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Fatty acid amide hydrolase is lower in young cannabis users

Maya R. Jacobson[1,2,4] | Jeremy J. Watts[1,2,4] | Tania Da Silva[1,4] | Rachel F. Tyndale[2,3,4] | Pablo M. Rusjan[1,3,4] | Sylvain Houle[1,3,4] | Alan A. Wilson[1,3] | Ruth A. Ross[2] | Isabelle Boileau[1,3,4] | Romina Mizrahi[1,2,3,4]

[1]Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

[2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

[3]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

[4]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Correspondence
Romina Mizrahi, PET Centre, Research Imaging Centre, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario M5T 1R8, Canada.
Email: romina.mizrahi@camhpet.ca

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Abstract

We have recently shown that levels of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide, are lower in the brains of adult cannabis users (CUs) (34 ± 11 years of age), tested during early abstinence. Here, we examine replication of the lower FAAH levels in a separate, younger cohort (23 ± 5 years of age). Eighteen healthy volunteers (HVs) and fourteen CUs underwent a positron emission tomography scan using the FAAH radioligand [^{11}C]CURB. Regional [^{11}C]CURB binding was calculated using an irreversible two-tissue compartment model with a metabolite-corrected arterial plasma input function. The FAAH C385A genetic polymorphism (rs324420) was included as a covariate. All CUs underwent a urine screen to confirm recent cannabis use and had serum cannabinoids measured. One CU screened negative for cannabinoids via serum and was removed from analysis. All HVs reported less than five lifetime cannabis exposures more than a month prior to study initiation. There was a significant effect of group ($F_{1,26}$ = 4.31; P = .048) when two A/A (rs324420) HVs were removed from analysis to match the genotype of the CU group (n = 16 HVs, n = 13 CUs). Overall, [^{11}C]CURB λk_3 was 12% lower in CU compared with HV. Exploratory correlations showed that lower brain [^{11}C]CURB binding was related to greater use of cannabis throughout the past year. We confirmed our previous report and extended these findings by detecting lower [^{11}C]CURB binding in a younger cohort with less cumulative cannabis exposure.

KEYWORDS

[^{11}C]CURB, addiction, anandamide, cannabis, fatty acid amide hydrolase, positron emission tomography

1 | INTRODUCTION

Cannabis is the most widely used illicit drug worldwide.[1] Its use is especially high among youth, with rates of use being 19% and 33% among youth aged 15 to 19 and 20 to 24, respectively, as compared with 13% in adults above 25 years of age, in Canada.[2] Meanwhile, the perceived risk of harm from cannabis use, by youth, has declined in recent years.[3] Roughly 10% of individuals who try cannabis become dependent.[4] However, approximately 15% of users become dependent if cannabis use is initiated during adolescence.[4] There are also reported associations between cannabis use and adverse effects including addiction, altered brain development, cognitive impairment, poor educational outcomes, and diminished life satisfaction.[4] Most of these findings appear to be more consistent when cannabis use is initiated in early adolescence.[4] Conversely, cannabis has been used for medicinal purposes in numerous ailments.[5]

The main psychoactive component of cannabis, (-)-trans-Δ 9-tetrahydrocannabinol (THC), is a partial agonist for cannabinoid

receptors 1 and 2 (CB1R and CB2R, respectively).[6,7] These receptors, along with their endogenous ligands and associated metabolizing enzymes, comprise the canonical endocannabinoid (eCB) system (although an extended eCB system has also been described).[8] Fatty acid amide hydrolase (FAAH), the catabolic enzyme responsible for controlling levels of fatty acid amides including anandamide, sets the tone of eCB signaling[9,10] and has garnered considerable attention as a potential therapeutic agent. Recently, D'Souza et al showed treatment with FAAH inhibitor PF-04457845 to reduce cannabis withdrawal symptoms in men with cannabis dependence, highlighting FAAH's role in cannabis use disorders (CUDs).[11]

Most,[12-15] but not all,[16] preclinical and postmortem studies detect a decrease in CB1R availability with cannabinoid usage. In humans, positron emission tomography (PET) studies have consistently detected a downregulation of CB1R in cannabis users (CUs)[17-19]; these findings include a 20% reduction in the neocortex and limbic cortex,[17] a 15% reduction across brain regions,[18] and a global 12% decrease in CB1R availability.[19] However, this effect may be transient and reversible, normalizing after 2 to 4 days of abstinence.[18]

Data derived from research on endocannabinoids and their metabolizing enzymes are inconsistent. One study, which divided its cannabis users into "frequent" and "infrequent" users (those who used more and less than 10 times per month, respectively), found lower levels of anandamide in the frequent compared with infrequent users, but neither group differed from controls.[20] An additional study detected no effect of cannabis use on cerebrospinal fluid anandamide levels.[21] Using the novel PET radioligand [^{11}C]CURB, our center reported a reduction of FAAH in the brains of adults (34 ± 11 years of age) with CUD who had been using cannabis for 18 ± 11 years.[22] Here, we aimed to replicate this finding in a younger cohort with daily cannabis use, irrespective of CUD status. We measured FAAH in CU relative to healthy controls (HV) using [^{11}C]CURB and a high-resolution research tomograph (HRRT). We hypothesized that [^{11}C]CURB binding would be lower in the CU compared with HV across brain regions when tested in early abstinence as in our previous study. We also explored the relationship between [^{11}C]CURB binding and the extent of cannabis use and peripheral levels of cannabis metabolites.

2 | MATERIALS AND METHODS

2.1 | Study participants

Eighteen HVs and 14 CUs completed all study procedures. All participants were recruited using local advertisements in the Greater Toronto Area. Participants did not meet criteria for any *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition* (*DSM-IV*), Axis I disorder, as determined by the Structured Clinical Interview for *DSM-IV* (SCID),[23] with the exception of nicotine abuse/dependence and cannabis abuse/dependence in CU. All participants were excluded for any of the following: first degree relatives with a psychotic disorder, significant current or past medical conditions, neurological illnesses or head trauma, use of medications that might affect the central nervous system, the presence of metal implants precluding a magnetic resonance imaging (MRI) scan, and/or pregnancy or breastfeeding. The Fagerstrom Test for Nicotine Dependence was used to assess nicotine dependence.[24]

Cannabis users were invited to participate if they smoked cannabis at least 4 days per week, had been using regularly for at least 1 year, and screened positive for cannabis use at baseline via urine toxicology. Hence, regular cannabis use, but not CUD, was required for inclusion criteria. However, *Diagnostic and Statistical Manual of Mental Disorders*, *Fifth Edition* (*DSM-5*),[25] was used to retrospectively determine probable CUD by using responses given on the SCID and the following questionnaires: Severity of Dependence Scale,[26] Drug Abuse Screening Test,[27] Marijuana Craving Questionnaire,[28] and Cannabis Withdrawal Scale.[29] The aforementioned scales were completed on scan day. All CUs were asked to abstain from cannabis overnight (approximately 12 hours) prior to the scheduled PET scan, as previously done by our group. At baseline, urine toxicology was used to rule out illicit drugs other than cannabis in CU. In addition, we collected blood samples for cannabinoid/metabolite analysis on the day of the PET scan (prescan). HVs were required to have zero past exposure to street drugs besides cannabis and no more than five lifetime cannabis uses, predating study initiation by at least 1 month.

This study was approved by the Research Ethics Board at the Centre for Addiction and Mental Health. All subjects provided written informed consent after being informed of all study procedures.

2.2 | PET and MRI data acquisition and analysis

[^{11}C]CURB PET data were acquired according to the validated method reported elsewhere.[30] [^{11}C]CURB was synthesized as previously described.[31] Briefly, participants underwent a transmission scan, followed by an intravenous bolus injection of [^{11}C]CURB (9.81 ± 0.72 mCi in HV, 9.50 ± 0.86 mCi in CU) and 60-minute PET scan using a 3-D HRRT brain tomograph (CPS/Siemens, Knoxville, TN, USA).[30] A 2-D filtered-back projection algorithm, with a HANN filter at Nyquist cutoff frequency, was applied to the 2-D sinograms to reconstruct the images. Arterial blood samples were collected automatically using an automated blood sampling system (ABSS; Model PBS-101, Veenstra Instruments, The Netherlands) for the first 22.25 minutes post injection at a rate of 2.5 mL/min, and samples were collected manually at 3, 7, 12, 20, 30, 45, and 60 minutes post injection, to measure radioactivity in blood and determine the relative proportion of radiolabeled metabolites. A metabolite-corrected input function was generated as previously described.[30] Blood-to-plasma radioactivity ratios were interpolated using a biexponential function, and parent plasma fraction by a Hill function. To permit delineation of regions of interest (ROIs), a standard proton density (PD) weighted brain MRI was acquired for each participant, using a Discovery MR750 3T MRI (General Electric, Milwaukee, WI, USA).

Time-activity curves for each ROI were extracted using an in-house imaging pipeline.[32] [^{11}C]CURB binding was quantified using the

composite parameter λk_3 ($\lambda = K_1/k_2$), as derived from an irreversible two-tissue compartment model, which is the validated method for quantifying [^{11}C]CURB binding in vivo.[33]

2.3 | rs324420 FAAH genotyping

The FAAH gene is polymorphic (rs324420, C385A), resulting in lower FAAH protein levels and associated [^{11}C]CURB binding[34] in carriers of one of more copies of the A allele. Thus, all participants were genotyped using a commercially available (Life Technologies, Burlington, Ontario, Canada) TaqMan assay as performed previously at our center.[34]

2.4 | Analysis of cannabinoid metabolites in serum

The cannabinoids multiplex assay (THC, THCOH, THCCOOH, and CBD) was performed at the CAMH Clinical Laboratory by gas chromatography coupled with mass spectrometry (GC-MS) as described in the Varian (Agilent Technologies) application note 00315 with slight analytical modifications and CBD addition to the assay.[35] Results' validation was based on three-level quality control (ACQ Science).

2.5 | Statistical analysis

Group differences in demographic measures were determined using independent sample *t* tests for continuous variable and Fisher's exact tests for categorical variables. Group difference in [^{11}C]CURB λk_3 was analyzed using a linear mixed models analysis, with group as a between-subjects factor, [^{11}C]CURB λk_3 as the dependent variable, ROI as a factor, and FAAH genotype as a covariate. The model tested for main effects of group, region, and genotype and a group × ROI interaction. ROIs (12) included in the model were cortical regions including prefrontal cortex (PFC), anterior cingulate cortex (ACC), temporal cortex, occipital cortex, parietal cortex, and insula and subcortical regions including dorsal striatum, ventral striatum, hippocampus, amygdala, thalamus, and cerebellum. Associations between [^{11}C]CURB λk_3 and cannabis use measures were tested using bivariate Pearson correlations. Further, we ran partial correlations to explore these associations including covariates (hours of cannabis abstinence, THC, and THCCOOH levels) to control for the potential confounding effects of recent use and cannabis use history. [^{11}C]CURB λk_3 values used in all correlations were adjusted for genotype by running a linear regression to generate the residuals of λk_3 values of each ROI on FAAH genotype. A genotype-corrected whole-brain [^{11}C]CURB λk_3 value was generated by calculating a weighted average value representative of all 12 ROIs. All statistical analyses were performed using Statistical Product and Service Solutions (SPSS) (version 24.0; International Business machines [IBM], Armonk, NY, USA), with $P < .05$ considered to be significant. Bonferroni corrections were completed for correlations in a priori regions by dividing the α level (.05) by the number of comparisons (4, corrected $\alpha = .013$).

3 | RESULTS

3.1 | Participant demographics

Eighteen HVs and 14 CUs performed all study procedures. One CU screened negative for serum THC/metabolites on scan day and was thus removed from analyses (n = 13 CUs). There was no significant difference in the frequencies of C385A FAAH genetic polymorphism (rs324420) between groups (n = 18 HVs, 13 CUs, $P = .71$). As previously demonstrated at our center,[34] there was a significant effect of genotype on [^{11}C]CURB binding ($P < .05$), whereby binding is lower in carriers of the C385A variant. Therefore, the two HVs who were homozygotes for the A allele were removed from analysis to improve the match to the CU group, which did not consist of any individuals who were homozygous for the A allele.

Group demographics are reported in Table 1 (n = 16 HVs, n = 13 CUs). The groups did not differ with respect to age, sex, ethnicity, body mass index (BMI), or total years of education (all $P > .05$). In addition, there were no significant differences between groups for any of the PET radiotracer parameters, including injected radioactivity, mass injected, and specific activity at the time of synthesis. There was a higher number of daily nicotine users in the CU group than the HV group; only one met criteria for nicotine dependence by scoring at least a 4 on the Fagerstrom Test for Nicotine Dependence. There was no difference in number of cannabis uses between the nicotine users and nonusers in the week ($P = .35$) or year ($P = .82$) prior to study.

All CUs screened positive for cannabis use via urine toxicology at baseline. Cannabis use is detailed in Table 2. THC (range: 1.3-35.5 ng/mL, mean: 10.6 ± 9.8 ng/mL, n = 13) and its metabolite 11-nor-9-carboxy-THC (THCCOOH; range: 10-308 ng/mL, mean: 101.9 ± 92.3 ng/mL, n = 13) were detected in the serum of 13 CUs on scan day. For the 13 CUs included in analysis, the average age of first consistent cannabis use (at least weekly) was 17.9 ± 5.5 (13-33 years of age), and they had been using cannabis regularly for an average of 4.8 ± 2.7 years (1-9 years). At the time of study, the 13 CUs reported using cannabis on average 6.9 ± 0.3 days (6-7) out of the past week and 28.2 ± 3.4 days (24-30) out of the past 30 days. We retrospectively determined that 6 of the 13 CUs met *DSM-5* criteria for CUD. All CUs reported fewer than 15 lifetime exposures to any other illicit drugs, with the most recent report being one instance of use, which occurred 1 month prior to the PET scan. Only two HVs reported one lifetime use of cannabis (more than 30 days prior to the time of study), and all HVs reported zero lifetime exposures to other illicit drugs. The average time of self-reported cannabis abstinence prior to the PET scan was 13.0 ± 2.5 hours (6.8-14.8 hours). There were no significant correlations between hours of abstinence and genotype-adjusted [^{11}C]CURB λk_3 any in ROI (all $P > .05$).

TABLE 1 Participant demographics

Measure	HV[a] Mean (SD)	CU[a] Mean (SD)	Result	P
n	16	13	-	-
Age	21.77 (1.93)	22.88 (4.58)	−0.87	.43
Sex				
Female	8	4	-	.45
Male	8	9		
FAAH Genotype (FAAH C385A polymorphism rs324420)				
CC	11	9	-	1.00
AC	5	4		
AA	0	0		
Daily nicotine users	0	5	-	.01
BMI	24.10 (4.78)	24.23 (4.60)	−0.40	.97
Years of education	14.78 (1.86)	13.50 (2.68)	1.33	.20
Ethnicity				
White	7	7	-	.71
Asian	5	2		
Black	4	4		
Radioactivity injected, mCi	9.86 (0.66)	9.50 (0.86)	1.76	.91
Molar activity at time of injection, mCi/μmol	2086.60			
(749.19)	1932.43			
(706.68)	0.74	.47		
Mass injected, μg	1.69 (0.72)	1.78 (0.85)	−0.36	.73

Abbreviations: BMI, body mass index; CU, cannabis user; HV, healthy volunteer.
[a]Demographics are shown for 16 HVs, after the removal of the two HVs with the A/A FAAH genotype, in order to better match groups. This cohort of HV was used for all calculations, unless otherwise stated. One CU was removed from analysis after screening negative for THC/metabolites in serum on scan day (n = 13).

TABLE 2 Cannabis use measures

Measure (n = 13)	Mean (SD)	Range
THC in serum, ng/mL	10.6 (9.8)	1.3-35.5
THCCOOH in serum, ng/mL	101.9 (92.3)	10-308
Age of first cannabis exposure	16.4 (4.3)	13-29
Age of first consistent cannabis use	17.9 (5.5)	13-33
Years of regular cannabis use	4.8 (2.7)	1-9
Estimated days of cannabis use (past year)	300.0 (70)	180-365
Estimated days of cannabis use (past month)	28.2 (3.4)	24-30
Cannabis smoked in the past week (grams)	10.8	4-25
Number of days of cannabis use in the past week	6.9 (0.3)	6-7
Severity of Dependence Scale (SDS)	3.1 (2.3)	0-7
Drug Abuse Screening Test (DAST)	6.5 (4.2)	0-15
Marijuana Craving Questionnaire (MCQ)	42.2 (9.6)	25-57
Compulsivity	6.2 (2.4)	3-10
Emotionality	8.8 (3.8)	3-15
Expectancy	11.5	6-17
Purposefulness	15.6 (5.4)	3-21
CU who were identified to have probable CUD (n)	6	

Note. Drug use profiles of CU who screened positive for cannabinoids in serum on screen day (n = 13). This sample was included in all analyses, unless otherwise stated.
Abbreviations: CU, cannabis user; CUD, cannabis use disorder.

3.2 | [^{11}C]CURB binding in cannabis users during early abstinence

We detected a significant effect of group ($F_{1,26}$ = 4.31; P = .048), genotype ($F_{1,26}$ = 6.54; P = .021), and ROI ($F_{11,297}$ = 41.84; P < .00) and no significant group × ROI interaction ($F_{11,297}$ = 1.057; P = .40), with the removal of the two A/A HVs. Overall, [^{11}C]CURB λk_3 was 12% lower in CUs (n = 13) compared with HVs (n = 16). In individual ROIs, the difference in [^{11}C]CURB λk_3 between CU and HV ranged from 8.5% in the insula to 16.0% in the hippocampus (Figure 1). There was no effect of sex ($F_{1,25}$ = 0.05; P = .83), tobacco use ($F_{1,25}$ = 1.218; P = .28), or past use of street drugs excluding cannabis ($F_{1,25}$ = 0.182; P = .67) on [^{11}C]CURB λk_3. There was no difference in [^{11}C]CURB λk_3 between the CUs who we determined to have CUD (n = 6) and those without (n=7), in this small sample (n = 13; $F_{1,10}$ = 0.10; P = .76). When the analysis was performed including the HVs with the A/A genotype (n = 18 HVs, n = 13 CUs), there was a trend towards lower [^{11}C]CURB binding in the CU compared with HV ($F_{1,28}$ = 3.50; P = .072), with an effect of ROI ($F_{11,319}$ = 43.14; P < .00) and genotype ($F_{1,28}$ = 20.69; P < .00), and nonsignificant group × ROI interaction ($F_{11,319}$ = 0.84; P = .60).

As expected, the sample was heterogeneous with respect to cannabis use. One participant displayed serum THC levels that indicated recent use (>25 ng/mL), although they did not report using cannabis closer to the scan. Another CU reported using cannabis approximately 6.5 hours prior to the scan, although they were instructed to abstain for 12 hours prior to the scan (serum THC levels, 17.4 ng/mL, were within the range of the rest of the group, 1.3-35.5 ng/mL). A sensitivity analysis found that with the removal of this individual (n = 12 CUs, n = 16 HVs), [^{11}C]CURB λk_3 was 8.5% lower in CU compared with HV, but this effect did not reach statistical significance ($F_{1,25}$ = 2.892; P = .10). Similarly, with removal of the individual with high levels of serum THC (>25 ng/mL), binding was 14.7% lower in CUs (n = 12) compared with HVs (n = 16), but the effect was not statistically

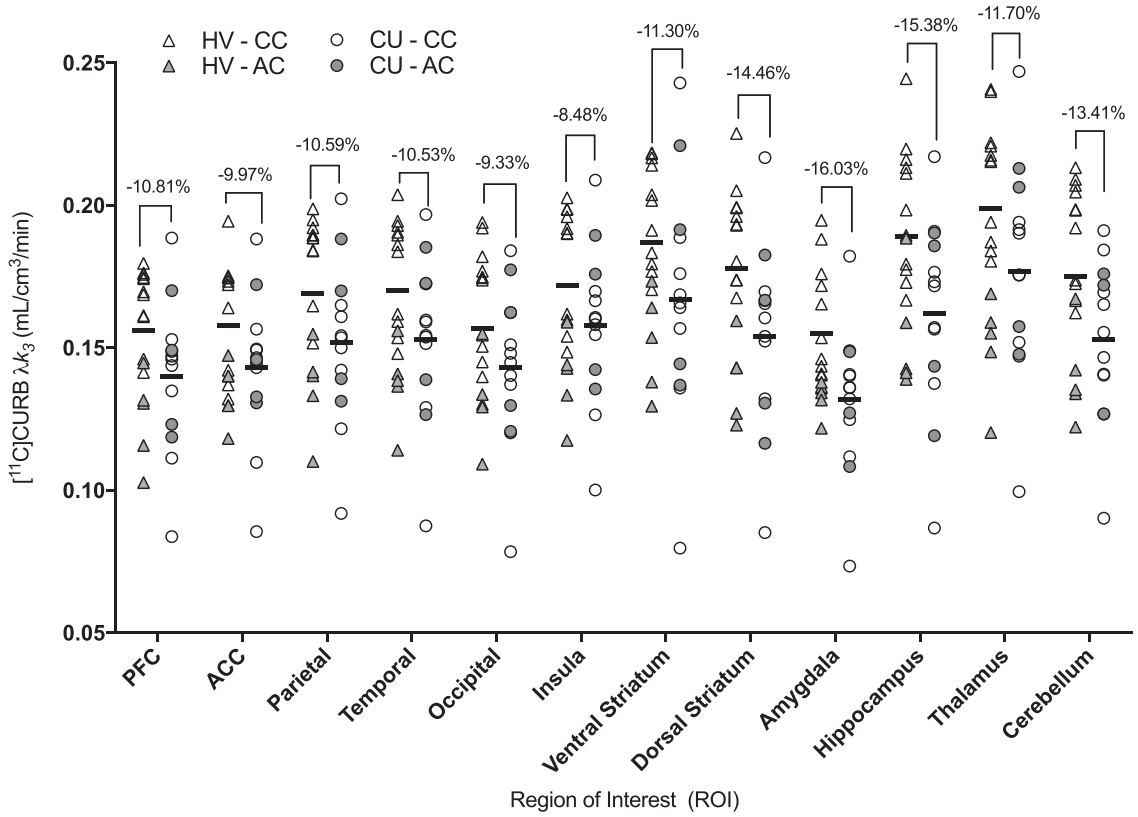

FIGURE 1 Brain [^{11}C]CURB λk_3 values, an index of [^{11}C]CURB binding to fatty acid amide hydrolase (FAAH), are presented for each participant in the healthy volunteer (HV; triangles) and cannabis user (CU; circles) groups. The C/C and A/C FAAH genotypes (C385A, rs324420) are indicated by white and gray symbols, respectively. The group means presented are the adjusted means to account for FAAH genotype. Percent differences are displayed for [^{11}C]CURB λk_3 averages in each region of interest (ROI) in CU (n = 13) versus HV (n = 16)

significant ($F_{1,25}$ = 3.922; P = .059), likely due to small sample size. There was no main effect of hours since cannabis use ($F_{1,11}$ = 2.557; P = .14) or serum levels of THC ($F_{1,11}$ = 0.803; P = .39) or THCCOOH ($F_{1,11}$ = 0.001; P = .98) on [^{11}C]CURB λk_3.

3.3 | Exploratory association between [^{11}C]CURB λk_3 and cannabis use

Correlations were run between cannabis use measures and [^{11}C]CURB λk_3 in a priori ROIs, including the amygdala, hippocampus, and ACC, as these regions were previously found to correlate with THC metabolites and the ventral striatum because of its relevance for drug abuse/dependence.[36] After a Bonferroni correction was performed for four comparisons (corrected α = .013), only the correlation between estimated number of days smoked in the past year, as determined using the Drug History Questionnaire, and [^{11}C]CURB λk_3 in the ventral striatum (r = –0.672; P = .012) remained significant (Figure 2; n = 13). This correlation remained significant after correcting for the hours since last cannabis use (r = –0.730; P = .007), the THC in serum (r = –0.767; P = .004), and THCCOOOH in serum (r = –0.772; P = .003). The correlation between number of days used in the past year and [^{11}C]CURB λk_3 in the amygdala was also significant when controlling for THC (r = –0.806; P = .002) and THCCOOH (r = –0.711; P = .006) in serum. An exploratory correlation was also detected between number of days smoked in the past year and an ROI-weighted whole-brain average λk_3 value (r = –0.628; P = .022), which remained significant (P < .05) when controlling for THC/THCCOOH/time since last use. There were correlations between [^{11}C]CURB binding and THCCOOH/THC (ratio, as validated in Huestis et al[37]) across regions, including the amygdala (r = 0.662; P = .031), ventral striatum (r = 0.599; P = .040), ACC (r = 0.597; P = .040), and hippocampus (r = 0.501; P = .097). These exploratory correlations were not corrected for multiple comparisons. There were no significant correlations between [^{11}C]CURB binding and any measures from questionnaires relating to cannabis use (craving/withdrawal).

4 | DISCUSSION

4.1 | FAAH in CU during early abstinence

Our current findings are in line with the prior study, which detected lower levels of [^{11}C]CURB binding (λk_3) in adults with CUD measured during early abstinence.[22] In the current study, we detected lower

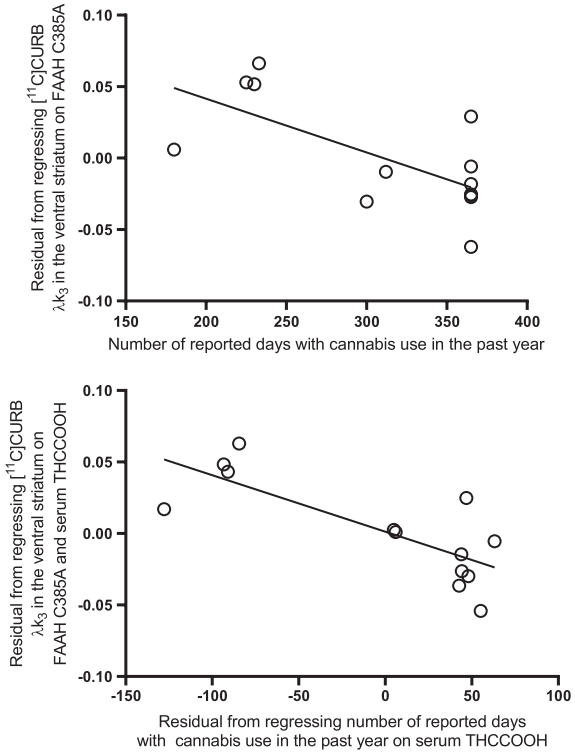

FIGURE 2 Correlation between the reported number of days with cannabis use in the past year and [^{11}C]CURB λk_3 in the ventral striatum (n = 13). All [^{11}C]CURB λk_3 values are all corrected for the FAAH C385A single nucleotide polymorphism. In the bottom plot, the values on the *x*-axis and *y*-axis reflect a partial correlation, which was corrected for serum THCCOOH levels

[^{11}C]CURB binding in CU compared with HV similarly tested in early abstinence. It is possible that recent cannabis use affects [^{11}C]CURB binding such that FAAH is lower with recent use but not after longer abstinence. This should be confirmed in larger studies designed to assess the impact of abstinence on [^{11}C]CURB binding. Notably, the percent difference in [^{11}C]CURB binding between groups in the current study (8.5%-16.0%, n = 13 CUs) is lesser than previously reported (14%-20%).[22]

The CU in the current and previous studies differed with respect to their ages and cumulative lifetime cannabis use. The subjects in the previous study[22] and the current study did not differ with respect to age of cannabis use initiation (17.9 ± 5.5 and 16.2 ± 4.2) or grams of cannabis used in the past week (10.8 ± 6.6 and 10.4 ± 6.4), past 90 days (85.7 ± 7 and 76.4 ± 15), or in the past year (300.0 ± 70 and 305.6 ± 60.1), for the current and previous studies, respectively. However, our cohort of CU was aged 23 ± 5 years, which is younger than the previous cohort that had a mean age of 34 ± 11 years (P = .010). In addition, the CU in the present study had on average 4.8 ± 2.7 years of regular cannabis use, less than the 17.5 ± 10.8 years of use in the previous cohort (P = .010). Also, unlike in the previous study, not all participants in the current study met *DSM-IV/5* criteria for CUD. In the current study, there was no difference in [^{11}C]CURB binding between CUs who were determined to meet *DSM-5* criteria for CUD (n = 6) versus those who did not (n = 7), though this is likely due to the small sample size. Because of the similar cannabis use reported in the two samples at the time of study, it is unlikely that frequency of use accounts for the difference in magnitudes between groups. Therefore, the discrepancies in results between the current and previous studies may be attributed to the differences in cumulative use over a number of years or CUD status. Furthermore, our sensitivity analysis highlights the importance of considering cannabis use patterns on [^{11}C]CURB binding. The different ages of the CU in the two studies might be another potential explanation for the discrepancy between the observed [^{11}C]CURB λk_3 group differences. Overall, our findings highlight the importance of considering the extent of cannabis use/CUD diagnosis when discussing the effects of cannabis use on brain FAAH, and future research should seek to disentangle the effects of age, CUD, and years of cannabis use on FAAH.

To our knowledge, there is limited research on how cumulative cannabis use affects the eCB system in humans. Hirvonen et al reported an inverse association between CB1R availability and years of cannabis use, with a greater reduction apparent in those with more years of use.[17] However, subsequent studies of CB1R in CU failed to detect such an association.[18,19] In rodents, the downregulation of CB1R in response to cannabinoid exposure is dose dependent.[12] It is possible that FAAH is sensitive to the amount of total cumulative cannabis use. In fact, Boileau et al detected an inverse association between levels of THC metabolites and [^{11}C]CURB λk_3, suggesting that heavier recent cannabis use is associated with lower FAAH levels. Coincidentally, we reported a negative association between past-year cannabis use and ventral striatum [^{11}C]CURB λk_3 (discussed below).

Prior to the first study at our center,[22] the existing literature regarding the relationship between FAAH and cannabis use was ambiguous. Studies that measured anandamide levels in the cerebrospinal fluid of CU detected no alteration compared with healthy volunteers.[20,21] Genotype studies reported that individuals with the FAAH A/A genotype, associated with lower FAAH expression/activity,[38,39] might be at the lowest risk of becoming cannabis dependent[40] and the high expression/activity C/C genotype has been linked with other cannabis-related problems.[41-43] Whereas these studies suggest that higher levels of FAAH might predispose individuals to cannabis dependence and associated problems, our finding suggests that FAAH levels are lower in CU in early abstinence.

A number of interactions in the eCB system could account for the lower observed FAAH in early abstinence CU. It is possible that in response to the decrease in CB1R availability in CU,[17-19] FAAH is reduced to increase anandamide and subsequent CB1R signaling. A second possibility is that the lower FAAH in CU is a compensatory mechanism to account for a downregulation of anandamide in CU. Alternatively, given that THC has been shown to suppress immune responses in cell models,[44] Boileau et al proposed that CU might exhibit lower FAAH alongside a decrease in microglia activity/density; however, findings from our group do not support this postulation.[45]

Although we failed to reproduce the correlations between serum levels of THC or THCCOOH and [^{11}C]CURB λk_3 as previously reported,[22] we were able to detect correlations between the THCCOOH/THC ratio and [^{11}C]CURB λk_3 across regions. A difference in methodology for the quantification of the THC/metabolites, using serum in the present study and whole blood in the previous study,[22] could contribute to the discrepancies in metabolite levels and relationships to [^{11}C]CURB binding. Importantly, we did not obtain a second serum sample (postscan), which could indicate whether CUs were in early abstinence, which was established when the previous relationship was observed.[22]

4.2 | Exploratory correlations with cannabis use measures

Here, we explored the relationship between cannabis use and [^{11}C]CURB binding. We observed negative correlations between estimated days smoked in the past year and [^{11}C]CURB λk_3 in the ventral striatum, and both the ventral striatum and amygdala when controlling for serum THC/THCCOOH levels. These correlations were corrected for four comparisons and may not survive additional corrections. Our results, which control for FAAH genotype, suggest that the observed lower levels of FAAH are potentially related to the extent of cannabis use in the preceding year and not solely as a result of recent use or of predisposition. This relationship between cumulative cannabis use and FAAH is especially interesting when considering the importance of cannabis dose/-extent of usage and its adverse effects. For example, meta-analysis revealed that the risk of psychotic outcomes from cannabis use is dependent on the extent of cumulative cannabis use.[46] Furthermore, frequency and amount of cannabis have been correlated to the degree of cognitive effects from cannabis, such as verbal learning and memory in adolescent CU.[47] Our finding, which associates heavier cannabis use with lower [^{11}C]CURB binding in the ventral striatum, is interesting when considering both the role of ventral striatum in reward/addiction[35] and FAAH as a target for CUD treatment.[11] All of the associations between days smoked in the past year and [^{11}C]CURB λk_3 are exploratory, given the small sample size and the number of correlations. Nevertheless, these findings highlight the importance of cumulative cannabis use on the eCB system.

4.3 | Limitations

Strengths of this study include the use of an HRRT and a radiotracer with excellent reproducibility and reliability.[33] Although we cannot confirm that [^{11}C]CURB λk_3 is representative of FAAH enzyme activity, [^{11}C]CURB binds to FAAH at the catalytic site for anandamide hydrolysis, so binding of [^{11}C]CURB to FAAH is reflective of available FAAH catalytic sites, suggesting a role in enzyme activity.[30,48] Further, validation from our center found [^{11}C]CURB binding (λk_3) to be independent of cerebral blood flow.[30] One limitation of this study is that CUs were not specifically screened for CUD using *DSM-5* (although we did retrospectively determine some participants to meet criteria for CUD using *DSM-5* and additional questionnaires). Nonetheless, all CUs reported high levels of cannabis use (using 6.9 ± 0.3 days in the past week) at the time of study. As with most studies in CU, lifetime and recent cannabis metrics were based on self-report and do not distinguish between cannabis strains and potencies. In addition, we obtained only one serum sample on scan day (prescan), and thus, we are not able to discern whether our study participants were in early abstinence (approximately 12 hours) as verbally reported. Also, participants were not excluded for use of caffeine, nicotine, and/or alcohol, and a detailed history of alcohol use was not obtained for each participant. However, there was no main effect of tobacco use on [^{11}C]CURB λk_3, and only one participant was nicotine dependent according to the Fagerstrom Test for Nicotine Dependence. In addition, no participants met *DSM-IV/5* criteria for alcohol use disorder. The sample size was also small and not fully matched between groups, which prompted the exclusion of the two A/A subjects from the HV group, to better match the groups. Although an a priori power analysis was not conducted as all participants were recruited as part of a larger study, a power analysis[22] based on the previously published study with effect size d = 0.96 suggests that a sample size of 38 (19 participants per group) would be required to detect a group difference with an α level of .05 and 80% power. We therefore decided that a small sample size would be sufficient to conduct this study given its replicative nature, while minimizing the number of volunteers exposed to radiation. Finally, since the results were obtained during (presumed) early abstinence (13.0 ± 2.5 hours), it is unknown if the reduction of FAAH remains over a longer period of abstinence.

5 | CONCLUSION

We extended previous findings[22] by performing the current study in a cohort that was younger and had less cumulative cannabis use than previously reported. We report a small group difference, yet significantly lower [^{11}C]CURB binding in CU compared with HV in the current sample. We highlight the importance of extent of cannabis use when considering its potential effects on the eCB system. Our results suggest that the eCB system could be altered in younger individuals who have less cumulative lifetime use of cannabis. While small, we believe that our findings are important, especially considering recent legalization of recreational cannabis use across the globe. Our sample captured individuals in their early to mid-twenties, an age where there is elevated risk for developing CUD[49] and psychotic disorder(s),[50] alongside widespread recreational cannabis use.

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AUTHOR CONTRIBUTIONS

RM designed the study. MJ, JW, TD, and RT conducted experiments. MJ and RM analyzed the data. MJ and RM wrote the manuscript, with input from IB, RT, and JW. All authors critically reviewed the content and approved the final version for publication.

DISCLOSURE/CONFLICT OF INTEREST

R.F.T has consulted for Quinn Emmanual and Apotex and is a member of numerous scientific advisory boards, on unrelated topics. All other authors report no biomedical financial interests or potential conflict of interest.

ORCID

Jeremy J. Watts https://orcid.org/0000-0003-1751-5409
Rachel F. Tyndale https://orcid.org/0000-0003-1297-2053
Romina Mizrahi https://orcid.org/0000-0001-6667-7928

REFERENCES

1. UNODC. *World Drugs Report*. Vienna: United Nations Publications; 2019.
2. Health Canada. Canadian Tobacco, Alcohol and Drugs Survey (CTADS): summary of results for 2017 website. https://www.canada.ca/en/health-canada/services/canadian-tobacco-alcohol-drugs-survey/2017-summary.html. Updated January 4, 2019. Accessed August 4, 2019.
3. Miech RA, Johnston LD, O'Malley PM, et al. *Monitoring the Future national survey results on drug use, 1975-2018: Volume I, Secondary school students*. Ann Arbor: Institute for Social Research, The University of Michigan; 2019;369-377.
4. Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. *NEngl J Med*. 2014;370:2219-2227.
5. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Population Health and Public Health Practice; Committee on the Health Effects of Marijuana: An Evidence Review and Research Agenda. *The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research*. Washington (DC): National Academies Press (US); 2017, Therapeutic Effects of Cannabis and Cannabinoids. Available from: https://www.ncbi.nlm.nih.gov/books/NBK425767/#
6. Pertwee RG. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol*. 2008;13:147-159.
7. Huestis MA, Gorelick DA, Heishman SJ, et al. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry*. 2001;58:322-328.
8. Di Marzo V, Piscitelli F. The endocannabinoid system and its modulation by phytocannabinoids. *Neurotherapeutics*. 2015;12:692-698.
9. Cravatt BF, Demarest K, Patricelli MP, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A*. 2001;98:9371-9376.
10. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature*. 1996;384:83-87.
11. D'Souza DC, Cortes-Briones J, Creatura G, et al. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *Lancet Psychiatry*. 2019;6:35-45.
12. Oviedo A, Glowa J, Herkenham M. Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. *Brain Res*. 1993;616:293-302.
13. Zhuang S, Kittler J, Grigorenko EV, et al. Effects of long-term exposure to Δ9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. *Brain Res Mol Brain Res*. 1998;62:141-149.
14. Romero J, Garcia-Palomero E, Berrendero F, et al. Atypical location of cannabinoid receptors in white matter areas during rat brain development. *Synapse*. 1997;26:317-323.
15. Villares J. Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience*. 2007;145:323-334.
16. Westlake TM, Howlett AC, Ali SF, Paule MG, Scallet AC, Slikker W Jr. Chronic exposure to Δ9-tetrahydrocannabinol fails to irreversibly alter brain cannabinoid receptors. *Brain Res*. 1991;544:145-149.
17. Hirvonen J, Goodwin RS, Li CT, et al. Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Mol Psychiatry*. 2012;17:642-649.
18. D'Souza DC, Cortes-Briones JA, Ranganathan M, et al. Rapid changes in CB1 receptor availability in cannabis dependent males after abstinence from cannabis. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2016;1:60-67.
19. Ceccarini J, Kuepper R, Kemels D, van Os J, Henquet C, Van Laere K. [18F]MK-9470 PET measurement of cannabinoid CB1 receptor availability in chronic cannabis users. *Addict Biol*. 2015;20:357-367.
20. Morgan CJ, Page E, Schaefer C, et al. Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *Br J Psychiatry*. 2013;202:381-382.
21. Leweke FM, Giuffrida A, Koethe D, et al. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr Res*. 2007;94:29-36.
22. Boileau I, Mansouri E, Williams B, et al. Fatty acid amide hydrolase binding in brain of cannabis users: imaging with the novel radiotracer [(11)C]CURB. *Biol Psychiatry*. 2016;80:691-701.
23. First MSR, Gibbon M, Williams J. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-Patient Edition*. (SCID-I/NP) New York: Biometrics Research, New York State Psychiatric Institute; 2002.
24. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*. 1991;86:1119-1127.
25. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.
26. Gossop M, Darke S, Griffiths P, et al. The Severity of Dependence Scale (SDS): psychometric properties of the SDS in English and Australian samples of heroin, cocaine and amphetamine users. *Addiction*. 1995;90:607-614.
27. Skinner HA. The Drug Abuse Screening Test. *Addict Behav*. 1982;7:363-371.
28. Heishman SJ, Evans RJ, Singleton EG, Levin KH, Copersino ML, Gorelick DA. Reliability and validity of a short form of the Marijuana Craving Questionnaire. *Drug Alcohol Depend*. 2009;102:35-40.

29. Allsop DJ, Norberg MM, Copeland J, Fu S, Budney AJ. The Cannabis Withdrawal Scale development: patterns and predictors of cannabis withdrawal and distress. *Drug Alcohol Depend*. 2011;119:123-129.
30. Rusjan PM, Wilson AA, Mizrahi R, et al. Mapping human brain fatty acid amide hydrolase activity with PET. *J Cereb Blood Flow Metab*. 2013;33:407-414.
31. Wilson AA, Hicks JW, Sadovski O, et al. Radiosynthesis and evaluation of [(1)(1)C-carbonyl]-labeled carbamates as fatty acid amide hydrolase radiotracers for positron emission tomography. *J Med Chem*. 2013;56:201-209.
32. Rusjan P, Mamo D, Ginovart N, et al. An automated method for the extraction of regional data from PET images. *Psychiatry Res*. 2006;147:79-89.
33. Boileau I, Rusjan PM, Williams B, et al. Blocking of fatty acid amide hydrolase activity with PF-04457845 in human brain: a positron emission tomography study with the novel radioligand [(11)C]CURB. *J Cereb Blood Flow Metab*. 2015;35:1827-1835.
34. Boileau I, Tyndale RF, Williams B, et al. The fatty acid amide hydrolase C385A variant affects brain binding of the positron emission tomography tracer [11C]CURB. *J Cereb Blood Flow Metab*. 2015;35:1237-1240.
35. Sears RM. Solid phase extraction of THC, THC-COOH and 11-OH-THC from whole blood. Agilent Technologies Application Note 00315. Retrieved from https://www.agilent.com/cs/library/applications/A02465.pdf
36. Nestor L, Hester R, Garavan H. Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage*. 2010;49:1133-1143.
37. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH). *J Anal Toxicol*. 1992;16:283-290.
38. Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum Mol Genet*. 2004;13:2113-2119.
39. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci U S A*. 2002;99:8394-8399.
40. Tyndale RF, Payne JI, Gerber AL, Sipe JC. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B:660-666.
41. Haughey HM, Marshall E, Schacht JP, Louis A, Hutchison KE. Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. *Addiction*. 2008;103:1678-1686.
42. Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. *Neuropsychopharmacology*. 2010;35:967-975.
43. Shollenbarger SG, Price J, Wieser J, Lisdahl K. Poorer frontolimbic white matter integrity is associated with chronic cannabis use, FAAH genotype, and increased depressive and apathy symptoms in adolescents and young adults. *Neuroimage Clin*. 2015;8:117-125.
44. Mecha M, Carrillo-Salinas FJ, Feliu A, Mestre L, Guaza C. Microglia activation states and cannabinoid system: therapeutic implications. *Pharmacol Ther*. 2016;166:40-55.
45. Da Silva T, Hafizi S, Watts J, et al. In vivo imaging of translocator protein in chronic cannabis users. *JAMA Psychiat*. 2019 Jun;76(12):1305-1313. [Accepted]
46. Moore TH, Zammit S, Lingford-Hughes A, et al. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*. 2007;370:319-328.
47. Solowij N, Jones KA, Rozman ME, et al. Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. *Psychopharmacology (Berl)*. 2011;216:131-144.
48. Mileni M, Kamtekar S, Wood DC, Benson TE, Cravatt BF. StevensRC. Crystal structures of fatty acid amide hydrolase bound to the carbamate inhibitor URB597: discovery of a deacylating water molecule and insight into enzyme inactivation. *J Mol Biol*. 2010;400:743-754.
49. Hasin DS, Kerridge BT, Saha TD, et al. Prevalence and correlates of DSM-5 cannabis use disorder, 2012-2013: findings from the National Epidemiologic Survey on Alcohol and Related Conditions-III. *Am J Psychiatry*. 2016;173:588-599.
50. McGrath JJ, Saha S, Al-Hamzawi AO, et al. Age of onset and lifetime projected risk of psychotic experiences: cross-national data from the World Mental Health Survey. *Schizophr Bull*. 2016;42:933-941.

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