex), controlling for age, were conducted. N = 30-32 (AUD group); N = 34 (control group). Overall effect of group is indicated by * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001. Abbreviations: AUD= Alcohol Use Disorder.

**Figure 3.** Analysis of cognitive performance in the AUD and control groups: (A) GCF, (B) Cognitive Domains: Visuospatial Cognition, Memory/Learning, EF. Results are presented as mean (SD). Two-way ANCOVAs (group and sex), controlling for age and education, were conducted. N= 33 (AUD group); N= 34 (Control group). Overall effect of group is indicated by * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001. Abbreviations: AUD= Alcohol Use Disorder; GCF= General Cognitive Functioning; Visuospatial = Visuospatial Cognition; EF= Executive Function.

**Figure 4.** ROC analysis for Hierarchical Logistic Regression models. (A) GCF deficit as a dependent variable in the AUD group; (B) Presence of AUD as a dependent variable in the entire sample. The graph shows the trade-off between sensitivity (y-axis) and 1-specificity (x-axis). Both axes of the graph include values between 0 and 1 (0% to 100%). The line drawn from point 0.0 to the point 1.0 is called the reference diagonal or the line of non-discrimination. Abbreviations: GCF= General Cognitive Functioning; AUC= Area Under the Curve; AUD= Alcohol Use Disorder; CI= Confidence Interval; SE= Standard Error.

**Figure 5.** Correlations between plasma apolipoproteins APOAI/APOM with inflammation and cognition in the AUD group. (A) APOAI with LPS (left) and GCF scores (right). (B) APOM with LPS (left) and GCF scores (right). Significantly different: **p<0.01; ****p < 0.0001. Abbreviations: GCF= General Cognitive Functioning; n.s: non-significant.
Figure 6. Correlations between plasma apolipoproteins APOAI/APOM with inflammation and cognition in the entire sample (AUD and control groups). (A) APOAI with LPS (left) and GCF scores (right). (B) APOM with LPS (left) and GCF scores (right). Significantly different: **p<0.01; ****p < 0.0001.

Abbreviations: AUD = Alcohol Use Disorder; GCF= General Cognitive Functioning.
Table 1. Characteristics of the sample.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUD (N= 33)</th>
<th>Control (N= 34)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age [mean (SD)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years</td>
<td>49.36 (7.41)</td>
<td>37.41 (12.61)</td>
<td><strong>0.000</strong>a</td>
</tr>
<tr>
<td><strong>BMI [mean (SD)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg/m²</td>
<td>26.44 (4.84)</td>
<td>24.44 (3.90)</td>
<td>0.066a</td>
</tr>
<tr>
<td><strong>Sex [N (%)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>9 (27.3)</td>
<td>18 (52.9)</td>
<td>0.050b</td>
</tr>
<tr>
<td>Men</td>
<td>24 (72.7)</td>
<td>16 (47.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Education [N (%)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic (No and yes high school degree)</td>
<td>12 (36.4)</td>
<td>5 (14.7)</td>
<td><strong>0.053</strong>b</td>
</tr>
<tr>
<td>Higher (College degree)</td>
<td>21 (63.6)</td>
<td>29 (85.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Current work status [N (%)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>26 (78.8)</td>
<td>32 (94.1)</td>
<td>0.083b</td>
</tr>
<tr>
<td>Unemployed</td>
<td>7 (21.2)</td>
<td>2 (5.9)</td>
<td></td>
</tr>
<tr>
<td>**Current smoking status [N (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20 (60.6)</td>
<td>16 (47.1)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>5 (15.2)</td>
<td>4 (11.8)</td>
<td>0.337c</td>
</tr>
<tr>
<td>No</td>
<td>8 (24.2)</td>
<td>14 (41.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Psychiatric medication use [N (%)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>16 (48.5)</td>
<td>no use</td>
<td>-</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>7 (21.2)</td>
<td>no use</td>
<td>-</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>14 (42.4)</td>
<td>no use</td>
<td>-</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>4 (12.1)</td>
<td>no use</td>
<td>-</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>29 (87.9)</td>
<td>no use</td>
<td>-</td>
</tr>
<tr>
<td><strong>Emotional symptomatology [mean (SD)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive (BDI-II)</td>
<td>11.61 (10.53)</td>
<td>4.76 (3.51)</td>
<td><strong>0.001</strong>a</td>
</tr>
<tr>
<td>Anxious (BAI)</td>
<td>11.21 (13.57)</td>
<td>4.09 (3.13)</td>
<td><strong>0.006</strong>a</td>
</tr>
</tbody>
</table>

Note. Anthropometric measures assessed: weight, height and body mass index (BMI=weight in kg/height squared). Abbreviations: [AUD: alcohol use disorder; BMI: body mass index; BDI-II: Beck Depression Inventory-II; BAI: Beck Anxiety Inventory; SD: standard deviation; N: total of cases]. Data are expressed as mean (SD). The significant values (p value < 0.05) are denoted by bold entries in the table: aStudent's t-test; bFisher's exact test; cChi-square test.
Table 2. Alcohol abuse outcomes and liver status in the AUD group

<table>
<thead>
<tr>
<th>Alcohol Abuse Variables</th>
<th>[Mean (SD)]</th>
<th>N=33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of alcohol abuse since the last relapse (weeks)</td>
<td>37.67 (19.28)</td>
<td></td>
</tr>
<tr>
<td>Age of first alcohol consumption (years old)</td>
<td>15.12 (3.72)</td>
<td></td>
</tr>
<tr>
<td>Age of problematic drinking initiation (years old)</td>
<td>29.91 (11.22)</td>
<td></td>
</tr>
<tr>
<td>Duration of abstinence since last consumption at recruitment (days)</td>
<td>44.94 (16.45)</td>
<td></td>
</tr>
<tr>
<td>Standard Drinking Units (SDU) per month</td>
<td>24.88 (13.02)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liver status markers</th>
<th>[Mean (SD)]</th>
<th>N= 30-33</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L) (n=33)</td>
<td>32.42 (26.36)</td>
<td></td>
</tr>
<tr>
<td>AST (U/L) (n=33)</td>
<td>30.09 (26.24)</td>
<td></td>
</tr>
<tr>
<td>GGT (U/L) (n=32)</td>
<td>54.78 (59.05)</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L) (n=30)</td>
<td>81.03 (23.28)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dL) (n=31)</td>
<td>0.57 (0.37)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The duration of alcohol abuse variable refers to the length of time the patient has been consuming alcohol since their last relapse. Standard Drinking Units: volume of alcohol in liters times the percentage of alcohol contained in the beverage times 0.8 (since 1 ml of alcohol contains 0.785 grams of alcohol). The European reference values (inner and upper limits) for each parameter (International Units/Litre, U/L) are: ALT: 5-45; AST: 5-33; GGT: 8-61; ALP: 40-130; Bilirubin: 0.2-1.0. Abbreviations: [AUD: Alcohol Use Disorder; N: total of cases; SDU: Standard Drinking Units; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; GGT: Gamma-Glutamyl Transferase; ALP: Alkaline Phosphatase; SD: standard deviation]. Data (n=30-33) is expressed as mean (SD).
Table 3. Hierarchical Logistic Regression model showing the association between biomarkers (apolipoproteins and inflammatory markers) with GCF deficit.

<table>
<thead>
<tr>
<th>Variables entered</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>OR (95 % CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.14</td>
<td>0.19</td>
<td>1.64</td>
<td>0.87 (0.70-1.08)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex</td>
<td>0.91</td>
<td>1.71</td>
<td>0.28</td>
<td>2.48 (0.09-70.23)</td>
<td>0.59</td>
</tr>
<tr>
<td>Education</td>
<td>2.77</td>
<td>1.72</td>
<td>2.59</td>
<td>15.92 (0.55-461.97)</td>
<td>0.11</td>
</tr>
<tr>
<td>APOM</td>
<td>-0.78</td>
<td>0.31</td>
<td>6.38</td>
<td>0.46 (0.25-0.84)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note. Hierarchical logistic regression model having included APOE, APOM and LPS, controlled by age, sex and educational level. Table shows final model (step1), with APOM showing significance. Abbreviations: [GCF: General Cognitive Functioning; B: coefficient; SE: standard error; Wald: Wald test; OR: Odds Ratio; CI: confidence interval]. The significant values (P-value < 0.05) are denoted by bold entries in the table.
Table 4. Hierarchical Logistic Regression model showing the association between biomarkers (apolipoproteins and inflammatory markers) and presence of AUD.

<table>
<thead>
<tr>
<th>Variables entered</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>OR (95 % CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.17</td>
<td>0.09</td>
<td>3.92</td>
<td>1.19 (1.00-1.40)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sex</td>
<td>0.74</td>
<td>1.50</td>
<td>0.24</td>
<td>2.10 (0.11-40.09)</td>
<td>0.62</td>
</tr>
<tr>
<td>Education</td>
<td>2.63</td>
<td>2.13</td>
<td>1.53</td>
<td>13.86 (0.21-896.84)</td>
<td>0.22</td>
</tr>
<tr>
<td>APOAI</td>
<td>0.34</td>
<td>0.16</td>
<td>4.43</td>
<td>1.40 (1.02-1.91)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>APOM</td>
<td>-0.62</td>
<td>0.31</td>
<td>3.97</td>
<td>0.53 (0.29-0.99)</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

Note. Hierarchical logistic regression model having included APOAI, APOB, APOE, APOJ, APOM and LPS, controlled by age, sex and educational level. Table shows final model (Step 2), with APOAI and APOM showing significance. Abbreviations: [AUD: Alcohol Use Disorder; B: coefficient; SE: standard error; Wald: Wald test; OR: Odds Ratio; CI: confidence interval]. The significant values (P-value < 0.05) are denoted by bold entries in the table.
Figure 3

A. General Cognitive Function (GCF)

B. Cognitive Domains
Figure 6

A

- $r = 0.422^{**}$
- $r = -0.426^{****}$

B

- $r = -0.557^{****}$
- $r = 0.566^{****}$