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Cannabis Use and Brain Volume in Adolescent and Young Adult Cannabis Users: Effects Moderated by Sex and Aerobic Fitness

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Abstract

Objectives: Studies examining the impact of adolescent and young adult cannabis use on structural outcomes have been heterogeneous. One already-identified moderator is sex, while a novel potential moderator is extent of aerobic fitness. Here, we sought to investigate the associations of cannabis use, sex, and aerobic fitness levels on brain volume. Second, we explored brain–behavior relationships to interpret these findings. **Methods:** Seventy-four adolescents and young adults (36 cannabis users and 38 controls) underwent 3 weeks of monitored cannabis abstinence, aerobic fitness testing, structural neuroimaging, and neuropsychological testing. Linear regressions examined cannabis use and its interaction with sex and aerobic fitness on whole-brain cortical volume and subcortical regions of interests. **Results:** No main-effect differences between cannabis users and nonusers were observed; however, cannabis-by-sex interactions identified differences in frontal, temporal, and paracentral volumes. Female cannabis users generally exhibited greater volume while male users exhibited less volume compared to same-sex controls. Positive associations between aerobic fitness and frontal, parietal, cerebellum, and caudate volumes were observed. Cannabis-by-fitness interaction was linked with left superior temporal volume. Preliminary brain–behavior correlations revealed that abnormal volumes were not advantageous in either male or female cannabis users. **Conclusions:** Aerobic fitness was linked with greater brain volume and sex moderated the effect of cannabis use on volume; preliminary brain–behavior correlations revealed that differences in cannabis users were not linked with advantageous cognitive performance. Implications of sex-specific subtleties and mechanisms of aerobic fitness require large-scale investigation. Furthermore, present findings and prior literature on aerobic exercise warrant examinations of aerobic fitness interventions that aimed at improving neurocognitive health in substance-using youth.

Keywords: Cannabis, Physical fitness, Sex factors, Gray matter, Neuroimaging, Marijuana use

INTRODUCTION

Within the United States, cannabis (CAN) is the second most commonly used substance among adolescents and young adults (Schulenberg et al., 2019). Approximately 52% of young adults (aged 18–25) (Han, Compton, Blanco, & Jones, 2019) and 31% of adolescents (Johnston et al., 2020) have used CAN within the past year. Repeated and regular CAN use within this age range is associated with adverse neurocognitive (Gonzalez, Pacheco-Colon, Duperrouzel, & Hawes, 2017; Meier et al., 2012) and brain structural and functional outcomes (Batalla et al., 2013; Lisdahl, Gilbart, Wright, & Shollenbarger, 2013); however, structural findings are not always consistent (Lisdahl et al., 2013). Thus, there is a call to investigate potential moderating factors, which could prove to be influential in these associations (Lorenzetti, Chye, Silva, Solowij, & Roberts, 2019).

Exogenous CAN acts on the endogenous cannabinoid system, primarily through binding to its cannabinoid receptor type 1 (CB1), which is principally involved in neuromodulation (Mechoulam & Parker, 2013) diffusely across the cerebral cortex (Eggan & Lewis, 2007). Chronic and regular CAN use can affect CB1 downregulation (Hirvonen et al., 2012) and binding (Villares, 2007) for at least a month. In relation to preclinical adolescent models, this developmental period influences the effects of CAN administration (Viveros, Llorente, Moreno, & Marco, 2005) with altered dopaminergic systems (Higuera-Matas et al., 2010) and frontal circuitry

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(Eggan, Mizoguchi, Stoyak, & Lewis, 2010) potentially resulting in structural brain changes (Renard, Rushlow, & Laviolette, 2016). One primary brain morphological index of continued interest is regional gray matter volume. Gray matter volume is known to be at its largest during childhood and, due to pruning, decreases in adolescence and then plateaus into young adulthood (Mills et al., 2016). Introduction of repeated CAN exposure during this developmental period may be associated with abnormal structure and downstream effects on neuropsychological functioning.

Aberrations in these volumetric indices related to CAN use in this age range include smaller medial orbitofrontal and inferior parietal cortices (Price et al., 2015), smaller left rostral anterior cingulate cortex (Maple, Thomas, Kangiser, & Lisdahl, 2019), larger cerebellar vermis (Medina, Nagel, & Tapert, 2010), and smaller bilateral hippocampal volumes (Ashtari et al., 2011). Some studies have reported that aberrant brain morphometry was linked to poorer executive functioning (Medina et al., 2009), long-delay recall (Jacobus et al., 2012), complex attention (Price et al., 2015), working memory (Bava, Jacobus, Mahmood, Yang, & Tapert, 2010), and affect discrimination (Maple et al., 2019) in CAN users. One potential reason underlying inconsistent findings is that potential moderators identifying at-risk or more resilient individuals are underspecified in the literature to date.

In healthy adolescent and young adult samples, regional gray matter volume development has exhibited trajectories that are sex specific (Vijayakumar et al., 2016). In addition, preclinical models have demonstrated sexual dimorphic CB1 diffusivity in the endocannabinoid system, with greater desensitization of these receptors shown in adolescent female rodents after tetrahydrocannabinol (THC) administration compared to males (Burston, Wiley, Craig, Selley, & Sim-Selley, 2010; Rodriguez de Fonseca, Ramos, Bonnin, & Fernandez-Ruiz, 1993). Furthermore, investigations into effects of sex within humans have indicated differences between CAN-using males and females and their nonusing same-sex counterparts within use patterns (Cuttler, Mischley, & Sexton, 2016; Khan et al., 2013) and neuropsychological performance (Crane, Schuster, Fusar-Poli, & Gonzalez, 2013; Crane, Schuster, Mermelstein, & Gonzalez, 2015). Specifically, male CAN users exhibit impairments on psychomotor and visuospatial performance (Crane et al., 2013) and age of regular CAN onset is associated with poorer episodic memory among female users (Crane et al., 2015). Furthermore, previous studies have demonstrated sex differences in the impact of CAN use on brain structure with female users exhibiting larger right amygdala volume (McQueeny et al., 2011), larger prefrontal volume (Medina et al., 2009), and larger cortical surface structure (Sullivan, Wallace, Wade, Swartz, & Lisdahl, 2020). Notably, most of the reviews examining outcomes indicate a majority of studies either skew or are predominantly male or did not examine sex differences (Crane et al., 2013; Lisdahl et al., 2013; Lisdahl, Wright, Kirchner-Medina, Maple, & Shollenbarger, 2014). Thus, there is an increased need to examine sex as a potential moderator to determine sex-specific associations linked to potential adverse outcomes related to CAN use (Rubino & Parolaro, 2015).

A novel factor to consider in CAN-related investigations is extent of aerobic fitness. Increased levels of aerobic fitness has been robustly related to positive brain outcomes in older adults (Bherer, Erickson, & Liu-Ambrose, 2013; Thomas, Dennis, Bandettini, & Johansen-Berg, 2012), and converging lines of research indicate that fitness is additionally beneficial within healthy adolescents and young adults (Chaddock, Pontifex, Hillman, & Kramer, 2011; Herting & Nagel, 2013; Pereira et al., 2007; Schwarb et al., 2017; Voss, Vivar, Kramer, & van Praag, 2013; Whiteman, Young, Budson, Stern, & Schon, 2016). Its link to regional gray matter volume may be due to a number of mechanisms, including, but not limited to: brain-derived neurotropic factors (BDNFs; Huang, Larsen, Ried-Larsen, Moller, & Andersen, 2014), vascular growth factors (VGFs; Fleenor, Marshall, Durrant, Lesniewski, & Seals, 2010), and neurogenesis (Nokia et al., 2016). As it pertains to CAN use, acute aerobic exercise releases endocannabinoids (Koltyn, Brellenthin, Cook, Sehgal, & Hillard, 2014), and thus it has been theorized this may counteract downregulation of CB1 receptors among exogenous CAN use (Lisdahl et al., 2013). Supporting this notion, CAN users who were more aerobically fit demonstrated superior neuropsychological performance compared to less aerobically fit users (Wade, Wallace, Swartz, & Lisdahl, 2019), and an intervention utilizing aerobic exercise found decreased craving and use among otherwise-sedentary users (Buchowski et al., 2011). Our group previously reported that aerobic fitness was positively related to temporal, parietal, and frontal cortical surface structure in a similar sample of CAN users and controls (Sullivan et al., 2020). Even though this emerging line of research presents fitness as a viable moderator, few studies have incorporated aerobic fitness into assessments of CAN-related consequences on neurocognition and doing so may elucidate aerobic fitness outcomes that potentially put adolescents and young adults at more or less of a risk for adverse effects of CAN use.

Determining the contribution of potential moderators (i.e., sex and aerobic fitness) to brain morphology of CAN users may elucidate subgroups at higher or lower risk for abnormal structural outcomes. Therefore, the aim of the present study is to examine the main effect of CAN use, and novel interactions with sex and aerobic fitness on volume and subcortical volume regions of interest (ROIs). We expect to see aerobic fitness associated with larger brain volume regardless of group, overall CAN group differences, and CAN-by-sex interactions. Exploratory analyses will examine the correlation between significant regions and neurocognitive performance (i.e., working memory, processing speed, and sustained attention) (Lisdahl & Price, 2012; Wade et al., 2019).

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METHODS

Participants

Seventy-four participants (CAN users = 36, controls = 38) were recruited through flyers and advertisements in the local community and college as part of a larger parent study, which examined the neurocognitive effects of CAN use in young adults (R01-DA030354; PI: Lisdahl). Participants in the present analysis were between the ages of 16 and 26 years ($M = 21.1$, $SD = 2.6$), were sex balanced (44.6% female), and racial identities consisted of predominantly: Caucasian (64.9%), Asian (10.8%), multiracial (10.8%), and African-American (8.1%) (see Table 1).

Participants in the parent study were included if they were right handed, spoke English, and were willing to abstain from substance use over a 3-week period. Exclusion criteria included having an independent DSM-IV Axis I (attention, mood, anxiety, or psychotic) disorder, current use of psychoactive medications, major medical or neurological disorders (including metabolic disorders), loss of consciousness >2 min, history of learning disability or intellectual disability, prenatal medical issues or premature birth (gestation <35 weeks), MRI contraindications (pregnancy, claustrophobia, and metal in body), reported significant prenatal alcohol exposure (≥4 drinks in a day or ≥6 drinks in a week), prenatal illicit drug exposure, or prenatal nicotine exposure (average >5 cigarettes per day longer than 1 month), elevated Physical Activity Readiness Questionnaire (Thomas, Reading, & Shephard, 1992) scores screening eligibility for VO_2 maximum (VO_2 max) testing, or excessive other illicit drug use (>20 times of lifetime use for each drug category, including CAN use for nonusing control participants). Based on the International Physical Activity Questionnaire (Fogelholm et al., 2006), participants were additionally balanced based on being active versus inactive to increase likelihood of adequate range in aerobic fitness within both groups.

CAN users in the present analysis were categorized as current users who used CAN at least 44 times in the last year (i.e., nearly weekly) and at least 100 lifetime uses. Nonusing controls in the present analysis used CAN no more than five times in the past year and less than 20 times in their lifetime (Lisdahl & Price, 2012; Wade et al., 2019; Wallace, Wade, Hatcher, & Lisdahl, 2019).

Procedures

All aspects of the protocol were approved by local Institutional Review Board (IRB) and in accordance with the Helsinki Declaration. Potential participants who expressed interest in the parent study were consented and interviewed with a detailed phone screen, along with a parental informant phone interview (explained further in Supplementary Materials).

Eligible participants came in for five study sessions over the course of 3 weeks. The first three sessions occurred 1 week apart and consisted of drug toxicology and a brief neuropsychological battery (for details, see Wallace, Wade, & Lisdahl, 2020). Sessions 4 and 5 occurred at least 1 week after Session 3 and consisted of ascertaining body composition, VO_2 max testing, a full neuropsychological battery, and then an MRI that occurred within 24–48 hr of each other.

During the entire study period, participants were asked to remain abstinent from alcohol, CAN, and other drug use (other than tobacco), which was confirmed through breath, urine, and sweat toxicology screening. If positive for illicit drug use, showed an increase in 11-nor-9-carboxy-THC (THCCOOH) levels, or had a breath alcohol concentration greater than .000 at the start of any subsequent session after baseline, participants were allowed to continue their involvement in the study from Session 1. Participants who used tobacco were asked to abstain from use an hour before the MRI scan to prevent interference with functional task data.

Measures

Past-year substance use

A modified version of the Timeline Follow-Back (TLFB) interviews was conducted by trained research assistants (RAs) to measure substance use patterns on a weekly basis for the past year while providing memory cues such as holidays and personal events (Lisdahl & Price, 2012; Sobell & Sobell, 1992). Substances were measured by standard units [alcohol (standard drinks), nicotine (number of cigarettes; occasions for chew/pipe/cigar/hookah), CAN (smoked/vaped flower, concentrates, edibles were measured, and dosing was converted to joints-based grams), ecstasy (tablets), sedatives (pills), stimulants (mg), hallucinogens (hits), heroin/opium (hits), and inhalants (hits)]. Days of CAN abstinence at scan were calculated from date of last CAN use based on the TLFB and date of scan.

Verifying drug abstinence

As participants were expected to remain abstinent from all alcohol and drugs (other than nicotine) throughout the course of the study, abstinence was evaluated at each session with the following: urine samples were tested using the ACCUTEST SplitCup 10 Panel drug test, which measures amphetamines, barbiturates, benzodiazepines, cocaine, ecstasy, methadone, methamphetamines, opiates, phencyclidine (PCP), and delta-9-THC; in addition, urine samples were tested using NicAlert to test cotinine level—a metabolite of nicotine; participants also wore PharmChek Drugs of Abuse Patches, which continuously monitor sweat toxicology for the presence of cocaine, benzoylecgonine, heroin, 6-Monoacetylmorphine (6MAM), morphine, codeine, amphetamines, methamphetamine, THC, and PCP, and gave quantified values of THCCOOH (a metabolite of THC); and participants underwent breathalyzer screens to test for alcohol use at the start of each session.

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Table 1. Demographic, substance use, and aerobic fitness characteristics

		CAN Users			Nonusing Controls		
	Whole sample	All	Male	Female	All	Male	Female
N	74	36	23	13	38	18	20
M (*SD*) or %							
Age	21.1 (2.6)	21.4 (2.3)	21.4 (2.4)	21.4 (2.0)	20.8 (2.8)	20.5 (3.1)	21.0 (2.6)
Race (% Caucasian)	64.9%	58.3%	65.2%	46.2%	71.1%	72.2%	70.0%
Ethnicity (% Non-Hispanic)	82.4%	77.8%	78.3%	76.9%	86.8%	94.4%	80.0%
Educational attainment	14.0 (2.0)	14.0 (1.6)	13.9 (1.8)	14.1 (1.3)	14.1 (2.4)	14.0 (2.9)	14.2 (1.9)
Past-year alcohol use[a]*	216.4 (270.2)	338.7 (300.8)	376.6 (306.2)	271.6 (290.5)	100.6 (173.6)	141.8 (225.1)	63.5 (101.6)
Past-year tobacco use[a]*	104.7 (351.8)	214.6 (483.7)	311.8 (585.1)	42.8 (68.1)	0.5 (2.0)	0.2 (0.4)	0.7 (2.7)
Cotinine level[b]*	1.6 (1.4)	2.0 (1.8)	2.3 (2.1)	1.5 (1.0)	1.1 (0.6)	1.1 (0.6)	1.1 (0.6)
Past-year CAN use[a]*	208.5 (373.3)	428.2 (440.4)	499.9 (510.7)	301.5 (245.4)	0.36 (1.2)	0.7 (1.6)	0.1 (0.2)
Lifetime CAN use[c]*	579.5 (1122.7)	1189.6 (1372.3)	1419.7 (1621.6)	782.5 (625.0)	1.5 (2.9)	1.2 (2.3)	1.8 (3.5)
Age at regular CAN use onset	–[d]	17.5 (1.7)	17.4 (1.9)	17.8 (1.3)	–[d]	–[d]	–[d]
CAN abstinence length[e]	–[d]	31.1 (22.9)	34.3 (27.9)	25.5 (6.5)	–[d]	–[d]	–[d]
VO_2 maximum (ml/kg/min)[f]	42.5 (9.4)	43.7 (9.0)	47.9 (6.6)	36.1 (7.7)	41.4 (9.8)	47.9 (8.8)	35.5 (6.3)
Body fat	20.4% (9.3)	19.1% (8.5)	15.6% (6.9)	25.3% (7.7)	21.6% (10.0)	13.6% (6.1)	28.7% (7.0)

Note. CAN: Cannabis.
[a] Measured in standard uses on TLFB (Sobell & Sobell, 1992).
[b] Measured at VO_2 maximum testing.
[c] Measured in standard uses on Customary Drinking and Drug Use Record (CDDR) (Brown et al., 1998).
[d] Not applicable.
[e] Calculated from TLFB last CAN use date and date of sMRI.
[f] Maximum rate of oxygen consumption—measured in milliliters of oxygen consumed per kilogram of body weight, per minute.
* $p < .01$.

Neuropsychological battery

Immediately prior to VO_2 max testing, participants were administered a full neuropsychological battery (see Wade et al., 2019, for further information), which included the Paced Auditory Serial Addition Task (PASAT), Ruff 2&7 Selective Attention Task, and Delis-Kaplan Executive Function System (D-KEFS) Trails Making Test-4 (i.e., switching). PASAT total raw scores were used as a measure of processing speed, concentration, and working memory (Diehr, Heaton, Miller, & Grant, 1998). Ruff 2&7 age-corrected total accuracy was used to measure selective and sustained attention (Lezak, Howieson, Loring, & Fischer, 2004; Ruff, Niemann, Allen, Farrow, & Wylie, 1992). Trails switching total time was used as a measure of executive control and working memory (Arbuthnott & Frank, 2010; Lezak et al., 2004; Sanchez-Cubillo et al., 2009).

Body fat percentage

An electrical bioimpedance analysis system was utilized to estimate body fat percentage [The Tanita Body Composition Analyzer, TBF-300 (Tanita Corporation, Tokyo, Japan)] with all pretesting requirements met, which was utilized to compare between-group differences to address attributions of adiposity on results within the present analysis (Schwartz et al., 2014).

VO_2 maximum

Participants were asked to refrain from food and caffeine for 4 hr prior to the exercise test. Exercise testing was completed using a calibrated ParvoMedics TrueOne 2400 metabolic measurement system (ParvoMedics, Salt Lake City, UT). Participants completed an incremental exercise test on a treadmill following the Bruce protocol until volitional fatigue (for full details, see Sullivan et al., 2020; Wade et al., 2019). Criteria for VO_2 max were based on Howley, Bassett, and Welch (1995).

MRI acquisition

Structural MRI (sMRI) scans were acquired on a 3T Signa LX MRI scanner (GE Healthcare, Waukesha, WI) using a 32-channel quadrature transmit/receive head coil. Anatomical images were acquired using a T1-weighted spoiled gradient-recalled at steady-state pulse sequence (TR = 8.2 ms, TE = 3.4 s, TI = 450, and flip angle of 12°). The in-plane resolution of the anatomical images was 256 × 256 with a square field of view of 256 mm. One hundred fifty slices were acquired at 1-mm thickness. This resulted in a 1 mm × 1 mm × 1 mm voxel resolution.

Processing pipeline

Participant structural scans were processed in a standard processing pipeline within FreeSurfer version 5.3 (explained further in Supplementary Materials).

Statistical Analysis

Differences in demographic variables were examined using ANOVAs and Chi-square tests in *R* (R Development Core Team, 2010). A series of multivariate regressions were run on whole-brain regional gray matter volume with CAN group, sex, VO_2 max levels, and their interactions (CAN × Sex and CAN × VO_2 max) as the independent variables of interest[1]; covariates included past-year alcohol and cotinine level at the time of aerobic fitness testing (see Supplementary Materials for results of a power analysis). Analyses were completed separately between each hemisphere (right and left) and smoothed with a global Gaussian blur at full width at half maximum (FWHM) of 15. Corrections for multiple comparisons were made using Monte Carlo simulations at a vertex-wise/cluster-forming threshold of $p < .05$ (i.e., 1.3) and cluster-wise probability (*cwp*) of $p = .05$, while correcting across both hemispheric spaces; no minimum number of voxels required to achieve significant cluster results were set (Greve & Fischl, 2018). Regional effect sizes (ESs) were computed through dividing the residual error standard deviation by the contrast ES for significant effects within the analyses.

A series of linear regressions were run in *R* which examined subcortical volume ROIs (hippocampus, amygdala, cerebellum, caudate, and putamen) with CAN group, sex, VO_2 max levels, and their interactions (CAN × Sex and CAN × VO_2 max) as the independent variables of interest[2]; covariates included past-year alcohol use and cotinine level at the time of aerobic fitness testing. Corrections for multiple comparisons using false discovery rate (FDR) were computed for the series of subcortical volume ROI linear regressions (Benjamini & Hochberg, 1995), both raw p-values and FDR-corrected p-values are reported below.

Follow-up exploratory analyses examined correlations between corrected significant clusters or subcortical ROIs and neuropsychological performance on aforementioned neuropsychological tests. Correlation matrices were computed using Pearson's *r* correlations. Decisions on reporting were made at $p < .05$. Correlations were run separately between users and nonusers for CAN × VO_2 max interactions, and between users and nonusers by sex for CAN × Sex interactions to interpret specific effects. Correlations for each group were compared using two-tailed Fisher's *z* computation (Ramseyer, 2015).

RESULTS

Demographic Data

There were no significant differences between CAN and nonuser groups in regard to age ($p = .27$), sex distribution

[1]One outlier (CAN-using male) was removed from the right hemisphere volume analyses due to an error in processing; this participant is included in all other analyses for purposes of maintaining power.

[2]One outlier (non-using male control) was removed from subcortical analyses due to subcortical values >3 *SD* above the mean, this participant is included in all other analyses for purposes of maintaining power.

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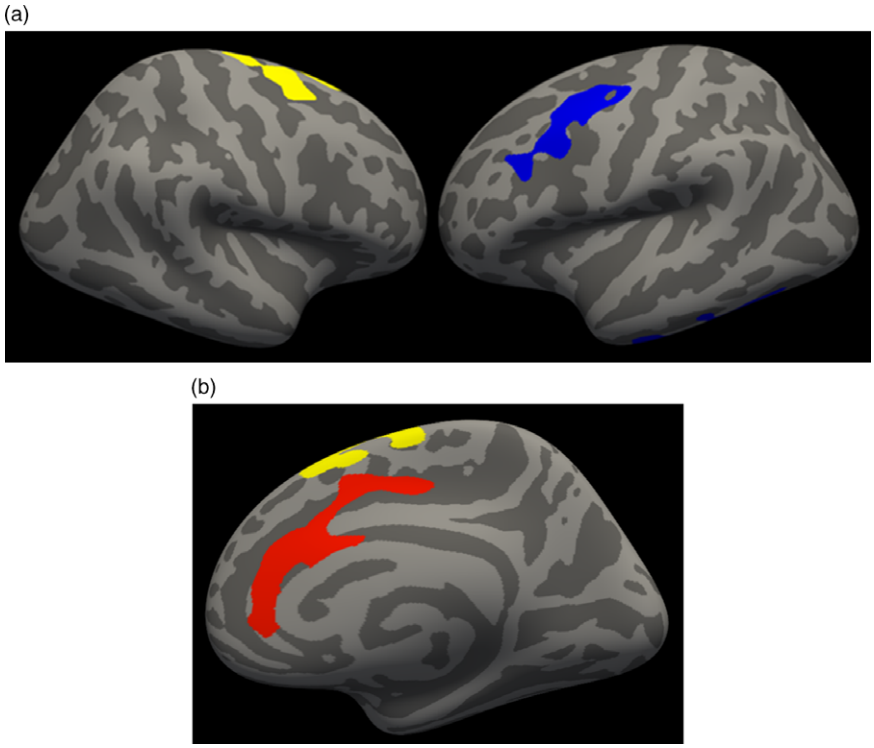

Fig. 1. *Cannabis* × *Sex findings*. Lateral view of CAN group and sex interactions observed in (a) left lateral orbitofrontal, left inferior temporal, left precuneus (not pictured), left caudal middle frontal, right superior frontal, and (b) right paracentral volumes. CAN-using males exhibited less volume compared to nonusing males. Contrarily, CAN-using females demonstrated more volume in aforementioned regions compared to nonusing females, except for left inferior temporal and right superior frontal volume where less volume was observed in CAN-using females compared to nonusing females.

($p = .23$), ethnicity ($p = .26$), race ($p = .44$), educational attainment ($p = .78$), VO_2 max ($p = .30$), and body fat percentage ($p = .27$). As expected, there were significant differences in lifetime ($p < .001$) and past-year CAN use ($p < .001$), past-year tobacco use ($p = .008$), cotinine levels at VO_2 max testing ($p = .003$), and alcohol consumed within the past year ($p < .001$); cotinine levels and alcohol use were included as a covariate in all analyses. Within the CAN users, there was no difference between sexes for past year ($p = .20$) or lifetime ($p = .19$) CAN use, days of CAN abstinence prior to sMRI ($p = .27$), or age of first regular CAN use onset ($p = .55$).

Primary Analyses

CAN findings

There were no significant CAN group findings observed in whole-brain or subcortical volume outcomes.

CAN × Sex findings

Whole-brain volume. Interactions were observed between CAN group and sex in the left lateral orbitofrontal [$t(58) = -3.99$, $ES = -.29$, $cwp = .019$], left inferior temporal [$t(58) = -2.73$, $ES = -.28$, $cwp = .017$], left precuneus [$t(58) = -2.67$, $ES = -.29$, $cwp = .034$], left caudal middle frontal [$t(58) = -2.40$, $ES = -.27$, $cwp = .0003$], right superior frontal [$t(57) = 4.42$, $ES = .30$, $cwp = .001$], and the right paracentral [$t(57) = 3.19$, $ES = .29$, $cwp = .005$] regions (see Figure 1). CAN-using females demonstrated greater volume in left lateral orbitofrontal, left precuneus, left caudal middle frontal, and right paracentral regions compared to nonusing females, whereas CAN-using males had reduced volume in these regions compared to nonusing males. However, in the left inferior temporal and right superior frontal, CAN-using females demonstrated less volume compared to nonusing females, similar to the relationship in males, yet CAN-using males demonstrated the most robust decrease in these regions compared to nonusing males (see Supplementary Table 1 and Supplementary Figure 2). *Subcortical volume.* Significant interactions between CAN group and sex were observed in right amygdala [$t(63) = -2.41$, $p = .019$, FDR = 0.13] and right caudate [$t(63) = -2.04$, $p = .046$, FDR = 0.23] regions. CAN-using males demonstrated smaller right amygdala volume compared to nonusing males, whereas CAN-using females exhibited larger volume in the right amygdala compared to nonusing females. Both male and female CAN users demonstrated less right caudate volume compared to male and female nonusing controls, respectively; yet, this was more robust for females compared to males. However, neither region survived correction for multiple comparisons.

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Table 2. Correlations between volume and neuropsychological performance in regions that differed in primary analyses

		CAN Users		Nonusing Controls		Fisher's *z* Comparison	
Neurocognitive Measure	Identified Region	*r*	*p*	*r*	*p*	*z*	*p*
Whole group							
Ruff 2&7	Left superior temporal[a]	.02	.91	.33*	.046	−1.32	.19
Males							
PASAT	Right paracentral[b]	.43*	.046	.39	.11	0.15	.88
Ruff 2&7	Left lateral orbitofrontal[b]	−.03	.89	.49*	.04	−1.65	.10
	Left inferior temporal[b]	.08	.73	.65*	.003	−2.05*	.04
	Left precuneus[b]	−.27	.22	.60*	.008	−2.83*	.005
	Left caudal middle frontal[b]	.44*	.036	.48*	.046	−0.13	.89
Females							
PASAT	Left inferior temporal[c]	.65*	.016	−.01	.95	1.99*	.046
Trails 4	Left precuneus[d]	−.68*	.011	−.52*	.02	−0.63	.53
	Right superior frontal[c]	.54	.057	−.19	.42	2.00*	.046

Notes. Fisher's *Z* comparisons were run to determine whether correlation coefficients significantly differed between CAN and controls. CAN: Cannabis users; PASAT: Paced Auditory Serial Addition Task.
[a] Region identified from Cannabis × VO_2 analysis; controls demonstrated a more robust positive relationship between VO_2 max and volume.
[b] Regions identified from Cannabis × Sex analyses where CAN-using males had smaller volume compared to nonusing males.
[c] Regions identified from Cannabis × Sex analyses where CAN-using females had smaller volume compared to non-using females.
[d] Regions identified from Cannabis × Sex analyses where CAN-using females had greater volume compared to nonusing females.
*$p < .05$.

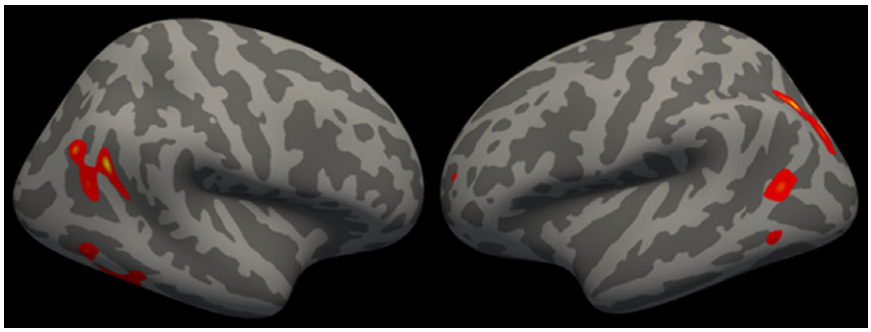

Fig. 2. *VO_2 findings.* Lateral view of VO_2 findings observed in left inferior parietal, left rostral middle frontal, right inferior parietal, right fusiform, and right precuneus (not pictured) volumes. Increased VO_2 was positively associated with more volume in these regions.

VO_2 findings

Whole-brain volume. A significant relationship between increased VO_2 max and larger volume was observed in two separate areas of the left inferior parietal [$t(58) = 5.21$, $ES = .37$, $cwp = .0001$; $t(58) = 4.31$, $ES = .32$, $cwp = .0001$], left rostral middle frontal [$t(58) = 2.89$, $ES = .27$, $cwp = .039$], right inferior parietal [$t(57) = 3.40$, $ES = .30$, $cwp = .035$], right fusiform [$t(57) = 3.11$, $ES = .29$, $cwp = .001$], and right precuneus [$t(57) = 3.06$, $ES = .29$, $cwp = .02$] regions (see Figure 2) (see Supplementary Table 1). *Subcortical volume.* There was a significant relationships between increased VO_2 max and larger volume in left [$t(63) = 3.04$, $p = .003$, FDR = 0.03] and right [$t(63) = 2.84$, $p = .006$, FDR = 0.046] caudate, and in left [$t(63) = 3.01$, $p = .004$, FDR = 0.03] and right [$t(63) = 2.48$, $p = .016$, FDR = 0.11] cerebellum, though the finding in the right cerebellum did not survive corrections.

CAN × VO_2 findings

Whole-brain volume. A significant interaction was observed between VO_2 max and CAN group in the left superior temporal region [$t(58) = -3.58$, $ES = -.30$, $cwp = .0001$] (see Supplementary Table 1). Nonusing controls demonstrated a positive relationship between increased VO_2 max and more volume, whereas no trend was observed for the CAN group (see Supplementary Figure 1). *Subcortical volume.* There were no VO_2 max-by-CAN group interactions observed for subcortical volume.

Exploratory Brain–Behavior Correlations

Correlations in VO_2 associated regions are located in Supplementary Materials. See Table 2 for correlation coefficients between brain volume and cognitive tasks in regions that differed according to CAN × VO_2 or CAN × Sex

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interactions. Fisher's z scores were calculated to determine whether correlation coefficients significantly differed by CAN group status for the males and females in the sample.

DISCUSSION

Given ongoing policy debates (Carliner, Brown, Sarvet, & Hasin, 2017) and prevalence of use in adolescents and young adults (Johnston et al., 2020), further characterizing brain structure as it relates to regular CAN use in this population is of continued importance. Yet, findings from the current literature are largely heterogeneous and there is a call to examine potential influencers (Lorenzetti et al., 2019). The current study sought to further elucidate this relationship by investigating two potentially moderating factors—sex and aerobic fitness—on the associations between CAN group and brain volume in a group of healthy adolescents and young adults who underwent 3 weeks of monitored abstinence. There were no main effects of CAN group on volume after accounting for sex, aerobic fitness, past-year alcohol use, and current nicotine use. However, CAN-by-sex interactions were observed in frontal, temporal, paracentral, and precuneus volumes. Males demonstrated smaller volumes, whereas female users generally had larger volumes compared to their nonusing same-sex counterparts. Exploratory and preliminary brain–behavior analyses largely demonstrated that the pattern of volume findings in both male and female CAN users was linked with disadvantageous neuropsychological performance. Whole-sample findings with aerobic fitness were diffusely observed with increased cortical volume; and furthermore, a CAN-by-aerobic fitness interaction was demonstrated in left superior temporal volume, with nonusers showing a positive association, whereas no relationship was observed for CAN users. Overall, aerobic fitness was linked with greater brain volume and was in turn associated with superior neuropsychological performance.

Notably, we did not find any main effects of CAN on volume in the present study. This is inconsistent with prior studies, which have demonstrated differences between CAN users and nonusers in several regions (Ashtari et al., 2011; Lisdahl et al., 2016; Maple et al., 2019; Matochik, Eldreth, Cadet, & Bolla, 2005; Medina et al., 2010; Medina, Schweinsburg, Cohen-Zion, Nagel, & Tapert, 2007; Price et al., 2015). The present null main-effect findings could be due to novel sampling of balanced aerobically fit and unfit CAN users, varying frequency of use to determine inclusion criteria, or due to the longer-than-average length of abstinence (3 weeks) our sample maintained (Batalla et al., 2013; Lisdahl et al., 2013; Scott et al., 2018), but nonetheless, lend further evidence to the overall heterogeneous aberrant volumetric findings in CAN users and potentially influential effect of moderators (Lorenzetti et al., 2019).

To that end, the present analyses revealed several CAN-by-sex interactions in left frontal, temporal, and precuneus volumes, and right frontal volume. It may be that null main-effect findings could be due to accounting for these significant interactions that are demonstrating opposing effects in several regions. For example, CAN-using males demonstrated smaller volume compared to nonusing males and CAN-using females generally showcased larger volume compared to nonusing females in left lateral orbitofrontal, left precuneus, left caudal middle frontal, and right paracentral regions. Yet, in the left inferior temporal and right superior frontal volume, both CAN-using males and females exhibited smaller volume compared to their nonusing same-sex counterparts; however, this difference was starker in males. This general trend in findings aligns with previous literature showcasing larger amygdala volume in CAN-using females (McQueeny et al., 2011) and CAN-by-sex interactions in prefrontal volume (Medina et al., 2009) in a somewhat younger cohort who also underwent 30 days of monitored abstinence.

In follow-up preliminary analyses to understand brain–behavior relationships in the male and female CAN users and nonusers, we found an overall pattern in the male CAN users that linked smaller volume in right paracentral and left caudal middle frontal regions with poorer sustained attention. Controls also demonstrated significantly more robust correlations between smaller volumes and poorer neuropsychological performance compared to male CAN users in left inferior temporal and left precuneus regions. Thus, consistent with prior studies, we again found that smaller volume in conjunction with CAN use is disadvantageously linked to neuropsychological function in males (Medina et al., 2009; Price et al., 2015). Among female CAN users, larger volumes in the left precuneus and smaller volumes in the right superior frontal region were correlated with worse sequencing and processing speed performance, whereas smaller volume in the left inferior temporal lobe was linked with poorer sustained attention. This pattern generally suggested that abnormal volumes observed in female CAN users compared to female nonusing controls were disadvantageous. Interestingly, female CAN users had more robust correlations between volume and neuropsychological performance in left inferior temporal and right superior frontal regions. However, it is notable that the smallest sample size was of female CAN users; thus, these findings need replication in larger samples as the magnitude of brain–behavior relationships is potentially smaller than previously recognized (Palmer et al., 2020). Importantly, previous research has demonstrated neuropsychological differences prior to CAN initiation, which represent a risk for use (Cheetham et al., 2012; Jackson et al., 2016; Tervo-Clemmens, Quach, Calabro, Foran, & Luna, 2020), and thus the present findings—particularly the preliminary brain–behavior relationships—are noted as associations rather than causal relationships. Even so, present findings suggest negative links with cognition associated with aberrant brain volume morphology between CAN -using and nonusing groups, which require further replication in large-scale studies.

More broadly, CAN-by-sex findings may be due to several factors. Sex-specific pruning patterns may be impacted by introducing exogenous CAN exposure into staggered developmental trajectories (Medina et al., 2009; Rubino & Parolaro,

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2015), which temporally differ between the sexes (Giedd et al., 1999; Lenroot et al., 2007). Furthermore, increased CB1 receptor density in males compared to females has also been observed in preclinical models (Burston et al., 2010; Rubino et al., 2008). In addition, male CAN users tend to use more frequently, severely, and with higher potency products (Cuttler et al., 2016), potentially contributing to a more consistent picture of reduced brain volume and cognitive deficits (Lisdahl & Price, 2012).

Investigating the associations between aerobic fitness and brain morphometry revealed robust positive associations between superior aerobic fitness and larger volume in bilateral inferior parietal, left rostral middle frontal, right fusiform, and right precuneus regions—regardless of CAN group status. In our Supplementary Material, we demonstrated a pattern of significant preliminary positive correlations between brain volume and cognitive functioning. Chiefly, these findings are supported by previous literature examining the relationship between increased aerobic fitness levels and brain morphometry (Herting & Chu, 2017; Herting & Keenan, 2017; Wittfeld et al., 2020) and cognitive function, particularly on sustained attention and psychomotor speed tasks in young adults (Hwang, Castelli, & Gonzalez-Lima, 2017; Lee et al., 2014; Wade et al., 2019). Moreover, associations were observed between superior aerobic fitness and larger left cerebellar and bilateral caudate volumes, which are consistent with previous findings demonstrating a positive link between physical exercise and subcortical volume in children (Ortega et al., 2019) and adults (Wittfeld et al., 2020). An interesting finding was an interaction between aerobic fitness and CAN group in left superior temporal volume, where nonusing controls exhibited a robust positive association and no trend was observed for CAN users. Left superior temporal volume was also positively related to sustained attention in nonusers, but not CAN users. Intriguingly, this region has previously been identified as a benefactor to increased aerobic fitness in healthy adults (Wittfeld et al., 2020); although present findings indicate this relationship may not be as evident for young adult CAN users, suggesting that CAN use may disrupt this benefit. Further, this is consistent with our prior study findings that CAN users did not have as robust of a relationship between fitness and cortical surface structure in cuneus and occipital regions (Sullivan et al., 2020). Taken together, this may suggest CAN users demonstrate gains in neurocognitive indices following aerobic activity; yet, these gains may not be as apparent in some brain regions; however, this needs to be confirmed in a clinical trial design.

Still, it is notable that regional links between aerobic fitness and brain volume exist across participants—regardless of CAN group membership—while accounting for sex, alcohol use, and cotinine level in a physically healthy cohort of adolescents and young adults. This represents a novel finding in the aerobic fitness literature. One possible mechanism underlying these findings is that recent studies have revealed that aerobic exercise releases endocannabinoids (Heyman et al., 2012; Hillard, 2018; Meyer, Crombie, Cook, Hillard, & Koltyn, 2019; Watkins, 2018). This may lessen the negative impact of repeated and regular exogenous CAN exposure in youth. Another proposed explanation for aerobic fitness main effects on brain structure in CAN users is that physical activity may metabolize exogenous cannabinoids out of the body at a faster rate, which has been previously examined experimentally (Westin, Mjones, Burchardt, Fuskevag, & Slordal, 2014; Wong et al., 2013); hence, reducing the time cannabinoids cycle through the body and perhaps diminishing their overall impact on brain morphometry. As aforementioned, engaging in aerobic exercise has been additionally linked with increased BDNF release (Huang et al., 2014), VGF (Fleenor et al., 2010), and neurogenesis (Nokia et al., 2016). Moreover, these structural findings add to our previous research in our lab that found superior performance on visual memory, psychomotor speed, and sequencing ability in more aerobically fit CAN users compared to nonfit users (Wade et al., 2019). Taken together, these findings suggest that aerobic fitness may be a moderating factor between CAN exposure and neurocognitive health, and this could be harnessed in prevention and treatment efforts. Future studies are needed to help elucidate potential underlying mechanisms explaining the relationship between aerobic fitness, brain structure, and neurocognition in CAN-using youth. Furthermore, understanding which types of physical activity (e.g., muscle strength, balance, and resistance training) influences fitness and, potentially, substance use and brain–behavior relationships may be an important future direction.

It is worth noting potential limitations of the present study. Causality cannot be determined from the present sample due to CAN use initiation occurring prior to study protocols. Moreover, although attempts were made to balance the sample according to active and sedentary individuals, the VO_2 max of our sample was lower than average age-based norms (Pescatello, 2014). Assessing a more representative sample of the population (i.e., a more aerobically fit group) may demonstrate stronger ameliorative associations between aerobic fitness and brain structure in CAN users. Although we did find sex differences, the smallest cell in the present analysis was the CAN-using females ($n = 13$), which limits our power; we expect that a larger sample size altogether could reveal more robust findings across and within sexes. In addition, there are other influential factors on the relationship between CAN use and brain morphometry, including, genetics (Filbey, Schacht, Myers, Chavez, & Hutchison, 2010; Shollenbarger, Price, Wieser, & Lisdahl, 2015; Verweij et al., 2010; Zinkstok et al., 2006) and psychopathological comorbidities (Crippa et al., 2009; Lev-Ran et al., 2014). The present study did not have the capacity to account for potentially influential effects of genetics and excluded for major Axis I disorder. Furthermore, prenatal substance use was measured through parental self-report, which may minimize reporting of use. Future investigations should prioritize specific characteristics of CAN use, including, but not limited to, age of first regular onset, severity of use, or CAN potency. In addition, despite CAN metabolites (i.e., THCCOOH) cycling out within a 3- to 4-week

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period (Goodwin et al., 2008), future studies are needed to determine whether subtle abnormalities would recover with longer periods of sustained abstinence.

The current analysis found that after 3 weeks of monitored abstinence, sex moderated the relationship between CAN use and brain volume. In CAN-using males, smaller volumes were observed in lateral orbitofrontal, superior frontal, caudal middle frontal, inferior temporal, precuneus, and paracentral volumes compared to nonusing males. CAN-using females generally exhibited larger volume in these areas compared to nonusing females, except for in the left inferior temporal and right superior frontal, where they also demonstrated smaller volumes. Preliminary brain–behavior correlations generally indicate that abnormal volumes were not advantageous in either the male or female CAN users. We also found robust associations between aerobic fitness and greater inferior parietal, rostral middle frontal, inferior parietal, fusiform, precuneus, cerebellum, and caudate volumes in both CAN users and nonusers. Greater volume in these regions was linked with superior neuropsychological performance. These findings, coupled with existing literature, suggest that aerobic interventions may be a potential low-cost ameliorative tool in the recovery of chronic and repeated CAN use. Taken together, we found that sex and aerobic fitness may be factors that help explain heterogeneity in findings and future studies examining the impact of CAN use on brain volume need to consider these significant factors. Additional prospective and longitudinal studies, such as the ABCD Study® (Lisdahl et al., 2018), are needed to confirm causality and replicate findings.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/S135561772100062X

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CONFLICTS OF INTEREST

There are no conflicts of interests to report.

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