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ARTICLE

Lower brain fatty acid amide hydrolase in treatment-seeking patients with alcohol use disorder: a positron emission tomography study with [C-11]CURB

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The endocannabinoid enzyme, fatty acid amide hydrolase (FAAH), has been proposed as a therapeutic target for alcohol use disorder (AUD) and co-morbid psychiatric illnesses. Investigating this target in the living human brain and its relationship to clinical outcome is a critical step of informed drug development. Our objective was to establish whether brain FAAH levels are low in individuals with AUD and related to drinking behavior. In this pilot study, treatment-seeking patients with AUD completed two PET scans with the FAAH radiotracer [C-11]CURB after 3–7 days ($n = 14$) and 2–4 weeks ($n = 9$) of monitored abstinence. Healthy controls ($n = 25$) completed one scan. FAAH genetic polymorphism (rs324420) and blood concentrations of anandamide and other *N*-acylethanolamines metabolized by FAAH were determined and AUD symptoms assessed. In AUD, brain FAAH levels were globally lower than controls during early abstinence ($F(1,36) = 5.447$; $p = 0.025$)) and FAAH substrates (anandamide, oleoylethanolamide, and *N*-docosahexaenoylethanolamide) were significantly elevated (30–67%). No significant differences in FAAH or FAAH substrates were noted after 2–4 weeks abstinence. FAAH levels negatively correlated with drinks per week ($r = -0.57$, $p = 0.032$) and plasma concentrations of the three FAAH substrates ($r > 0.57$; $p < 0.04$)). Our findings suggest that early abstinence from alcohol in AUD is associated with transiently low brain FAAH levels, which are inversely related to heavier alcohol use and elevated plasma levels of FAAH substrates. Whether low FAAH is an adaptive beneficial response to chronic alcohol is unknown. Therapeutic strategies focusing on FAAH inhibition should consider the possibility that low FAAH during early abstinence may be related to drinking.

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INTRODUCTION

Alcohol use disorder (AUD) is common and imposes significant burden worldwide; however, current therapeutics are limited. Increasing evidence suggests that the endocannabinoid system is involved in features of AUD including positive reinforcement, anxiety or stress-induced craving, and relapse [1]. Therefore, its constituents—including the G-protein-coupled cannabinoid receptor CB1, its endogenous ligands (anandamide (arachidonoylethanolamide, AEA) and 2-aracidonoylglycerol), and their metabolizing enzymes (fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase, respectively)—are considered potential targets for AUD pharmacotherapy but also specifically for conditions of the associated with AUD (e.g., posttraumatic stress and anxiety disorders). In particular, the enzyme FAAH is of interest due to its critical role in regulating concentrations of AEA and other *N*-acylethanolamines (NAEs) [2]. Pharmacological and genetic inactivation of FAAH in preclinical models dampens anxious behavior and abnormal stress responses [3], but also increases alcohol-seeking in some studies [4, 5], making the translation of FAAH inhibitors for the treatment of AUD and co-morbid conditions promising but complex.

Alcohol-induced changes in brain FAAH levels have been investigated in preclinical and limited clinical investigations. Overall, preclinical studies suggest that chronic alcohol exposure might (region-dependently) increase AEA concentrations and reduce FAAH activity, whereby enhanced endocannabinoid signaling may promote alcohol consumption [6]. In addition, a single study in humans with a loss-of-function FAAH mutation [7]

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(rs324420, C385A [8]), as well as knock-in mouse models of this polymorphism [9], found increased alcohol binge consumption and severity of alcohol dependence. Together, these data suggest that acquired or inherited reductions in FAAH and corresponding AEA increases may influence pathological drinking and could serve as a biomarker of AUD severity and/or risk. However, human brain investigations in AUD are limited to two inconsistent postmortem brain studies [10, 11].

We previously reported that FAAH levels can be measured in vivo using the positron emission tomography (PET) radioligand [C-11]CURB ([11C-carbonyl]-6-hydroxy-[1,10-biphenyl]-3-yl cyclohexylcarbamate (URB694)) [12]. The aim of the present study was to use [C-11]CURB PET imaging to establish whether brain FAAH levels in individuals with AUD would be lower relative to those in healthy controls (HCs) and related to drinking behavior, as predicted by some preclinical findings.

MATERIALS AND METHODS

Participants

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board. AUD subjects were recruited from the CAMH Alcohol Research and Treatment Clinic, and met criteria for AUD as per the DSM-IV. Following written informed consent, subjects completed a comprehensive interview to rule out the past or present significant medical conditions, neurological illnesses, or head trauma, other Axis I psychiatric disorders, magnetic resonance imaging (MRI) and PET contraindications. Subject sex and ethnicity were obtained by self-report. Urine and scalp hair samples (United States Drug Testing Laboratories, Des Plaines, IL, USA) were used to exclude for drugs of abuse. AUD patients were permitted short-acting benzodiazepines to aid with withdrawal from alcohol. Current and past (2 year) medication history was recorded (Table 1). Prescription medication use remained constant throughout the study participation.

HCs were recruited from the community and completed one PET scan with [C-11]CURB, whereas AUD patients completed two: in early abstinence 3–7 days after cessation of ongoing drinking (mean: 4.7 ± 3.77 days) and after 2–4 weeks of monitored abstinence (mean: 24.56 ± 10.56 days) (Table 1). Nicotine smokers were instructed not to smoke on scan days. PET intake assessments included breath alcohol concentration measurement (0.0 g/L required for scanning); urine toxicology and pregnancy test; expired carbon monoxide (<10 p.p.m. to rule out recent nicotine inhalation); and mood, craving, withdrawal, and alcohol-use questionnaires in AUD patients only (Penn Alcohol Craving Scale [13], Clinical Institute Withdrawal Assessment for Alcohol [14], and the Timeline Follow-back Method [15]). AUD patients were provided short-acting benzodiazepines to aid with alcohol withdrawal if requested; on PET scan day, 5 of 14 AUD patients tested positive for benzodiazepines. Participants with AUD came to the lab approximately every 3–4 days (mean: 4.2 ± 3.8) to monitor abstinence. AUD patients completed self-report questionnaires and the Timeline Followback. Urine samples were obtained for testing of ethylglucuronide, an alcohol metabolite formed after alcohol consumption (or extraneous exposure to alcohol). Those who tested positively for ethylglucuronide on at least one occasion between scans were classified as "non-abstinent." Non-abstinent subjects were not excluded from the study; instead, they were invited to complete the study after later achieving 2–4 weeks of continued abstinence.

Table 1. Subject demographic information.

Characteristic	Controls ($n = 25$)[a]	Alcohol users (Scan 1, $n = 14$; Scan 2, $n = 9$)[a]	p-Value	χ^2
Sex (females/males), n	12/13	1/13	**0.005**	7.85
Age, years	36.64 (12.54)	46.93 (10.87)	**0.014**	–
Ethnicity (White/Black/Asian/Hispanic), n	16/6/1/2	13/1/0/0	0.250	4.12
Body mass index	24.82 (3.30)	27.66 (5.71)	0.056	–
Genetics (rs324420, C385A), n	18(CC), 7(AC)	10(CC), 4(AC)	0.970	0.001
Education, years	15.48 (1.66)	16.07 (3.73)	0.497	–
Current alcohol use/week, n Standard drinks	2.41 (4.02)	75.29 (42.6)	**<0.0001**	–
Years of alcohol use, n	18.58 (12.21)	28.9 (12.1)	**0.022**	–
Cigarette smokers, n	4 of 25	5 of 14	0.161	1.97
Current cigarettes/day, n	20 (4.6)	15 (9.5)	0.354	–
Fagerstrom test of nicotine dependence	4.8 (3.27)	4.0 (3.22)	0.682	–
Medications, past 3 months, n	–	Blood pressure, 4 of 14 Naproxen, 1 of 14 Effexor 225 mg, 1 of 14 Antibiotic, 1 of 14 Daily Humalog 100 U/mL, 1 of 14		
Barratt Impulsiveness Scale–total	57.50 (10.77)	61.29 (6.41)	0.245	–
Marin Apathy Scale	26.05 (15.84)	35.6 (7.2)	**0.042**	–
Beck Depression Inventory	3.43 (6.05)	15.64 (8.51)	**0.00002**	–
Obsessive Compulsive Drinking Scale				
Obsessive	–	5.71 (3.93)	–	
Compulsive	–	14.86 (3.59)	–	
Amount injected (mCi)				
Scan 1	9.42 (0.79)	9.25 (0.86)	0.545	
Scan 2	–	9.66 (0.88)	–	
Specific activity (mCi/umol)				
Scan 1	2972.5 (1238.98)	3329.05 (918.92)	0.356	
Scan 2	–	3296.40 (1553.03)	–	
Mass injected (μg)				
Scan 1	1.17 (0.93)	0.93 (0.26)	0.103	
Scan 2		1.12 (0.53)	–	
Days of abstinence prior to scan				
Scan 1	–	4.71 (3.77)	–	
Scan 2	–	24.56 (10.56)	–	
Penn Alcohol Craving Scale				
Scan 1	–	13.29 (5.80)	–	
Scan 2	–	14.82 (4.92)	–	
Clinical Institute Withdrawal Assessment for Alcohol				
Scan 1	–	2.07 (2.09)	–	
Scan 2	–	1.36 (1.75)	–	
Alcohol Dependence Scale	–	16.21 (7.20)	–	

[a]Values represent mean (SD) unless otherwise indicated
"–" indicates values not reported
Statistically significant p-values are in bold

Image acquisition and reconstruction

[C-11]CURB radiosynthesis was described previously [12]. PET image acquisition was performed with a HRRT brain tomograph (CPS/Siemens, Knoxville, TN, USA) (details previously published [16]). Briefly, subjects were supine and head secured by a thermoplastic mask. Following injection of 342 ± 33 MBq (9.3 ± 0.9 mCi) of [C-11]CURB, emission data were acquired over 1 h in sequential frames of increasing duration. Images were reconstructed from two-dimensional (2D) sinograms with a 2D filtered-back projection algorithm, with a HANN filter at Nyquist cut-off

frequency. Radioactivity in arterial blood was determined via automatic blood sampling (Model PBS-101, Veenstra Instruments, The Netherlands) for the first 22.5 min post injection. The radioactivity in plasma and metabolization was studied from arterial blood samples extracted at 3, 7, 12, 20, 30, 45, and 60 min post injection. A metabolite-corrected plasma curve was generated and used as the input function for the kinetic analysis [16]. Blood-to-plasma radioactivity ratios were interpolated by a biexponential function and parent plasma fraction by a Hill function.

A standard proton density-weighted brain MRI scan was completed for each subject, for delineation and volume determination of regions of interest (ROIs), using a Discovery MR750 3T MRI scanner (General Electric, Milwaukee, WI, USA).

FAAH genotype and NAE and endocannabinoid concentrations

Venous blood samples at PET scans allowed quantification of plasma concentrations of NAEs and endocannabinoids: AEA, oleoylethanolamide (OEA), and *N*- docosahexaenoylethanolaide (DHEA, a.k.a. "synaptamide") using high-performance liquid chromatography-tandem mass spectrometry [17] (see Supplementary Methods), and genotyping for the FAAH polymorphism (rs324420) according to published procedures [18] as this affects [C-11] CURB quantification.

ROI, kinetic, and statistical analyses

Time–activity curves [16] were extracted using ROMI [19] and the composite parameter λk_3 ($\lambda k_3 = k_3{*}K_1/k_2$) was calculated from a two-tissue compartment model with irreversible binding in the second compartment [16]. We showed previously that λk_3 is sensitive to changes in FAAH levels [16].

To investigate whether FAAH levels were different between HC and AUD, differences in [C-11]CURB λk_3 were investigated in 10 a priori determined ROIs (see Fig. 1) using repeated-measures analysis of covariance (RM-ANCOVAs) with ROI as a within-subject factor and FAAH genotype as a covariate [18] (SPSS 21.0, SPSS, Inc., Chicago, IL, USA). Post-hoc *t*-tests were applied to investigate regional differences in λk_3. Pearson's product–moment correlations were used to investigate relationships between whole brain λk_3 and demographic factors, craving/withdrawal/mood scores, as well as peripheral plasma AEA and NAE concentrations. Level of significance was set at $p < 0.05$.

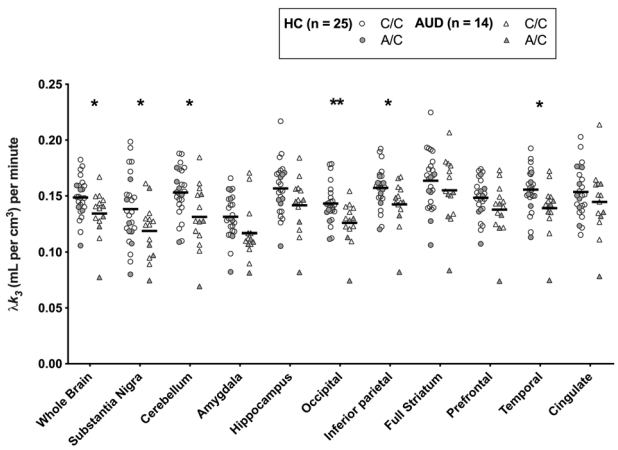

Fig. 1 FAAH is lower in AUD patients in early abstinence compared with healthy controls. Comparison of brain [C-11]CURB λk_3 values, an index of fatty acid amide hydrolase (FAAH) activity, between individuals with alcohol use disorder in early abstinence (AUD) ($n = 14$, triangles) during early abstinence and matched healthy control subjects (HC) ($n = 25$, circles). FAAH genotypes (rs324420, C385A) of the subjects are indicated (open, C/C; gray, C/A). Statistically significant differences in binding are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$. Percent difference in binding in AUD compared with HC across the whole brain is −9%. Percent differences vary by region of interest (ROI): substantia nigra (−13%), cerebellum (−13%), amygdala (−9%), hippocampus (−9%), occipital cortex (−12%), inferior parietal cortex (−8%), striatum (−3%), prefrontal cortex (−6%), temporal cortex (−10%), and the cingulate cortex (−6%).

RESULTS

HC data (60%) have been previously published [18]. Twenty-five HC subjects and fourteen AUD patients were scanned. Three AUD patients were excluded or withdrew after the first PET scan. Age was higher in AUD, sex proportions differed significantly, and FAAH genotype proportions did not differ between groups. Demographic information is reported in Table 1.

[C-11]CURB binding (λk_3) in AUD patients during early abstinence

A RM-ANCOVA (ROI [10] × Group [2], with FAAH C385A genotype (rs324420) as a covariate), investigating differences in λk_3 between HC and AUD, revealed a significant main effect of group ($F(1,36) = 5.447$; $p = 0.025$), a trending effect of genotype ($F(1,36) = 3.936$; $p = 0.055$) and no significant interaction ($F(4.25,152.8) = 0.822$; $p = 0.52$). The between-group difference indicated significantly lower λk_3 in AU relative to HC (−9%; Fig. 1; overall Cohen's $d = 0.78$). This finding is not explained by group differences in age or body mass index (BMI) (Age: $F(1,35) = 0.049$, $p = 0.827$; BMI: $F(1,35) = 0.007$, $p = 0.16$). Further, λk_3 in HC was not correlated with age ($r = -0.24$, $p = 0.25$) or BMI ($r < 0.001$, $p > 0.99$). Group differences in sex revealed a trend for higher FAAH levels in females ($F(1,35) = 3.365$, $p = 0.08$) but did not influence the main effect. RM-ANOVA tests revealed no significant differences between AUD and HC in regional brain volume ($F(1,37) = 2.320$, $p > 0.1$); λk_3 did not differ between AUD patients who took short-acting benzodiazepines ($n = 5$) and those who did not ($F(1,12) < 0.001$, $p > 0.9$).

Relation between [C-11]CURB binding (λk_3) at scan 1 (early abstinence) and clinical features of AUD

We investigated whether low λk_3 in AUD at scan 1 correlated with self-reported alcohol use, days of abstinence, craving, withdrawal symptoms, or mood scores. Whole brain λk_3 values in early abstinence was correlated negatively with number of standard drinks per week ($r = -0.574$; $p = 0.032$ Fig. 2a) but did not correlate with craving, withdrawal severity, or days of abstinence (all $p > 0.10$). Self-reported mood (Beck depression inventory (BDI) scores) did not correlate with whole brain λk_3 ($r = 0.09$; $p = 0.76$). In AU, self-reported drinks per week did not differ between FAAH C385A variants ($p = 0.25$).

We investigated whether λk_3 at scan 1 was related to non-abstinence before scan 2. Eleven AUD patients completed the second PET scan: five provided urine samples positive for ethylglucuronide (non-abstinent) during the monitored abstinence period and six AU (abstinent) tested negative. An RM-ANCOVA, using FAAH genotype as a co-factor, revealed that λk_3 at scan 1 was nonsignificantly lower (10–24%) across brain regions in non-abstinent patients compared with abstinent ($F(1,8) = 3.321$; $p = 0.106$, Cohen's $d = 1.29$) (Supplementary Fig. S1). Although relapse groups did not significantly differ in age, FAAH C385A (rs324420) genotype, or days of abstinence at scan 1, non-abstinent patients self-reported heavier alcohol binge consumption prior to scan 1 (Supplementary Table S1).

[C-11]CURB binding (λk_3) in AUD patients after 2–4 weeks of abstinence

Eleven AUD patients completed a second [C-11]CURB PET scan after 24.4 ± 10.7 days of abstinence; two were excluded from analyses due to ethylglucuronide-positive urine on scan day (Table 1). After 2–4 weeks of abstinence, λk_3 values in AUD ($n = 9$) were nonsignificantly increased (mean: +5%) from scan 1 ($F(1,7) = 4.438$; $p = 0.073$, Cohen's $d = 1.59$) and not significantly different

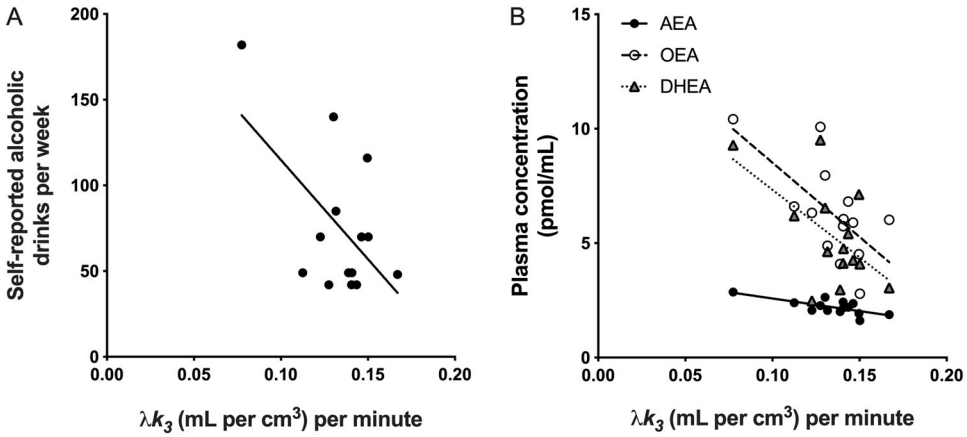

Fig. 2 Correlations with whole brain FAAH in AUD patients in early abstinence. a Brain FAAH levels across the whole brain, as indicated by λk_3, are negatively correlated with self-reported recent number of alcoholic drinks per week in AUD patients. $r = -0.574$; $p = 0.032$). **b** Whole brain λk_3 levels, representing FAAH levels in the brain, are negatively correlated with peripheral concentrations of FAAH substrates anandamide (AEA, closed circles, $r = -0.723$, $p = 0.003$), oleyolyethanolamine (OEA, open circles, $r = -0.632$, $p = 0.015$) and synaptamide (DHEA, triangle, $r = -0.569$, $p = 0.034$).

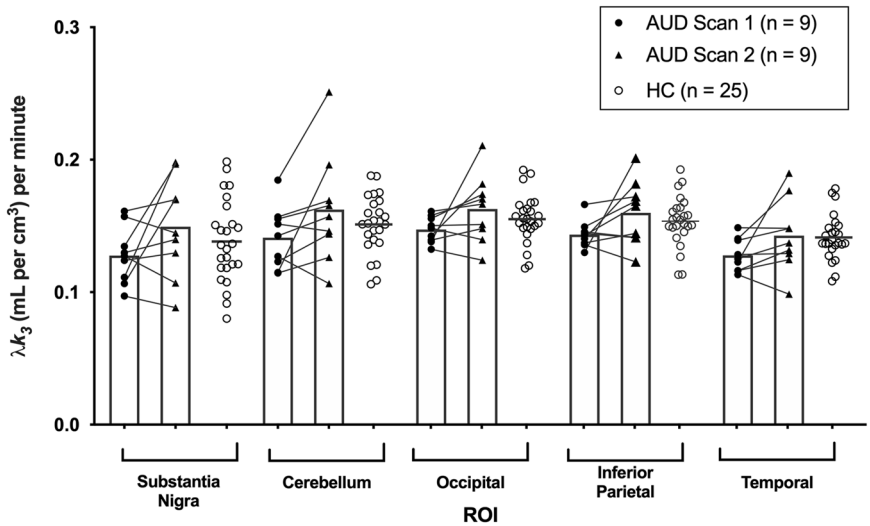

Fig. 3 FAAH brain levels in early and longer abstinence in alcohol use disorder compared to controls. Comparison of brain [C-11]-CURB λk_3 values, an index of fatty acid amide hydrolase (FAAH) activity in AUD patients ($n = 9$) at two time points: Scan 1 (closed circles) during early abstinence (3–7 days post alcohol) and Scan 2 (triangles) in longer abstinence (2–4 weeks post alcohol). Within-subject changes from early to longer abstinence are indicated by the connecting line. No significant changes in FAAH levels from scan 1 to scan 2. Matched healthy control subjects are also shown ($n = 25$, circles). There is no statistically significant difference in FAAH levels between HC and AUD at scan 2.

from HC ($F(1) = 0.816$; $p = 0.373$) (Fig. 3). Five of nine subjects showed whole brain increases in λk_3 values > 10% between scan 1 and 2; one subject decreased and three subjects showed no change (<10%). The percent change between scans was not related to clinical features.

NAE and endocannabinoid concentrations in AUD and relationship to brain FAAH

Independent-sample *t*-tests revealed significantly higher plasma concentrations of FAAH substrates in AUD in early abstinence compared with HC ($n = 22$) (AEA: +30%, $F(34) = 2.501$, $p = 0.002$; OEA: +56%, $F(34) = 0.027$, $p = 0.001$; DHEA: +67%, $F(34) = 0.07$, $p = 0.01$) (Fig. 4). Plasma concentrations of NAE were negatively correlated with whole brain λk_3 in AUD early abstinence (Fig. 2b; AEA: $r = -0.723$, $p = 0.003$; OEA: $r = -0.632$, $p = 0.015$; DHEA $r = -0.569$, $p = 0.034$), but not in longer abstinence. There were no significant changes in levels of these compounds from early to longer abstinence in AUD ($p > 0.4$; Fig. 4). AEA, OEA, and DHEA concentrations were elevated compared with controls during longer abstinence but differences were not significant (Fig. 4). Plasma concentrations of AEA and DHEA in early abstinence were positively related to the number of alcohol drinks per week, although not significantly (AEA: $r = 0.48$; $p = 0.07$; DHEA: $r = 0.67$, $p = 0.06$). In addition, peripheral concentrations of FAAH substrates were higher in non-abstinent compared with abstinent AUD patients during early abstinence (AEA: +12%, $p = 0.16$, OEA: +41%, $p = 0.04$, DHEA: +96%, $p = 0.006$) (Supplementary Fig. S2). Plasma concentrations of these FAAH substrates did not relate to any other clinical features of AUD or mood symptoms in early or longer abstinence. The percent change in NAE from early to longer abstinence did not correlate with the percent change in λk_3 or with changes in clinical features of AUD or mood symptoms.

DISCUSSION

In this pilot study we show that brain [C-11]CURB λk_3, an indicator of FAAH levels, is modestly lower in recently abstinent AUD patients compared with controls and correlates with increased

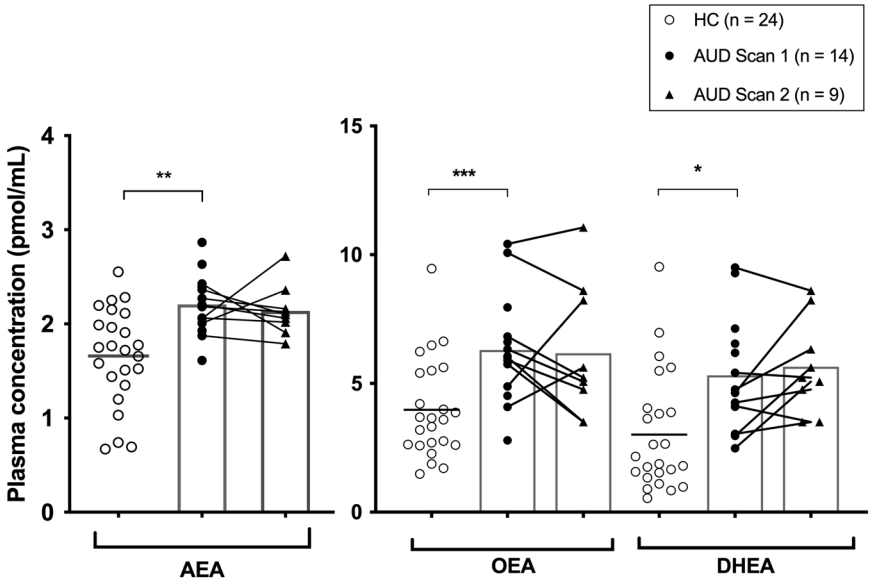

Fig. 4 Peripheral concentrations of FAAH substrates are elevated in AUD patients in abstinence. Comparison of peripheral plasma concentrations of FAAH substrates anandamide (AEA), oleoylethanolamine (OEA), and synaptamide (DHEA) in AUD patients at two time points: Scan 1 (closed circles) during early abstinence (3–7 days post alcohol) and Scan 2 (triangles) in longer abstinence (2–4 weeks post alcohol). Within-subject changes from early to longer abstinence are indicated by the connecting line. Matched healthy control subjects are also shown ($n = 25$, circles). Percent differences in AUD in early abstinence (Scan 1) compared with HC vary by FAAH substrate: AEA + 30%, OEA + 56%, DHEA + 67%.

plasma concentrations of FAAH substrates AEA, OEA, and DHEA, and with heavier recent alcohol use. Our study contributes to existing literature, suggesting that FAAH and FAAH-metabolized endocannabinoids and NAE might be involved in AUD.

FAAH is low during early abstinence from alcohol

The current study aligns with rodent studies reporting reduced FAAH levels and mRNA levels during withdrawal, following forced continuous or intermittent exposure to alcohol (see review [6]). Our findings are somewhat consistent with those of a postmortem study [10] reporting decreased FAAH in ventral striatum of subjects with AUD compared with non-AUD controls (but not another, which reports no change in FAAH in the prefrontal cortex [11]). Unlike preclinical studies, which have suggested a region-dependant effect on FAAH levels (i.e., primarily in the cortex), our data suggest a global change. This difference may depend on the pattern of use in humans (escalating and chronic) vs. alcohol administration in animals (e.g., acute sustained ethanol vapor).

To our knowledge, there are no preclinical studies investigating longitudinal changes in FAAH in animal models of AUD. We found that five of nine individuals with AUD had small increases in FAAH above test–retest variability [20] from early to longer abstinence, suggesting that FAAH levels may normalize with abstinence in a sub-population of AUD patients. A larger scale longitudinal study is required to better understand changes in FAAH and FAAH substrates during longer abstinence.

Anandamide and NAE concentrations are elevated in AUD and correlate with brain levels of FAAH

Although the literature is largely focused on AEA, some evidence suggests that other NAE might also be involved in AUD. OEA plays a role in the intake of fat-containing foods, is structurally similar to AEA, but does not activate CB1 receptors. Rather, OEA interacts with the peroxisome proliferator-activated receptor-α (PPAR-α)—which has been reported to attenuate alcohol reinforcement [21]—as well as with the transient receptor potential channel and the G-protein orphan receptors GPR110 and GPR55 [22]. In contrast, DHEA (a.k.a., synaptamide), a major polyunsaturated NAE with known neuroprotective/neurotrophic effects in the brain, has in vitro affinity for the CB1 receptor [23]. Despite their presumed protective role, only one study suggests that OEA may decrease motivation to use alcohol (via PPAR-α) and there are limited preclinical studies investigating the effects of alcohol exposure on OEA and DHEA concentrations [22, 24]. In the few clinical investigations, elevated plasma concentrations of AEA, OEA, and DHEA were found in AUD abstinent humans for a period of at least 4 weeks [25], but not another [26]. Here we report for the first time in humans, that lower FAAH brain levels during early abstinence from alcohol correlated with plasma concentrations of AEA, OEA, and DHEA in AUD, suggesting that impairment in brain endocannabinoid and NAE degradation may, in part, account for elevated endocannabinoids noted in most, although not all, preclinical studies [6]. However, the brain–blood correlation might not be causal; plasma levels of FAAH substrates could be increased due to low FAAH in the peripheral organs (e.g., the liver) or a mechanism independent of FAAH entirely.

Low FAAH linked to heavier recent alcohol consumption

We report a negative association between brain FAAH levels and weekly alcohol consumption. Although we found a trending effect of FAAH genotype on FAAH levels in the brain, replicating the previous PET study showing that FAAH brain levels are lower in individuals with the A/C or A/A genotype, in our small sample, we did not find that genotype was associated with clinical features (data not shown). In parallel, we also find that non-abstinent AUD subjects, who reported nonsignificantly heavier drinking, had nonsignificantly lower brain FAAH levels and increased plasma FAAH substrates as compared with non-abstinent AUD subjects. These findings converge with preclinical studies, suggesting endocannabinoid involvement in alcohol-seeking behaviours [6, 27] and, more specifically, that decreased endocannabinoid metabolism might promote increased drinking or reflect an adaptation to alcohol consumption. In this regard, decreased FAAH expression and activity, and increased AEA have been noted in alcohol-naive rats bred to like alcohol (Sardinian [sP] Alko [AA] alcohol-preferring rats) [28]. Similarly, animals with a knock-in C385A FAAH polymorphism causing lower FAAH levels have greater rates of alcohol self-administration [9] and pharmacological

inhibition of enzymatic activity or increasing AEA promotes drinking in some [29, 30] but not all investigations [31, 32]. Translation of these concepts into humans has shown that carriers of the known FAAH C385A polymorphism, who have lower levels of FAAH, have increased alcohol intake, higher alcohol dependence severity (higher scores on the Alcohol Use Disorders Identification Test), and increased risk for AUD [7]. Together with our findings, these data suggest that differences in FAAH and increased endocannabinoid tone may be related to motivation to use alcohol or increased tolerance to the effects of alcohol. The exact mechanism behind this effect is unknown but may involve increases in mesolimbic dopamine signaling as reported in mice models [33, 34] but not rats [35]. Future investigation of potential endocannabinoid-based mechanisms of risk for developing alcohol use problems is necessary.

Mechanism explaining lower FAAH in AUD

The mechanism behind low FAAH and high endocannabinoids in AUD remains elusive. It is unknown whether chronic alcohol use elevates endocannabinoids and NAE initially via increased biosynthesis, which then decreases enzymatic degradation (by blocking activity), or whether reduced FAAH activity and/or gene expression causes elevation of its substrates.

In line with the former, alcohol can activate phospholipase A2, promoting the synthesis of AEA [6, 36], and can increase endocannabinoids by increasing the production of proinflammatory cytokines (e.g., tumoe necrosis factor-α) known to boost AEA production. A reduction in FAAH subsequent to elevated endocannabinoid release has been shown in preclinical studies in which AEA inhibits its own degradation by blocking FAAH through a lipoxygenase route [37]. Although this explanation does not account for reduced FAAH protein levels in animal models of AUD, inhibition of FAAH by a bioactive lipid product following alcohol intake cannot entirely be ruled out in humans.

Low FAAH in AUD could also occur via upstream changes in endocannabinoids as a compensatory response to decreased CB1 stimulation. In this regard, preclinical and PET studies suggest that CB1 receptors are persistently desensitized/reduced in AUD at the same time points during abstinence [38–40]. Thus, lower FAAH may be a homeostatic effort to increase endocannabinoid tone and restore CB1 activity. The trigger for lower FAAH levels is unknown. Speculatively, *N*-arachidonoyl-glycine, produced from AEA oxidation by alcohol dehydrogenase (the main metabolite of alcohol), could inhibit the activity of FAAH [41]. Whether low FAAH is an acute compensatory response to chronic alcohol consumption or an inherited or acquired biological vulnerability, which predates drinking, is unknown.

Limitations

The present study contains a few limitations, including the small sample size and group differences in BMI, age, and sex, which could have contributed to the finding. FAAH levels have been reported to fluctuate with age [42] and may differ according to sex [43], and in people with high BMI (BMI > 30). Nonetheless, we did not find that these factors, when entered as covariates, affected results. Furthermore, in a large sample of HCs ($n = 25 + n = 36$ healthy heavy drinking youth), we do not find that BMI or age were related to Ik3, or that a significant difference in FAAH brain levels exist between males and females (Supplementary Fig. S3). The lack of an age effect is also consistent with our observation that levels of FAAH protein are stable in the prefrontal cortex of a small sample of autopsied brains from 20- to 50-year-old humans (Tong, Boileau, and Kish, unpublished). Other factors that may have affected our findings include variabilities in diet (including content, quantity and frequency of food consumed) and use of drugs, which can affect tissue concentrations of endocannabinoids and NAEs. Nicotine use was slightly more severe in our AUD subjects; however, we found no difference in FAAH between healthy smokers and non-smokers. Scores on the BDI differed significantly between AUD and HC (Table 1). Although we cannot rule out the potential relationship between depression/depression symptoms and the endogenous cannabinoid system, BDI scores did not correlate with brain FAAH in any ROI, nor with FAAH substrate levels. Finally, days of abstinence prior to scanning ranged between AUD subjects, which may have contributed to the large variability seen at this time point.

CONCLUSIONS

The clinical significance of our data is uncertain and likely to be complex. Nevertheless, our novel findings in AUD of changes in brain FAAH levels linked to differences in levels of substances metabolized by FAAH provide new support in humans for an involvement of the endocannabinoid system in AUD. Although FAAH inhibitors could be useful in mitigating negative reinforcement and stress-induced relapse in AUD, treatment approaches in AUD and in co-morbid illnesses need to consider that increased endocannabinoid tone during early abstinence could drive drinking. Longitudinal studies comparing FAAH and FAAH substrate levels vs. clinical symptoms in AUD should help in understanding whether FAAH might represent a useful therapeutic target.

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ADDITIONAL INFORMATION

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