Archival Report

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The Predictive Value of Plasma Bioactive Lipids on Craving in Human Volunteers With Alcohol Use Disorder

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ABSTRACT

BACKGROUND: Alcohol use disorder (AUD) is a chronic relapsing disorder characterized by alcohol seeking and consumption despite negative consequences. Despite the availability of multiple treatments, patients continue to exhibit high relapse rates. Thus, biomarkers that can identify patients at risk for heightened craving are urgently needed. Mounting preclinical and clinical evidence implicates perturbations in bioactive lipid signaling in the neurobiology of craving in AUD. We hypothesize that these lipids are potential biomarkers for predicting alcohol craving in patients with AUD.

METHODS: This study used archival deidentified clinical data and corresponding plasma specimens from 157 participants in 3 clinical studies of AUD. We evaluated plasma levels of 8 lipid species as predictors of craving in response to in vivo alcohol and affective cues during abstinence.

RESULTS: Participants were 109 men and 48 women who met DSM-5 criteria for severe AUD. We found that plasma levels of 12- and 15-HETE, 12/15-lipoxygenase–produced proinflammatory lipids, and palmitoylethanolamide, an anti-inflammatory fatty acid amide hydrolase–regulated lipid metabolite, were differentially correlated with alcohol craving during abstinence, predicting higher craving independent of demographics, alcohol use history, and multiple therapeutic treatments.

CONCLUSIONS: Our findings highlight the promise of these lipid metabolites as biomarkers of heightened alcohol craving. The results open a novel opportunity for further research and clinical evaluation of these biomarkers to optimize existing treatments and develop new therapeutics for AUD.

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Alcohol use disorder (AUD) is a chronic relapsing disorder characterized by the compulsion to seek out and consume alcohol despite the negative consequences of continued use (1). Front-line therapeutics such as naltrexone and acamprosate have regulatory approval for the treatment of AUD as part of a comprehensive treatment plan (2,3). They are effective and important aids in the treatment of people with this condition. Given the diverse biological processes that contribute to AUD, new medications are needed to provide a broader spectrum of treatment options. Some people may respond to a medication that helps with craving, whereas others may respond to a medication that relieves impulsivity, and others may respond to a medication that reverses the negative emotional states of early abstinence and protracted withdrawal. Just like any other medical condition, people with substance use disorders deserve to have a range of treatment options available to them. Notably, the emergence of negative affect (e.g., anxiety, depression, and pain) during abstinence is known to promote increased alcohol craving and relapse (4). To address this issue, biomarkers that can predict heightened craving and relapse risk before the beginning of treatment are urgently needed to identify and help vulnerable patients during treatment.

Mounting preclinical and clinical evidence implicates perturbations in bioactive lipid signaling in the neurobiology of negative affect in AUD. For example, the enzyme fatty acid amide hydrolase (FAAH), in addition to facilitating the metabolism of anandamide, tightly regulates endogenous levels of anti-inflammatory and antinociceptive *N*-acyl ethanolamides by metabolizing these lipid signals into inactive fatty acids. Preclinical studies indicate that therapeutic administration of FAAH inhibitors decreases alcohol consumption in both mouse and rat models of AUD (5,6) and reduces alcohol reinstatement in rats (5,6). During abstinence, patients with AUD have elevated plasma levels of the endocannabinoid (eCB) anandamide (AEA), as well as other *N*-acyl ethanolamides including oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (7), suggesting that these lipids may counteract negative affective states that emerge during protracted withdrawal. Additionally, one study that profiled circulating levels of eCBs in 12 patients with AUD showed a positive correlation of AEA with alcohol craving (8). However, the predictive value of

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circulating *N*-acyl ethanolamides on craving in patients with AUD has not been fully evaluated. This is important given that the arachidonic acid moiety contributes to the production of many other lipid species that are relevant to inflammation and disease, such as eicosanoids (9).

Proinflammatory lipids can induce negative affective symptoms and liver toxicity. Notably, overexpression of 12/15-lipoxygenases (12/15-LOX) can lead to negative affective symptoms similar to those observed in individuals with AUD, such as anxiety (10), pain (11), and cognitive impairments (12). Furthermore, alcohol consumption leads to increased 12/15-LOX activity in the liver, where genetic or chemical inactivation of 12/15-LOX reduces liver damage in mice (13). Together, 12/15-LOXs exert their proinflammatory and pronociceptive effects through the production of multiple lipid species including 15-HETE, 12-HETE, 13-HODE, and 9-HODE (14,15). In humans, circulating levels of 12/15-LOX metabolites have been found to be elevated in both cocaine-using adolescents during abstinence (16) and patients with alcohol-related liver disease (17), suggesting that plasma measurements of these lipids may reflect the neurobiological effects of AUD. Thus, their role in AUD should be explored further.

Despite mounting evidence of the important role of lipid signaling in AUD, no studies have directly evaluated the viability of these bioactive lipid species as potential biomarker(s) for predicting heightened alcohol craving during abstinence in clinical populations. Thus, the goal of the current study was to explore the relationship between circulating bioactive lipids in a clinical model of craving in participants with AUD. We demonstrated that bioactive lipid level assessments made prior to the initiation of treatment predicted alcohol craving during abstinence and may do so independently of baseline clinical characteristics and treatments (18–24).

METHODS AND MATERIALS

Study Participants and Research Material

This analysis was conducted using archival, deidentified clinical data and corresponding plasma specimens collected under research protocols originally approved by the Institutional Review Board of the Scripps Research Institute. Participants were non–treatment-seeking volunteers with AUD who participated in one of the 3 previous human laboratory studies (21,23,24) with similar research designs and admission criteria. Study samples were integrated to form the patient database for the current analysis ($N = 157$). Selection criteria for these 3 studies included male and female volunteers ages 18 to 65 who met DSM-5 criteria for AUD of moderate or greater severity. These 3 previous studies were all double-blind and placebo-controlled, with random assignment either to placebo or one of the 3 treatments: the glucocorticoid receptor antagonist mifepristone 600 mg/day (orally) (21), the phosphodiesterase 4 inhibitor apremilast 90 mg/day (orally) (23), and the peroxisome proliferator activated receptor alpha (PPARα) agonist fenofibrate 145 mg/day (orally) (24). Dosing duration was based on time to achieve steady-state drug plasma concentration or maintenance dosing and was never <1 week or longer than 2 weeks. Participants were permitted ad libitum drinking while on drug, except for required abstinence on the last 3 days of the dosing period to test the effect of the study drug on responsivity to alcohol and affective cues in the lab when motivational signs of early abstinence, that is, craving, are manifested. Plasma samples from these participants were collected at baseline and evaluated for the current analysis as described below.

Assessment of Alcohol Use

The Timeline Followback Interview (25) was used to assess daily alcohol use and was obtained at baseline (T1, prior to initiation of treatment) and on the last day of drug dosing (T2, defined as the day of in vivo alcohol cue exposure testing that occurred following 3 days of abstinence for all participants) as previously published and described in the Supplement.

Measurement of Alcohol Craving

Craving severity during abstinence was measured using in vivo laboratory outcomes on 4 visual analog scale (VAS) items as previously published (21). Briefly, following affective priming with positive, negative, or neutral images from the International Affective Picture System (26), craving was calculated as the sum of the responses to the 4 VAS items collected after each affect $\times$ beverage exposure. For details, see the Supplement.

Measurement of Lipids in Plasma Samples

Blood samples were collected from participants at baseline (T1) on the same day as clinical assessments, processed into plasma, and stored at -80 °C until lipid analysis by liquid chromatography–mass spectrometry as described in the Supplement.

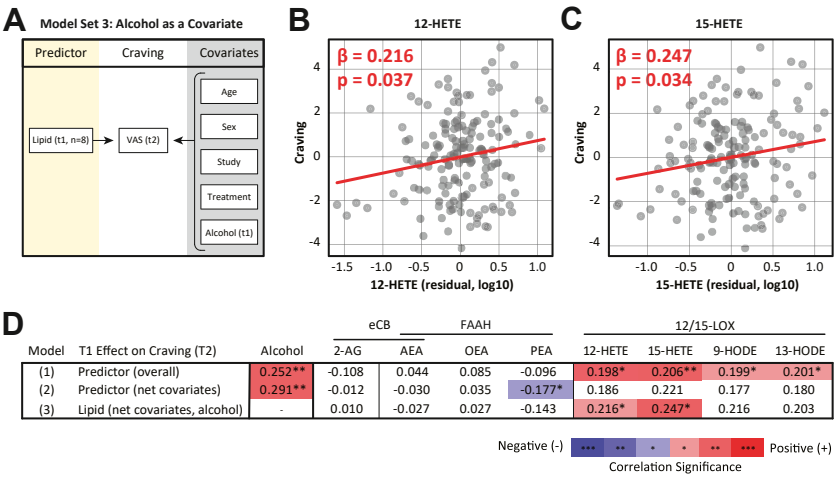
Statistical Analysis

Log Transformation of Non-Gaussian Data. Because alcohol use and plasma lipid measurements at T1 were substantially skewed toward high positive values, data were log base 10 transformed (hereafter $\log_{10}$) to address non-normal data distributions. Alcohol use was winsorized (27,28) at the 4th and 98th percentiles due to the presence of extreme outliers even after $\log_{10}$ transformation. Values higher than the 98th percentile and lower than the 4th percentile were recorded back to the 4th and 98th percentile values, respectively. Craving measurements exhibited a Gaussian distribution without obvious outliers, so no data transformations were implemented for craving VAS scores.

Covariates. Regression models included covariates to control for pretreatment alcohol consumption, age, sex/gender, and study group assignment. The study group effect consists of 6 separate groups, a treatment group in each of the 3 studies and a placebo group in each of the 3 studies. Accordingly, we coded this as 5 separate dummy variable contrasts, with the placebo group in the apremilast study serving as the omitted reference category.

Model Analysis

We estimated a series of model sets, with each subsequent model set built on the previous ones in terms of complexity and control of potential covariates. We did this to provide a baseline assessment of how each lipid might predict craving



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			eCB			FAAH	12/15-LOX			
Model	T1 Effect on Craving (T2)	Alcohol	2-AG	AEA	OEA	PEA	12-HETE	15-HETE	9-HODE	13-HODE
(1)	Predictor (overall)	0.252**	-0.108	0.044	0.085	-0.096	0.198*	0.206**	0.199*	0.201*
(2)	Predictor (net covariates)	0.291**	-0.012	-0.030	0.035	-0.177*	0.186	0.221	0.177	0.180
(3)	Lipid (net covariates, alcohol)	-	0.010	-0.027	0.027	-0.143	0.216*	0.247*	0.216	0.203

Negative (-) ••• •• • • •• ••• Positive (+)
Correlation Significance

Figure 1. Individual lipid models showed that plasma 12-HETE and 15-HETE predict alcohol craving during abstinence. The predictive effect of plasma lipids was compared with pretreatment baseline alcohol consumption in a series of models. **(A)** Model set 3 evaluated the effect of each lipid species on craving after accounting for the influence of other covariates (including age, sex, study, treatment, and pretreatment baseline alcohol consumption). Scatterplot illustrating a significant positive relationship between baseline **(B)** 12-HETE or **(C)** 15-HETE levels and alcohol craving during abstinence in model 3. For graphical representation, missing data points for 25 participants with partial data were generated by multiple imputation. **(D)** Table of Pearson correlations between all baseline predictor variables in model set 1 (line 1), model set 2 (line 2), and model set 3 (line 3). Positive effects are indicated in red, and negative effects are indicated in blue. Significance indicated by $^{*}p < .05$ and $^{**}p < .01$. AEA, anandamide; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; VAS, visual analog scale.

before evaluating a single overall regression on all lipids (and control variables). The first set of models (model set 1) (Figure S2A) was a model for a single predictor, either alcohol use (T1) or a plasma lipid (T1) with no other control variables. Model 1 for baseline alcohol use (T1) was not of direct theoretical interest but served as a useful benchmark for judging the strength of lipid effects on craving. The second set of models (model set 2) (Figure S2B) added all the covariates to each corresponding model in model set 1 for alcohol and each plasma lipid. The third set of models (model set 3) (Figure 1A) added alcohol use as a control variable to the corresponding model in model set 2 for each plasma lipid. Again, the alcohol effect in competition with the lipid was not of direct theoretical interest but is reported as a benchmark for judging the size of the lipid effect. Based on the plausibility of the idea that these lipid signaling pathways could biologically interact, we also estimated several final models that combined multiple T1 plasma lipids in competition with alcohol and other covariates to ultimately produce an integrated 3-lipid model (Figure 2A).

RESULTS

Participants

The sample comprised a total of 157 non–treatment-seeking participants with current AUD. Sixteen participants (10.2%) dropped out prior to cue reactivity testing (T2).

Participants were 39.3 (SD = 13.4) years of age on average. Participants self-identified their sex as female (n = 48; 30.6%) or male (n = 109; 69.4%); their race as American Indian/Alaska Native (2.6%), Asian (10.3%), Black (7.7%), multiracial (9.0%), Native Hawaiian/Other Pacific Islander (1.3%), White (67.7%), or unknown/decline to answer (1.3%); and their ethnicity as Hispanic/Latino (12.3%) or non-Hispanic/Latino (86.6%).

Participants met criteria for 6.4 (SD = 2.1) DSM-5 AUD symptoms, indicating an overall level of severe AUD. On average, participants drank 47.4 drinks per week in the 90 days prior to study participation (T1). Participants reported having their first drink at age 15.7 (SD = 3.1) years, had been drinking heavily for 16.2 (SD = 9.7) years prior to study participation, and could drink 7.2 (SD = 3.9) drinks before feeling intoxicated.

Table 1 depicts participants' T1 plasma lipid levels and T2 VAS craving scores.

Alcohol Intake Is Correlated With All Plasma Lipids Except PEA

Alcohol intake was significantly related to all plasma lipids except PEA (r = 0.066), which had the smallest lipid-alcohol correlation. All lipids were positively correlated with alcohol intake except 2-AG (r = −0.297), which was negatively correlated and had the largest lipid-alcohol correlation. Similar to the lipid-lipid correlations, alcohol correlations with 12/15-LOX lipids were much more consistent than those with FAAH-regulated metabolites.

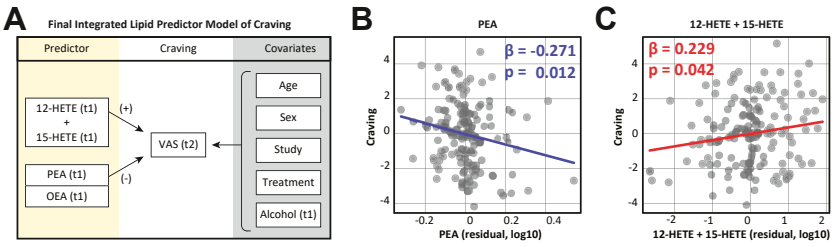

Figure 2. The final integrated model showed that baseline plasma level of 12/15-LOX lipids (12-HETE + 15-HETE) and PEA predict alcohol craving during abstinence. **(A)** An integrated 4 lipid model (sum of 12- and 15-HETE, PEA, and OEA) for predicting alcohol craving. Scatterplots of the **(B)** negative effect of PEA and **(C)** positive effect of 12-HETE + 15-HETE vs. craving after residualizing for the effects of OEA, age, gender, study, and group assignment. For graphical representation, missing data points for 25 participants with partial data were generated by multiple imputation. OEA, oleoylethanolamide; PEA, palmitoylethanolamide; VAS, visual analog scale.

Table 1. Descriptive Statistics for Covariates, Predictors, and VAS Craving Outcome

	Mean	SD	Skewness	Kurtosis	*n*
Sex, Self-Identified					
Female	30.6%	0.46%	0.85	−1.29	157
Male	69.4%	0.46%	−0.85	−1.29	157
Age, Years	39.3	13.4	0.5	−0.6	157
Standard Drinks per Week[a]	47.4	34.6	3.3	16.0	157
Craving VAS Scores[b]	36.5	21.1	0.2	−0.9	141
Plasma Lipids, pg/mL					
2-AG	2145.0	2229.6	1.9	3.4	141
AEA	132.3	64.2	1.0	2.2	141
OEA	1014.1	428.5	1.0	1.7	141
PEA	5639.3	2835.7	2.5	10.6	141
12-HETE	9976.4	11,551.3	2.1	5.7	141
15-HETE	5038.3	5634.1	1.5	1.8	141
9-HODE	12,834.3	14,396.6	1.6	2.7	141
13-HODE	16,289.8	16,634.5	1.7	2.9	141

AEA, anandamide; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; VAS, visual analog scale.

[a]A standard drink is defined as 0.6 fluid ounces (14 g) of pure alcohol, such that a 12-oz beer is equivalent to 5 oz of wine or 1.5 oz of distilled spirits. The mean and SD summarize the 90-day period prior to baseline (T1).

[b]All variables were collected at baseline (T1) except for the VAS, which was measured in response to in vivo alcohol and affective cues on the last day of drug dosing and after 3 days of verified abstinence (T2). Higher VAS scores indicate greater craving.

Minimal Interdependence Between FAAH and 12/15-LOX Lipid Levels in Plasma

Bioactive lipids share similar fatty acid precursors and enzymatic pathways, and thus individual lipid species may coregulate each other to influence their individual impact on our model for predicting alcohol craving. To investigate the relationship between our proposed pre-abstinence clinical and molecular predictors for craving, we estimated correlations using full-information maximum likelihood on baseline alcohol and all lipid predictors (Figure S1). Consistent with the influence of a common enzymatic 12/15-LOX biosynthetic pathway, all 12/15-LOX metabolites that were measured (12-HETE, 15-HETE, 9-HODE, 13-HODE) exhibited very strong and significant positive correlations with each other ($p < .05$), with Pearson coefficients ranging between 0.91 and 0.98. Likewise, lipids regulated by the common FAAH enzymatic degradation pathway (AEA, OEA, PEA) all had positive correlations with each other ($p < .05$) but exhibited greater variability, with Pearson coefficients ranging between 0.626 and 0.896. In contrast, the eCB lipid 2-AG exhibited a negative correlation ($p < .05$) with all FAAH and 12/15-LOX regulated metabolites and substantially lower and more variable Pearson coefficients ranging from −0.169 to −0.369.

Plasma Lipids Are Correlated With Alcohol Craving During Abstinence

Initially, we estimated our first set of models (model set 1) (Figure S2A) to evaluate baseline plasma lipid levels as predictors of alcohol craving during abstinence. Here, separate models for each lipid were developed with no other control variables. All predictor effects were standardized (in units of standard deviation) to facilitate more direct comparison of effect sizes. In this context, the standardized effect is equivalent to a correlation, which estimates the number of standard deviations of change in craving that is associated with 1 standard deviation change in the plasma level of a signal lipid species. We also evaluated the effect of baseline alcohol use on craving during abstinence as a benchmark for judging the relative strength of each lipid effect. In model set 1, alcohol and all 4 12/15-LOX lipids had significant positive correlations with craving (Figure 1D), indicating that increased craving at T2 could be predicted by an elevation in baseline 12/15-LOX lipid levels (or T1 alcohol use). Although craving exhibited the largest correlation with alcohol use in model set 1 ($r = 0.252$), the correlations with 15-LOX lipids were significant and ranged between 0.198 and 0.206. None of the correlations between craving and eCBs, or other FAAH-regulated lipid species, were significant in model set 1 (Figure 1D).

To further refine our predictions, we estimated model set 2 (Figure S2B) to control for potential confounding influences of other covariates in predicting craving. Specifically, model set 2 added covariates (sex/gender, age, group assignment, and study cohort) to the separate models for alcohol use and each bioactive lipid estimated in model set 1, which increased the overall effect size of alcohol use in predicting craving. The 12/15-LOX lipids exhibited a diminished correlation that fell just below significance; however, the effect of PEA ($r = −0.177$, $p < .05$) increased in magnitude in predicting craving with covariate control in model set 2 (Figure 1D). In contrast with the positive correlations with 12/15-LOX lipids, the effect of PEA on craving during abstinence was negative, and thus decreased baseline PEA levels predicted greater craving. Collectively, these findings indicate unique roles for plasma 15-HETE and PEA levels as potential predictors of alcohol craving.

Plasma 15-HETE and PEA Predict Craving Independent of Effect of Alcohol Use

Next, we wanted to investigate the possibility that lipid levels predict craving during abstinence independent of baseline alcohol use. To perform this analysis, a third set of models (model set 3) (Figure 1A) controlled for the amount of baseline alcohol use for each of the separate lipid predictors in model 2 to assess the joint effects of alcohol and each analyte on craving, net of covariates. Here, 12-HETE (Figure 1B) and 15-HETE (Figure 1C) were both significant ($p < .05$) predictors of craving, and 3 lipid species (PEA, $p = .080$; 9-HODE, $p = .064$; 13-HODE, $p = .063$) (Figure 1D) trended toward significance, with effects that were consistent in direction with previous models. When controlling for each lipid as a covariate, the alcohol effects on craving (Figure 2C, D) were all significant, indicating that no individual lipid species eliminated the significant effect of baseline alcohol consumption on craving during abstinence. The joint effects for alcohol, 12-HETE, 15-HETE, 9-HODE, and 13-HODE were slightly larger than their solitary effects in model set 2. In contrast, when PEA and alcohol competed to predict craving in model

set 3, their joint effects were reduced compared with their solitary effects in model set 2. Overall, results for model sets 1, 2, and 3 indicated that 15-HETE, 12-HETE, and PEA may each predict craving during abstinence independent of alcohol use history, although the PEA mechanism is probably distinct from the mechanism for 12- or 15-HETE.

To further understand how other eCB-related lipid species may influence PEA to predict craving, we ran 3 models that combined PEA with each of the 3 other lipid species (OEA, AEA, 2-AG; each pair analyzed separately) while controlling for alcohol and other covariates. The integrated model that included both PEA and OEA had substantially enhanced effects compared with model 3 (when each lipid was the sole predictor), with PEA having a larger standardized effect size ($\beta = -0.281$, $p = .008$) (Figure S3) than OEA; OEA had an effect size approaching significance ($\beta = 0.209$, $p = .059$) but in a direction opposite to that of PEA. The enhancement of effects in the opposite direction for both predictors suggests that a complex ratio of these lipids may be the biologically important predictor of craving. Similar models were performed using 12- and 15-HETE, each paired with the other 3 12/15-LOX lipids, but these analyses did not yield significant results ($p > .300$), most likely due to the high level of intercorrelation that may signal biological redundancy among these lipids with respect to predicting craving.

Thus, craving during abstinence could be most effectively predicted using a single model integrating 4 individual lipid species: PEA, OEA, 12-HETE, and 15-HETE (Figure 2A). Because 12-HETE and 15-HETE both similarly predict craving (Figure 1B, C), exhibit high intercorrelation (Figure S1), and can be produced by the same enzymes, we simplified our final integrated 4-lipid model by summing these 2 lipids (12-HETE + 15-HETE). Although the OEA effect was not significant ($\beta = 0.173$, $p = .114$), inclusion in this 4-lipid model substantially enhanced the PEA effect ($\beta = -0.271$, $p = .012$) (Figure 2B) as previously described. Finally, the 12-HETE + 15-HETE effect was also significant ($\beta = 0.229$, $p = .042$) (Figure 2C). For comparison to the lipid effects, the baseline alcohol standardized effect was 0.262 ($p = .004$). The likelihood ratio test of the joint effects of the 3 lipid predictions simultaneously was also significant ($\chi^2_3 = 10.36$, $p = .016$), and the 3 lipid predictors increased the model R^2 from 0.11 to 0.17, thus jointly accounting for an additional 6% of the variance in craving over and above baseline covariates. Using this approach, the diminished levels of antinociceptive PEA and elevated levels of pronociceptive 12-HETE + 15-HETE both predicted an increase in alcohol craving.

DISCUSSION

Our study evaluated the plasma collected from volunteer participants with AUD in 3 independent clinical trials (21,23,24) and demonstrated that alcohol craving during abstinence could be predicted independently of multiple therapeutic treatments and baseline clinical characteristics using bioactive lipid level assessments that were done prior to the initiation of treatment and abstinence. Specifically, in patients with AUD, we found that pronociceptive 12-HETE and 15-HETE were positively correlated, while antinociceptive PEA was negatively correlated, with alcohol craving during abstinence, and a regression analysis further revealed that the combination of information deriving from all 3 bioactive lipids best predicted alcohol craving independently of the history of alcohol use (Figure 2). While the standardized regression effects in the final individual models were modest due in part to the variability in clinical assessments, we propose that there is predictive value in measuring multiple circulating lipids in combination to tailor the treatment of patients with AUD who are at most risk for experiencing intense cravings during abstinence.

Preclinical studies have implicated PEA deficits as a potential mechanism that drives craving during drug abstinence. For example, alcohol intake is reduced in rodent models of dependence treated with a fenofibrate, an agonist of the PEA receptor PPARα (29). Likewise, multiple clinical trials have established the analgesic properties of therapeutic administration of oral PEA supplements for treating chronic pain conditions (30–32) and thus may represent a viable approach for addressing withdrawal-induced emotional responses that drive relapse in individuals with AUD. Inactivation of FAAH can also decrease alcohol consumption in rodent models of AUD (5,6); however, multiple effects produced by FAAH inhibitors beyond elevating PEA complicate the mechanism of action. In addition to promoting PEA signaling onto nuclear receptors, elevations in AEA levels may have implications for maintaining eCB tone (33).

While preclinical studies have previously implicated 12/15-LOX enzymes in alcohol-induced liver toxicity (13), the current study is novel in linking this pathway to the neurobiology of AUD. Our data indicate that plasma 12-HETE and 15-HETE levels in patients with AUD were positively correlated with craving during abstinence, with an effect size similar to but independent from the amount of alcohol consumed. Proinflammatory 12/15-LOX lipids have been identified as endogenous activators of TRPV1 and TRPA1 receptors, which have well-established roles in chronic pain (34–36) and AUD (36,37). Despite their importance, the development of drugs to mitigate TRPV1 and TRPA1 has historically been unsuccessful due to problematic side-effect profiles and pharmacokinetic challenges (38). In contrast, reducing levels of 12-HETE and 15-HETE may be more clinically tolerable using selective inhibitors of 12/15-LOX enzymes. This family of enzymes has largely been overlooked as a therapeutic target, but short-acting 12-LOX selective inhibitors have been developed and are currently in phase 2 clinical trials as potential treatment for heparin-induced thrombocytopenia (39). Thus, targeting lipid production using selective inhibitors of these enzymes may be more clinically effective than receptor antagonists, and future work is needed to establish their specific role in AUD.

Finally, our results could offer a strategy to improve AUD outcomes by identifying individuals at greater risk of relapse, thus informing treatment planning regarding a need for closer monitoring or greater treatment intensity. Although plasma is commonly used for clinical biomarker analysis, future studies could evaluate bioactive lipid levels in more bioaccessible fluids including saliva (40), sweat (41), and urine (42).

Limitations

Our regression analyses included observations that were flagged as below the limit of quantification (see Methods and

Materials), including PEA (15%) and 15-HETE (12%), due to our conservative limit of quantification, which was set to 3 times higher than the lipid concentration detected in blank extraction controls. Thus, the predictive relations that we observed for PEA and 15-HETE could have been somewhat attenuated by errors of measurement at the low end of the respective distributions. Measurement errors in predictors are known to attenuate slope estimates and reduce statistical power compared to the same scenario when the predictor is measured with very high precision. Thus, our pooled sample (n = 157) was likely sufficiently large to help overcome the loss of statistical power to detect significant effects for PEA and 15-HETE.

We did not observe a previously reported correlation between plasma anandamide levels and craving for alcohol during abstinence (8). Our study had a larger sample size but did not have a nondependent social drinking group, suggesting that the influence of anandamide on alcohol craving may be altered by the level of consumption and dependence. The goal of the current work was to determine the predictive value of our bioactive lipid screen in patients with AUD entering treatment. Future work that includes a healthy control group may help establish the relative impact of anandamide and other bioactive lipids as peripheral biomarkers for specific aspects of AUD. Additionally, we did not assess placebo versus treatment or response versus no response to treatment effects in relation to craving and lipid levels due to the small sample size; future investigations specifically designed and powered to address this gap should be conducted. Furthermore, our study was underpowered to examine sex as a biological variable, and future studies are warranted to explore potential sex differences in the plasma levels of these lipids.

Summary

Plasma levels of 12-HETE, 15-HETE, and the relative level of PEA to OEA represent viable potential biomarkers for predicting alcohol craving in human subjects with AUD. Importantly, plasma levels of these lipids were more effective predictors of craving than the level of previous alcohol use and functioned independently from specific therapeutic treatments, further supporting their potential clinical utility. Future research should aim to replicate these findings in a larger sample and fully evaluate these bioactive lipid biomarkers in clinical settings to help develop new treatments for AUD.

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CM, MWB, and BJM were responsible for investigation. CM and BJM were responsible for methodology. LAN, AMG, MWB, and BJM were responsible for conceptualization. SQ was responsible for data curation. MS was responsible for formal analysis, validation, and visualization. MWB and BJM were responsible for supervision and preparation of the original draft of the manuscript. BJM was responsible for funding acquisition, investigation, project administration, and resources. All authors were responsible for reviewing and editing the manuscript.

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Data will be made available upon request. Archival deidentified data from NCT02158273, NCT01548417, and NCT03175549.

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