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ORIGINAL ARTICLE

Associations Between Microbiota, Mitochondrial Function, and Cognition in Chronic Marijuana Users

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Abstract Marijuana (MJ) use is associated with cognitive deficits. Both mitochondrial (mt) dysfunction and gut dysbiosis also affect cognition. We examined whether cognition is related to peripheral blood mononuclear cells' (PBMCs) mt function and fecal microbiota in chronic MJ users. Nineteen chronic MJ users and 20 non-users were evaluated using the Cognition Battery in NIH Toolbox, their mt function for ATP production, and basal and maximal respirations were measured in PBMCs using the Seahorse XFe96 Analyzer, and the abundances of *Prevotella* and *Bacteroides* (associated with plant-based and animal product-based diet, respectively) were calculated from stool microbiota analysis. Average *Prevotella*:*Bacteroides* ratio was ~13-fold higher in nonusers than users. Lifetime MJ use correlated inversely with *Prevotella*:*Bacteroides* ratio (p = 0.05), mt function (p = 0.0027–0.0057), and Flanker Inhibitory Control and Attention (p = 0.041). *Prevotella* abundance correlated positively, while *Bacteroides* abundance correlated inversely, with mt function across all participants (p = 0.0004–0.06). *Prevotella* abundance also correlated positively with scores of Fluid Cognition, Flanker Inhibitory Control and Attention, List Sorting, and Dimension Change Card Sort in MJ users, but not in non-users (interaction-p = 0.018–0.05). Similarly, mt function correlated positively with scores of Fluid Cognition and Flanker Inhibitory Control and Attention in MJ users, but not in non-users (interaction-p = 0.0018–0.08). These preliminary findings suggest that MJ use is associated with alterations of gut microbiota and mt function, which may further contribute to cognitive deficits. We posited that MJ-associated low vegetable/fruit intake may contribute to these changes. Future studies are needed to delineate the relationships among diet, microbiota, mt function, and cognition in MJ users.

Keywords Marijuana · Mitochondria · Microbiota · Cognition · Diet · *Prevotella* · *Bacteroides*

Introduction

More than 180 million people use marijuana (MJ) worldwide (UNODC 2016). In the United States, legalized MJ production has become the fastest-growing industry (Ferner 2015), which is likely contributing to the continued rise in MJ use (NIDA 2014; SAMHSA 2014). Multiple studies have shown that both acute and chronic MJ use are associated with deficits in memory, attention, and some executive functions (Broyd et al. 2016). Recently, cannabinoid CB1 receptor was found in neuronal mitochondria (mt) membrane, where it mediated the inhibitory effect of Δ^9-tetrahydrocannabinol (THC) on mt respiration and energy production (Benard et al. 2012). Animal and *in vitro* models also showed that exposure to MJ smoke, THC, and cannabidiol (CBD) decreased mt membrane potential, cellular ATP level, and cell viability (Sarafian et al. 2005; Sarafian et al. 2006; Mato et al. 2010; Shrivastava et al. 2011). Furthermore, oxidative damages to nuclear and mt DNAs were found in postmortem brains of individuals with mild cognitive impairment (Wang et al. 2006), and mt structural decay were shown in the hippocampi of old rats (Liu

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et al. 2002). However, whether chronic MJ is associated with changes in mt function in human is unknown.

Due to their prokaryotic origins (Degli Esposti et al. 2014), mt share some common structure and function with bacteria, and microbiota can interact with host cells through regulating mt biogenesis (Saint-Georges-Chaumet and Edeas 2016). Gut dysbiosis is associated with both mt dysfunction and neurological disorders (Moos et al. 2016). Among the hundreds of genera of human microbiota, *Prevotella* and *Bacteroides* are the main determinants of human enterotypes (Arumugam et al. 2011), and their abundances are associated with long-term dietary patterns, with *Prevotella* enriched by plant-based diet and *Bacteroides* by animal product-based diet (Wu et al. 2011). Several studies found that people who used MJ tended to have lower intake of fruits and vegetables, but higher intake of fat and animal products (Farrow et al. 1987; Smit and Crespo 2001; Arcan et al. 2011; Hahn et al. 2014). Therefore, chronic MJ use may be associated with altered abundances of *Prevotella* and *Bacteroides*, which in turn may affect systemic mt function and cognition.

We carried out a pilot study in a small cohort of subjects as a first step to examine the possible relationships among microbiota composition, mt respiration, and cognitive function in the context of chronic MJ use. We recruited chronic MJ users and age- and sex-matched controls, and assessed their cognitive function using NIH Tolbox®, systemic mt oxygen consumption by measuring ATP production and basal and maximal respiration rates in PBMCs, and the abundances of *Prevotella* and *Bacteroides* from stool microbiota analysis.

Participant Recruitment Criteria, Materials and Methods

Participant Criteria

Thirty-nine participants (19 MJ users and 20 nonusers) were recruited from the local community and enrolled in the study after they met the study criteria. Both men and women were included in the study, if they were older than 18 years of age and able to provide written informed consent. Chronic MJ-users were also required to have used MJ for at least 3 times per week for at least 3 years, and had urine toxicology positive for THC but negative for cocaine, ampheatmines, barbiturates, opiates, and benzodiazepine. Nonusers were required to have <10 times lifetime MJ use, and the last MJ use was at least 6 months prior to the enrollment; they were also required to have negative urine toxicology screen for THC, cocaine, amphetamines, barbiturates, benzodiazepines, and opiates. Recreational alcohol and cigarette use was allowed for all subjects. All participants with any confounding conditions (including major psychiatric illnesses, significant head trauma, severe chronic medical disorders, and current or history of other moderate to severe substance use disorders) were excluded. The study protocol was approved by the Human Studies Program of the University of Hawaii.

Study Visits

During the first visit, after informed consents were obtained and signed, the participants were evaluated by a physician, which included a standardized physical and neuropsychiatric evaluation, detailed substance use and medical history, and urine toxicology screen. Those who met the study criteria were sent home with a stool collection kit (ALPCO, Salem, NH), and were asked to collect a stool sample within 24 h of the second visit, typically within 2 weeks of the first visit, and to keep the sample refrigerated before delivery. The stool samples were stored in a − 80°C freezer upon receipt. All participants were asked to fast for 12 h before the second visit, and MJ users were also asked to not use MJ within 12 h of the visit to avoid acute MJ effect on the neuropsychological tests. All participants received a second urine toxicology test, and blood samples were collected through venipuncture only in those who had a negative urine toxicology test (positive THC result was allowed for MJ users). After the blood draw, the participants were provided with refreshments, and allowed to rest for 15 min before taking a battery of neuropsychological tests using the computer-based NIH Toolbox®.

Neuropsychological Tests

Five tests were selected from the Cognition Battery of the NIH Toolbox® for this study: 1) the Flanker Task measured both inhibitory control and attention; 2) the List Sorting Task assessed working memory; 3) the Dimensional Change Card Sort Test evaluated cognitive flexibility; 4) the Picture Sequence Memory Test assessed episodic memory; 5) the Pattern Comparison Test measured speed of processing. The Fluid Cognition Composite score was calculated by NIH Toolbox® based on the scores of these 5 tests (Heaton et al. 2014).

PBMC Preparation

PBMCs were prepared from whole blood within 1 h of blood draw. Briefly, blood was collected in EDTA tubes and PBMCs were isolated over a Ficoll-Paque and washed three times with phosphate-buffered saline per IMPAACT/ACTG protocol (HANC 2014). Cells were viably cryopreserved at a concentration of 1.0–1.5 million cells per 1.5 mL aliquot. The cryopreservation medium contained 10% (*v*/v) DMSO and 90% heat inactivated FBS. The aliquots were collected in a Mr. Frosty™ freezer container before transferred into a − 80°C freezer, and the cells were frozen at a speed of −1°C per

min. The frozen aliquots were transferred to liquid nitrogen for storage within a few days.

PBMC Mt Respiration Measurements

PBMC viability was determined using acridine orange/propidium iodide staining. PBMCs were seeded at a density of $5.0e^5$ live cells per well in duplicate on cell culture plates treated with poly-L-lysine. PBMCs' mt oxygen consumption rate (OCR) was assessed using the Mito Stress test and Seahorse XFe96 (Agilent Technologies, Santa Clara, CA), which uses high-throughput oximetry to simultaneously measure OCR and extracellular acidification (ECAR) rates as we have described (Takemoto et al. 2017).

Microbiota Study

Stool samples were sent to the Genetics Core Facility at the Hawaii Institute of Marine Biology, University of Hawaii at Manoa (Honolulu, HI) for microbiome study. The 16 s rRNA was sequenced using Ilumina GAIIX sequencing platform, amplicon libraries were generated according to Illumina TruSeq protocols. The relative abundances of *Prevotella* and *Bacteroides* were calculated as the ratio of the number of reads of the target genus over the total number of reads of all genera identified in the sample.

Statistical Analyses

Univariate analyses were conducted to compare variables between users and nonuser groups. For continues variables, Student's t-test (i.e., for normally distributed outcomes), or Wilcoxon rank-sum test (i.e., for non-normally distributed outcomes) were used appropriately, whereas Chi-squared test was used for categorical variables. Analysis of covariance (ANCOVA) based on linear models were conducted to evaluate additive main effects and interaction effects (i.e., with user/nonuser groups) of a set of covariates on an outcome variable of interest. For covariate that showed a significant group interaction on the response variable based on ANCOVA analysis, group specific correlation analysis was performed and visualized the association using interaction plots. The Spearman's correlation coefficient (r) was used to quantify the strength of linear associations. A p-value (p) ≤ 0.05 was considered as statistically significant, while a larger p-value, ranging between 0.05 and 0.1 was considered as borderline significant. All the statistical analyses were performed using SAS version 9.3 (SAS institute Inc., Cary, NC, USA).

The composite score of fluid cognition was used as the primary measurement of NPT, and the five subdomains of the composite score (see Methods) were considered as secondary measurements of NPT. ATP production was used as the primary measurement of mt function, and basal and maximal respiration rates were considered as the secondary measurements of mt function. Due to the wide dispersion of the Prevotella percentage, and length of MJ use, log-transformed values of the corresponding variables were used in analyses.

Results

Participant Characteristics

Table 1 shows that race, ethnicity, and sex frequency distributions between two groups did not differ significantly. Besides, means of age, education, and BMI (further corroborated with waist/hip ratio and neck circumference) were not significantly different. All MJ users smoked MJ daily (7 days/week). Compared with non-users, MJ users were more likely to have used alcohol in the past-month (+30%, $p = 0.035$), had first use of alcohol and tobacco at an earlier age (alcohol: −3.3 years, $p = 0.043$; tobacco: −14.6 years, $p = 0.0002$), and had longer period of tobacco use (+4 years, $p = 0.036$). However, the lifetime alcohol and tobacco use are not different between MJ users and non-users.

Table 2 shows that as a group, all MJ users had similar cognitive function as the non-users. However, compared with non-users, MJ users had non-significantly higher (+20–30%) mt ATP production, and basal and maximal respiration rates.

Prevotella Abundance Correlated with Cognitive Function in MJ Users

The abundance of log-transformed % *Prevotella* and the abundance of *Bacteroides* correlated inversely across all participants ($r = -0.38$, $p = 0.012$, Fig. 1a). As expected, the ratio of *Prevotella*:*Bacteroides* was approximately 13-fold higher in the nonusers than in the users ($p = 0.34$, Wilcoxon rank-sum test, data not shown). Greater lifetime MJ use (log transformed) tended to associate with lower ratio of *Prevotella*:*Bacteroides* ($r = -0.45$, $p = 0.052$, Fig. 1b).

Amongst MJ users, the abundance of Log-*Prevotella*% correlated positively with the primary cognitive measurement, i.e. the composite score of fluid cognition ($r = 0.51$, $p = 0.03$, Fig. 1**c**). We then further examined the 5 subdomains of the composite score, and found that three of the subdomains also correlated positively with the abundance of *Prevotella*, including the flanker inhibitory control and attention score ($r = 0.46$, $p = 0.047$, Fig. 1d), list sorting working memory score ($r = 0.57$, p = 0.01, Fig. 1e), and dimension change card sort score ($r = 0.53$, $p = 0.02$, Fig. 1f). However, such correlations were not found among nonusers, leading to the group-by-*Prevotella* abundance interactions (interaction-$p = 0.018$–0.05, Fig. 1c-f). Variables that showed a difference in group characteristics (Table-1) such as ages of first alcohol or

Table 1 Participant characteristics

	Marijuana Users (n = 19)	Non-Users (n = 20)	p value (Chi-square or t-test)
Age (years)	28.0 (22.0–32.0)	27.0 (21.5–36.0)	0.94
Sex (Female/Male)	7/12	7/13	0.90
% Race (Asian/ Black / Mixed / Pacific Islander/ White)	10.5 / 0 / 26.3 / 10.5 / 52.6	30 / 0 / 20 / 0 / 50	0.25
% Ethnicity (Hispanic / Non-Hispanic)	26.3 / 73.7	20 / 80	0.64
Education (years)	15.0 (12.0–16.0)	14.8 (14.0–16.5)	0.29
Body Mass Index (kg/m^2)	23.6 (21.7–27.9)	26.3 (23.1–29.7)	0.42
Waist/Hip circumference ratio	0.85 (0.82–0.88)	0.86 (0.82–0.92)	0.88
Neck circumference (cm)	36.5 (33.5–38.0)	36.5 (34.5–39.0)	0.67
Substance Use Patterns			
Marijuana (MJ) Use			
# used MJ in past month (%)	19/19 (100%)		
Age of first MJ use (year)	16.0 (15.0–19.0)		
Daily average MJ used (g)	1.0 (0.5–2.0)		
Duration of MJ use (year)	7.5 (4.0–11.0)		
Total lifetime MJ used (kg)	2.7 (1.5–4.9)		
Alcohol Use			
# used alcohol in past month (%)	17/19 (90%)	12/20 (60%)	**0.035**
Age of first alcohol use (year)	18.5 (16.0–22.0)	21.0 (20.0–21.0)	**0.043**
Daily average alcohol used (ml)	7.7 (2.4–13.1)	1.6 (0.4–7.3)	0.061
Duration of alcohol use (year)	9.0 (4.0–11.0)	2.0 (1.0–8.0)	0.091
Alcohol use abstinence (day)	4.0 (1.0–9.0)	14.0 (4.0–30.0)	0.14
Lifetime alcohol used (L)	20.0 (9.8–33.3)	0.6 (0.2–20.1)	0.074
Tobacco Use			
# used tobacco in past month (%)	3/19 (16%)	3/20 (15%)	0.38
Age of first tobacco use (year)	16.0 (15.0–18.0)	29.0 (26.0–36.0)	**0.0002**
Daily average nicotine smoked (mg)	0 (0–68.2)	0 (0–18.0)	0.98
Duration of tobacco use (year)	0 (0–12.0)	0 (0–1.0)	**0.036**
Nicotine abstinence (day)	7.0 (0–730.0)	11.0 (0–62.0)	0.86
Lifetime nicotine used (g)	0 (0–196.6)	0 (0–39.3)	0.55

Data are shown in number, %, or Median (Interquartile Range)

Bold font shows statistical significance

tobacco use, % past month alcohol use, and duration of tobacco use did not show significant main effects on primary and secondary outcomes when examined by ANCOVA analysis. No significant correlations were found between any of the NPT scores and the abundance of *Bacteroides*.

Mt Oxygen Consumption Correlated with *Prevotella* Abundance and Lifetime MJ Use

Log-*Prevotella*% correlated positively with the primary measurement of mt function, i.e. ATP production across all participants (r = 0.46, p = 0.016, Fig. 2a). ANOVA analysis showed that there is a significant group (p = 0.029) and Log-*Prevotella*% (p = 0.0011) main effects, and also a group-by-Log-*Prevotella*% interaction on the ATP Production (p = 0.016). For the observed range of Log-*Prevotella*% (i.e., approximately −3 to 2), ATP production of MJ users remains higher than nonusers, given a Log-*Prevotella*% value (Fig. 2a). In contrast, regardless of MJ status, participants who had higher abundance of *Bacteroides* had lower level of mt ATP production (r = −0.40, p = 0.039, Fig. 2b). Similar to ATP production, mt basal respiration rate also correlated positively with *Prevotella* abundance (r = 0.47, p = 0.012, Fig. 2c) and inversely with *Bacteroides* abundance (r = −0.37, p = 0.06, Fig. 2d). Meanwhile, mt maximal respiration rate correlated with the abundance of Log-*Prevotella*% (r = 0.54, p = 0.035, data not shown), but not with that of *Bacteroides*.

Greater Log lifetime MJ use was associated with lower mt ATP production, (r = −0.72, p = 0.0057, Fig. 2e). Notably, among MJ users with relatively lower lifetime MJ use (i.e., < 3 kg), their mt ATP production was higher than the mean value of nonusers, while heavier MJ users had lower mt ATP production than the mean value of nonusers (Fig. 2e). Similarly, both basal respiration (r = −0.76, p = 0.0027) and

Table 2 Cognitive function and mitochondrial respiration in peripheral blood mononuclear cells (PBMC) of marijuana (MJ) users and non-users

Cognitive Function			
	MJ Users (n = 19)	Non-Users (n = 20)	p value (t-test)
Flanker Task	85.5 (79.7–91.9)	83.0 (80.6–87.1)	0.71
List Sorting Working Memory Test	105.2 (95.1–112.0)	110.8 (97.1–116.0)	0.70
Dimensional Card Sort Test	91.2 (85.7–95.8)	89.7 (82.8–93.2)	0.27
Picture Sequence Memory Test	99.7 (88.6–112.8)	99.9 (86.0–114.6)	0.86
Pattern Comparison Processing Speed	89.5 (79.8–101.0)	97.3 (78.2–105.1)	0.57
Fluid Cognition Composite Score	91.9 (79.7–99.7)	88.9 (78.9–99.0)	0.56
PBMC mt oxygen consumption rate (OCR)			
	MJ Users (n = 13)	Non-Users (n = 13)	p value (t-test)
ATP production (pMol/min)	93.8 (67.8–115.2)	65.0 (58.3–97.6)	0.19
Basal respiration rate (pMol/min)	92.6 (70.6–123.5)	80.6 (56.6–105.2)	0.23
Maximal respiration rate (pMol/min)	82.0 (51.3–149.1)	64.3 (34.0–125.8)	0.29

Data are shown in Median (Interquartile Range)

maximal respiration (r = −0.72, p = 0.0054) correlated inversely with Log lifetime MJ use (data not shown). Note that mt ATP production, basal and maximal respiration rates correlated closely with each other; a representative correlation between two of these variables is shown (Fig. 2f).

Mt Respiration Correlated with Cognitive Function in MJ Users

We further analyzed the relationship between mt function and cognitive scores. Fluid cognition composite score correlated positively with all three measurements of mt oxygen consumption (ATP production, r = 0.62, p = 0.019; basal respiration, r = 0.73, p = 0.003; maximal respiration, r = 0.54, p = 0.048) in MJ users, but not in nonusers (interaction-p = 0.0031 to 0.047, Fig. 3a-c). Among the five cognitive subdomains, flanker inhibitory control and attention score correlated positively with mt ATP production (r = 0.70, p = 0.0054), basal respiration (r = 0.61, p = 0.019), and max respiration (r = 0.55, p = 0.043) for MJ users, but not for nonusers, with significant group-by-mitochondrial function interactions for ATP production (interaction-p = 0.0018) and

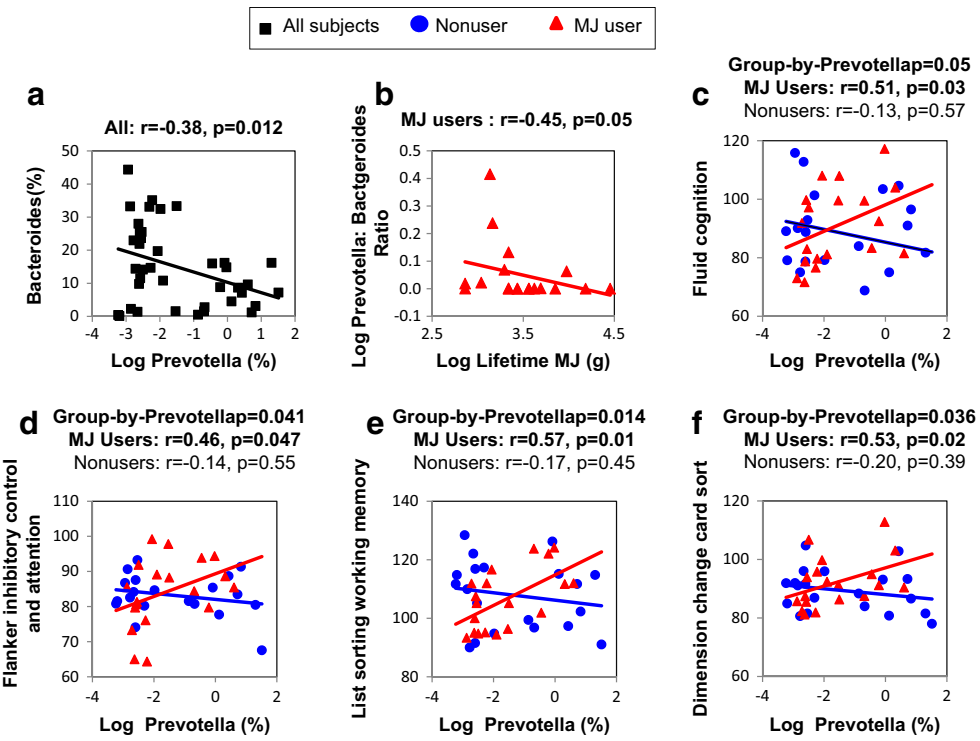

Fig. 1 Correlations between gut microbiota, lifetime MJ use, and cognitive function. **a** Inverse correlation between *Prevotella* abundance and *Bacteroides* abundance in fecal microbiota in all participants. **b** Lower *Prevotella:Bacteroides* ratio was associated with greater lifetime MJ use among MJ users. **c-f** Lower *Prevotella* abundance was associated with poorer Fluid Cognition Composite score, Flanker Inhibitory Control and Attention score, List Sorting working memory score, and Dimensional Change Card Sort score in MJ users, but not in nonusers. Statistical significance is noted in bold font

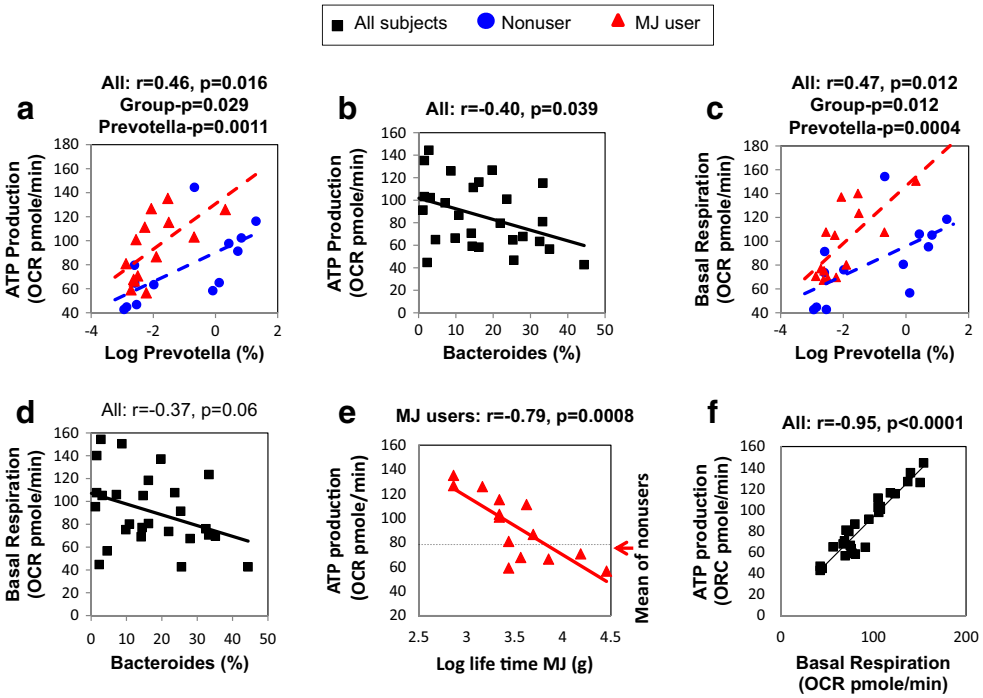

Fig. 2 Mitochondrial oxygen consumption in PBMCs correlated positively with the abundance of *Prevotella*, and inversely with the abundance of *Bacteroides*, and with lifetime marijuana use. **a-d** Mt ATP production and basal respiration rate correlated positively with *Prevotella* abundance (A & C; since the main effect of "group" was significant, trend lines were drawn for each group (red dashed line for user group, and blue dashed line for nonuser group); but since group-by-*Prevotella* interaction was not significant, all subjects were combined for correlation analyses), but correlated inversely with *Bacteroides* abundance (B & D) across all participants. **e** Mt ATP production inversely correlated with lifetime MJ use. **f** A representative figure demonstrating strong correlations among mt ATP production and basal (shown) or maximal (not shown) respiration rates. OCR, oxygen consumption rate. Statistical significance is noted in bold font

basal respiration rate (interaction-p = 0.011), Fig. 3d-f. But such interactions were not found for the other 4 subdomains. Variables that showed a difference in group characteristics (Table-1) such as ages of first alcohol or tobacco use, % past month alcohol use, and duration of tobacco use did not show significant main effects on primary and secondary outcomes when examined by ANCOVA analysis. Furthermore, Flanker Inhibitory Control and Attention score (but not other cognitive scores) correlated inversely with Log lifetime MJ use (r = −0.47, p = 0.041, Fig. 3g).

Discussion

This pilot study showed associations amongst mt function, microbiota composition, and cognitive function in chronic MJ users. We found that the composite score of fluid cognition and its subdomain Flanker inhibitory control and attention positively correlated with both *Prevotella* abundance in microbiota and systemic mt function only in MJ users, and not in nonusers. In addition, greater lifetime MJ use was associated with lower mt function and Flanker inhibitory control and attention scores in MJ users. However, regardless of MJ use status, mt function positively correlated with the abundance of *Prevotella*, but inversely correlated with that of *Bacteroides*.These findings provide a preliminary framework for further explorations of the possible relationships between MJ use, gut microbiota, mt function and cognitive function.

MJ Use, Dietary Patterns, and Prevotella

Prevotella and *Bacteroides* are two dominant and antagonistic genera of phylum Bacteroidetes, with the former associated with plant-based diet high in fruits and fiber, and the latter associated with animal-based diet high in protein and fat (Ley 2016). Consistent with previous reports (Kovatcheva-Datchary et al. 2015; Martinez et al. 2015), we observed an inverse correlation between the abundances of *Prevotella* and *Bacteroides* in this pilot study.

Individuals with plant-based diet showed greater *Prevotella*:*Bacteroides* ratio than those with animal-based diet (Wu et al. 2011). Moreover, MJ use was associated with lower fruit and vegetable consumption and greater animal-based food consumption in both adults (Smit and Crespo 2001; Hahn et al. 2014) and adolescents (Farrow et al. 1987; Arcan et al. 2011). Therefore, the 13-fold lower *Prevotella*:*Bacteroides* ratio in our MJ users may in part be due to their lower dietary intake of fruits and vegetables compared to nonusers. Furthermore, since the *Prevotella*:*Bacteroides* ratio tended to be lower with greater amount of lifetime MJ used in our participants, constituents in

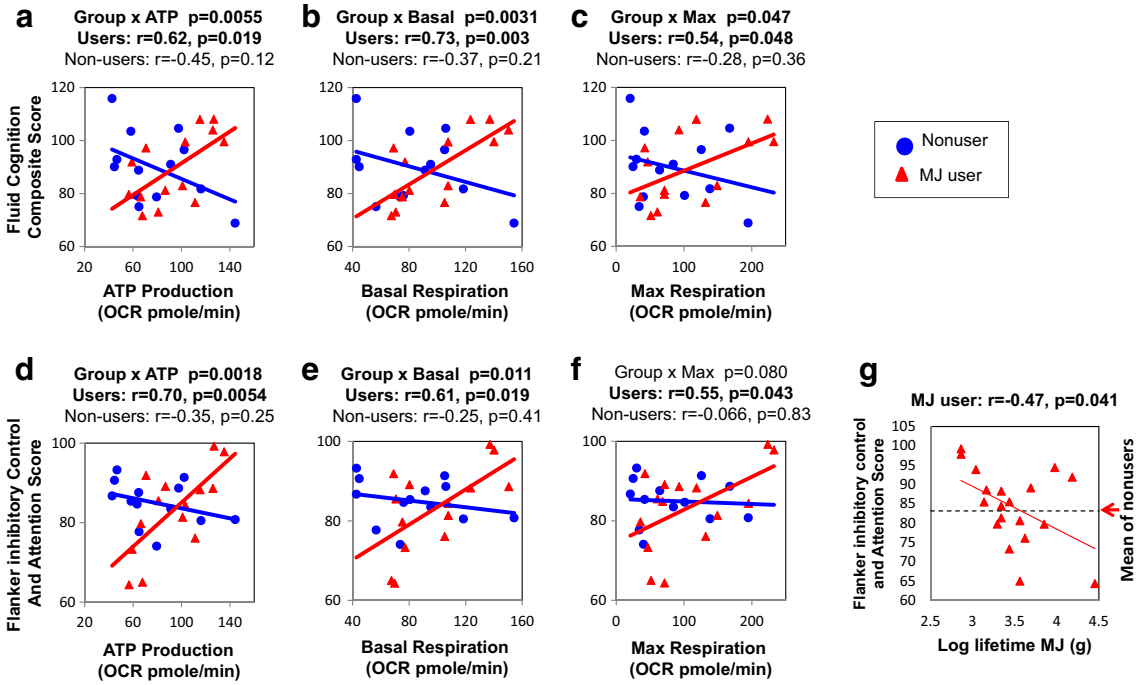

Fig. 3 PBMCs' mitochondrial oxygen consumption correlated positively with cognitive function and inversely with lifetime marijuana use among marijuana users. Fluid cognition composite score (**a-c**) and flanker inhibitory control and attention score (**d-f**) correlated positively with mt ATP production and basal and maximal respiration rates only in MJ users, but not in nonusers. Flanker inhibitory control and attention score correlated inversely with lifetime MJ use (**g**). OCR, oxygen consumption rate. Statistical significance is noted in bold font

MJ may additionally have direct effects on the levels of *Prevotella* and *Bacteroides*. In support of this hypothesis, THC administration prevented high-fat diet mediated increase in the Firmicutes:Bacteroidetes ratio in mice (Cluny et al. 2015).

Correlations Between Prevotella and Cognitive Function in MJ Users

In our MJ users, *Prevotella* abundance correlated positively with the composite score of fluid cognition and with three of its subdomain scores. Similarly, gut *Prevotella* was associated with improved cognition in patients without hepatic encephalopathy recurrence after lactulose withdrawal (Bajaj et al. 2012). However, the mechanism by which *Prevotella* influences brain function is unknown. As discussed above, the abundance of *Prevotella* may serve as a marker of dietary intake of fruits and vegetables, which are the main sources of dietary fiber. In the gut, dietary fiber is converted to short chain fatty acids (SCFA) through microbial fermentation (Simpson and Campbell 2015). Among the SCFA, butyrate is particularly known for its beneficial effects on neuronal health and cognitive function (Stilling et al. 2016), and reduction of dietary fiber intake resulted in lower fecal butyrate concentration (Duncan et al. 2007; Brinkworth et al. 2009). Therefore, the correlations between *Prevotella* and cognitive functions in MJ users may be mediated by gut SCFA, especially butyrate. Future studies should evaluate butyrate levels in MJ users to determine whether they have lower levels that might contribute to the cognitive deficits.

Possible Relationships Between Mt Function and Microbiota

Prevotella abundance (as a marker of greater plant-based food intake) correlated positively with mt ATP production and basal respiration, across all participants. Since mitochondria are the major sites of reactive oxygen species (ROS) production, sufficient antioxidant protection is required to maintain optimal function (Feniouk and Skulachev 2016). Plant-based foods typically contain higher levels of antioxidants than animal food products, which may explain why higher levels of *Prevotella,* associated with higher dietary intake of plant-derived antioxidants, may provide protection against oxidative stress and lead to greater mt function.

Conversely, the inverse correlations between *Bacteroides* abundance and mt activities may be related to relatively lower levels of antioxidants in animal food products. *Bacteriodetes* also produces propionate (Macfabe 2012), which interferes with mt tricarboxylic acid metabolism (Frye et al. 2013). Intraventricular administration of propionate induced cognitive deficits and brain mt

dysfunction in rats (MacFabe et al. 2007). Therefore the lower mt function in those with greater *Bacteroides* abundance might also be mediated by propionate production.

THC or Cannabis Effects on PBMC Mt Oxygen Consumption and Cognitive Function

Although our MJ users as a group had higher mt ATP production than nonusers, those who had greater lifetime MJ use had lower ATP production. These findings suggest a possible nonlinear dose-dependent effect of MJ use on mt function; for instance, a positive effect with low or moderate usage but a negative effect with greater usage. *In vitro* studies showed that THC exposure led to decreased mt membrane potential (Sarafian et al. 2005), alteration of mt membrane fluidity (Zaccagnino et al. 2012), and inhibition of mt respiratory chain function and increased hydrogen peroxide (H_2O_2) production (Wolff et al. 2015). In addition, CBD was shown to alter mt membrane potential, increase intracellular Ca^{2+} and ROS, and activate apoptosis (Mato et al. 2010; Shrivastava et al. 2011). Notably many of these *in vitro* studies used supraphysiological doses of cannabinoids, which is consistent with our observation that heavier MJ use was associated with lower systemic mt function.

In our relatively small sample, although MJ users and nonusers had similar cognitive performance at the group level, significant correlations between measures of mt function and cognitive performance were found only in the MJ user group. All measures of mt function (ATP production, basal or maximum respiration) correlated with both the Fluid Cognition composite score and the Flanker Inhibitory Control and Attention scores in the MJ users. These findings are consistent with prior reports that found greater oxidative damage in mt DNA from postmortem brains of individuals with mild cognitive impairment (Wang et al. 2006), and mt injury in patients with other cognitive disorders, including HIV-associated neurocognitive disorder (Var et al. 2016) and Alzheimer's disease (Dragicevic et al. 2010). In contrast, age-associated cognitive dysfunction was improved by dietary supplement of "mt nutrients" in rodents (Liu et al. 2002; Liu 2008). Taken together, mt dysfunction may contribute to MJ-associated cognitive deficit as reviewed previously (Volkow et al. 2016).

Limitations

This study has several limitations. (1) This is a pilot study in a relatively small cohort ($n \leq 20$ per group), and the mt function was measured only in ~70% of the subjects due to limited amount of PBMCs ($n = 13$ per group). Therefore, the limited statistical power did not allow for reliable mediation analyses among the different variables. (2) A detail dietary evaluation was not available to determine whether MJ use was associated with an altered dietary pattern that might be related to the altered *Prevotella* abundance or *Prevotella: Bacteroides* ratio. (3) All MJ users in this study used MJ daily; hence, the results may not be generalizable to occasional recreational MJ users. (4) The individual p values calculated in each analysis were not corrected for multiple comparisons, which may lead to a higher probability of type I errors (false positives) in the overall study.

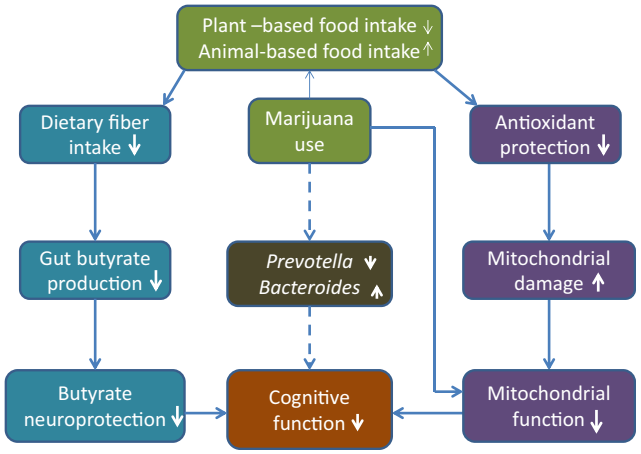

Fig. 4 Hypothetical network among chronic MJ use, dietary pattern, gut microbiota, mt respiration, and cognitive function

Summary

This study found that the *Prevotella*:*Bacteroides* ratio in fecal microbiota was lower in MJ users than in nonusers. Furthermore, lower *Prevotella* abundance was associated with lower systemic mt function in MJ users, which in turn might lead to poorer cognitive function. We hypothesize that MJ-associated dietary change may contribute to microbiota alterations (e.g., lower *Prevotella*:*Bacteroides* ratio), which together with lower dietary intake of antioxidants and fiber, in turn may alter mt antioxidant protection and gut SCFA (especially butyrate) production, and ultimately may lead to cognitive deficits, as shown in our hypothetical pathway (Fig. 4).

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Compliance with Ethical Standards

Conflict of Interest All authors reported no biomedical financial interests or potential conflicts of interest.

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