DISSERTATION

CANNABIS USE IN PEOPLE WITH MULTIPLE SCLEROSIS: THE HIGHWAY TO LOWER DISABILITY?

Submitted by

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ABSTRACT

CANNABIS USE IN PEOPLE WITH MULTIPLE SCLEROSIS: THE HIGHWAY TO LOWER DISABILITY?

The following dissertation describes a series of investigations designed to identify possible effects of cannabis use in people with Multiple Sclerosis. The specific aims of the three projects were: 1) to determine the proportion of people with Parkinson's Disease and Multiple Sclerosis currently using cannabis and collect self-reported measures of disability, to include physical function, balance, and fatigue; 2) to determine if people with Multiple Sclerosis using cannabis perform better on functional tasks compared to individuals who are not using cannabis; 3) to determine if resting brain glucose uptake is altered in people with Multiple Sclerosis using cannabis compared to people not using cannabis.

In Project 1 we found that a large portion of people with Parkinson's disease and Multiple Sclerosis responding to our survey are currently using cannabis. These individuals are also reporting lower levels of neurological disability, especially within the realms of mood, memory, and fatigue. A large majority of participants also reported reducing the amount of prescription medications since starting cannabis use. In project 2 we compared objective and subjective measurements of neurological disability between current cannabis users and data taken from a previous investigation investigating predictors/correlates of physical activity in people with Multiple Sclerosis. When we compared the users versus the non-users we found that users reported higher levels of fatigue as assessed by the fatigue severity scale questionnaire. We also found

that people with Multiple Sclerosis using cannabis performed worse on the Paced Auditory Serial Addition Test, which is a measure of cognitive function.

Project 3 utilized Positron Emission Tomography to measure brain glucose uptake with the glucose analog tracer [^{18}F]-Fluorodeoxyglucose. Higher levels of glucose uptake were beneficially correlated with disability status, fatigue, and pain in our sample. These findings agree with previous studies and indicated that brain glucose uptake can be used as a biomarker in people with multiple sclerosis. When our sample was dichotomized into current cannabis users and non-users measures of disability were similar, except that cannabis users performed more poorly during cognitive function testing. Even though most measures of disability were similar between the groups, cannabis users were found to have greater glucose uptake throughout areas of the frontal and temporal lobes. This suggests that cannabis may provide beneficial effects in maintaining nervous system glucose uptake but may also be accompanied by negative effects on cognition in people with multiple sclerosis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 – INTRODUCTION/EXPERIMENTAL AIMS

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system characterized by neuronal demyelination leading to neurodegeneration. This pathology results in interrupted signal transmission within the nervous system and between the nervous system and the periphery. Current estimates put the global prevalence of MS at 2012/100,000 (Global Burden of Disease 2015), and regionally the Colorado / Wyoming Chapter of the National Multiple Sclerosis Society estimates about 1 in 420 people. The most visible symptom of MS is impaired mobility, but other common symptoms include: pain, fatigue, spasticity, balance and cognitive impairments. Most individuals are diagnosed with MS in their 20's and 30's and live a normal lifespan. This means that individuals live with the disease for decades which brings a high cost to the burden of their disease. People with MS (PwMS) are estimated to have direct medical costs that are 5.1 times higher than the general population, even when controlling for all chronic conditions (Campbell et al. 2014). Current pharmaceutical treatments work fairly well at controlling the worsening of MS, but fail to adequately control symptoms such as pain, spasticity, and fatigue (Bethoux and Marrie 2016, Rudroff et al. 2016, Rønning and Tornes 2017).

Within the last couple of decades medical research has begun to highlight the possible importance of the human endocannabinoid system in the health and function of central nervous system as well as other systems. The cannabinoid receptor 1 (CBR_1) is the most abundant receptor within the brain, and is concentrated in regions responsible for mood, memory, and motor functions (Zanettini et al. 2011, Callén et al. 2012). Another endocannabinoid receptor of note is also the cannabinoid receptor 2 (CBR_2),

which is found within cells of the immune system. A review by Rom and Persidsky (2013) highlighted the potential of manipulating the CBR_2 in immunomodulation and neuroinflammation. In fact, therapies that target the endocannabinoid system, at both the receptor and ligand levels have been postulated to improve conditions ranging from MS (Baker and Pryce 2008, DiMarzo et al. 2000) to chronic pain (Chiou et al. 2013) to various movement and neurodegenerative disorders (Iuvone et al. 2009, Kluger et al. 2015).

Several FDA approved pharmaceuticals exist that contain compounds that interact/modulate the innate endocannabinoid system, but by far and large the most easily acquired product is the *Cannabis sativa* plant. Cannabis contains over 100 unique compounds that interact to provide effects on multiple human systems and behaviors. The two main phytocannabinoids, i.e. plant based cannabinoid compounds, are Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD). Both compounds interact with the CBR_1 and CBR_2, but can have opposite, additive, or synergistic effects dependent upon their bioavailable ratios (Pertwee 1997, 2008, Svíženská et al. 2008). The current body of literature is mostly prejudiced against cannabis use as the negative effects of cannabis on adolescent/adult cognitive function are touted by United States federal agencies. Despite this bias, several studies have shown that cannabis may be effective in the management of pain and spasticity in PwMS but may negatively affect cognitive function (Zajicek et al. 2003, 2005, Honarmand et al. 2011).

Currently 29 States and the District of Columbia have passed some form of medical cannabis law, and an additional 16 states have specific laws authorizing CBD use for specific conditions (NORML). Even with acceptance of medicinal cannabis at a

record high, with some polls reporting as high as 80% acceptance (Yahoo News/Marist), much uncertainty remains about the safety, efficacy, dosing, and long term consequences of medicinal cannabis use in MS and other conditions. Even though there is a lack of empirical evidence for or against medicinal cannabis use, a large portion of PwMS, 16% (Clark et al. 2004, Cofield et al. 2015) are currently using cannabis as a treatment for their signs and symptoms. Current federal regulations have severely restricted research in the past and continue to limit research into the beneficial and harmful effects of cannabis use in PwMS. As cannabis use is legal in a majority of states it becomes even more important to elucidate cannabis' effects so that both patients and care providers can make informed decisions about the start, continued use, or disuse, of cannabis as an adjunct therapy. Therefore, in this series of projects we wanted to measure and compare physical and cognitive function, psychological wellbeing, and brain health in PwMS currently or not using cannabis.

Overall Hypothesis: People with MS currently using cannabis will have greater measures of disability and perform worse on physical and cognitive tasks compared to non-users with MS based on the published literature performed in healthy individuals.

Specific Aim for Study 1: Determine areas of self-reported neurological disability that differ between individuals with neurological diseases currently using cannabis and those who do not.

Specific Aim for Study 2: Compare measures of disability between current cannabis users and non-users with MS.

Specific Aim for Study 3: Measure and compare brain glucose uptake and disability in current cannabis users and non-users with MS.

CHAPTER II – MANUSCRIPT I[1]

CANNABIS USE IN PEOPLE WITH PARKINSON'S DISEASE AND MULTIPLE SCLEROSIS: A WEB-BASED INVESTIGATION

Summary

Cannabis has been used for medicinal purpose for thousands of years; however the positive and negative effects of cannabis use in Parkinson's disease (PD) and Multiple Sclerosis (MS) are mostly unknown. Our aim was to assess cannabis use in PD and MS and compare results of self-reported assessments of neurological disability between current cannabis users and non-users. An anonymous web-based survey was hosted on the Michael J. Fox Foundation and the National Multiple Sclerosis Society webpages from 15 February to 15 October 2016. The survey collected demographic and cannabis use information, and used standardized questionnaires to assess neurological function, fatigue, balance, and physical activity participation. Analysis of variance and chi-square tests were used for the analysis. The survey was viewed 801 times, and 595 participants were in the final data set. Seventy-six percent and 24% of the respondents reported PD and MS respectively. Current users reported high efficacy of cannabis, 6.4 (SD 1.8) on a scale from 0-7 and 59% reported reducing prescription medication since beginning cannabis use. Current cannabis users were younger and less likely to be classified as obese ($P < 0.035$). Cannabis users reported lower levels of disability, specifically in domains of mood, memory, and fatigue ($P < 0.040$). Cannabis may have positive impacts on mood, memory, fatigue, and obesity status in people with

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PD and MS. Further studies using clinically and longitudinally assessed measurements of these domains are needed to establish if these associations are causal and determine the long-term benefits and consequences of cannabis use in people with PD and MS.

Introduction

Cannabis sativa has been used for medicinal purposes for several thousand years (Pain 2015). Compounds within the cannabis plant interact with what is now known as the endocannabinoid system, which is comprised of a group of receptors and ligands synthesized within the human body. The cannabinoid receptors are found throughout the body, but with higher densities within the central nervous and immune systems. It has been suggested that cannabis may be a natural therapy for combating neuro-inflammatory and neuro-degenerative conditions due to the high density of cannabinoid receptors in the central nervous system (Bisogno and Di Marzo 2010). Published reports suggest that people with Parkinson's disease (PD) and multiple sclerosis (MS) may experience relief of some of their symptoms, such as spasticity and pain, when using cannabis (Arjmand et al. 2015, Chagas et al. 2014, Di Marzo et al 2000, Iuvone et al. 2009, Saito et al. 2012, Zajicek et al. 2003, 2005). Under certain conditions cannabis has been shown to have neuroprotective effects (Sarne et al. 2011). However, negative effects, such as cognitive impairment, are prevalent as well (Honarmand et al. 2011).

Several surveys have looked into cannabis use in Parkinson's disease (PD, Finseth et al. 2015, Venderova et al. 2004) and Multiple sclerosis (MS, Banwell et al.

2016, Clark et al. 2004). While most studies reported some efficacy of cannabis, none of these studies compared symptoms or disability status between the cannabis users and the non-cannabis users. With the legal status of cannabis use currently in flux, we created an anonymous web based survey to: (1) investigate patterns of cannabis use among people with PD and MS and (2) compare self-reported measures of disability between the cannabis users and non-users.

Materials and Methods

Ethical Statement

All procedures and methods were approved by the Colorado State University Institutional Review Board. An acknowledgement of consent was displayed once a prospective participant accessed the survey, and acceptance of this consent was required before an individual could begin the survey.

Measures

The anonymous survey consisted of the following validated scales: Guy's Neurological Status Scale (GNDS, Rossier and Wade 2002), Nottingham Health Profile (NHP, Hunt et al. 1981), Fatigue Severity Scale (FSS, Krupp et al. 1989), Activities of Balance Confidence (ABC, Powell and Myers 1995), and the International Physical Activities Questionnaire (IPAQ, Booth 2000). Demographic (e.g. age, sex, body mass index (BMI)), disease diagnosis, and cannabis use (e.g. past/current use status, times per week, methods of cannabis use) were also assessed. Cannabis use related questions were collapsed into a dichotomous variable (current users vs. non-users).

Cannabis efficacy was assessed using an 8 point Likert scale (0: Not helpful - 7: Very Helpful).

Each of the scales were digitized and entered into the on-line survey host Qualtrics. The survey was tested in house by the authors to ensure proper: order, adaptive questioning, and required question enforcement. Adaptive questioning was used to hide questions when previous answers would make subsequent questions irrelevant, e.g. when a participant answered no to current cannabis use no further cannabis use questions were presented. Survey testing was conducted for approximately 3 months, after which an anonymous link was created by the survey host. This link was then posted to the websites of the Michael J. Fox Foundation and the National Multiple Sclerosis Society. These websites are recognized as prominent sources of information about their respective diseases and offer portals to view research opportunities that visitors can partake in. In total, the survey consisted of 185 items, although the length of each survey varied per person depending responses to adaptive questions.

Sampling

The anonymous online hyperlink to the web-based survey was posted to the research recruitment pages on the websites of the Michael J. Fox Foundation and the National Multiple Sclerosis Society from 15 Feb 2016 until 15 Oct 2016. The survey was also advertised through the participant databases of the investigators and posted to our laboratory webpages. This was a voluntary open survey allowing anyone with access to these websites to participate. There were no incentives offered for participation. Investigator contact information was also made available to prospective participants.

Participants were able to contact the investigators via email or through the websites directly if they had questions about the survey. IP address verification was performed to remove duplicate records from individuals who may have filled out the survey multiple times.

Statistics

Means and standard deviations were calculated for continuous variables. Individual variables are reported and listwise deletion variables were excluded if information was not provided. No statistical corrections for missing data were performed. Demographic comparisons between PD and MS respondents were performed using Students' T-Tests for continuous data (e.g. Age, BMI) and chi-square tests (e.g. sex, obesity status) for categorical data. The effect of cannabis use on self-reported scales (GNDS, NHP, ABC, FSS, IPAQ) was examined using a between-subjects two-way (Current Cannabis Use × Disease Diagnosis) analysis of variance (ANOVA). The main effects of disease are only reported in the tables, as it is expected that people with PD and MS will have varying levels of disability due to their differing disease diagnosis and symptoms. Chi-square values were used to test the associations of cannabis use status with categorical variables (e.g. sex and obesity status). Obesity status was defined as having a BMI ≥ 30 and education status was defined as possessing at least a 4 year degree. All analyses were two-sided with significance set to $\alpha < 0.05$ and performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp, Armonk, N.Y., USA).

Results

Sample Demographics

The survey was viewed a total of 801 times. The participation/recruitment rate was 96.1%, with 31 records not providing consent. Forty-one records were removed after IP address verification, and 92 records were removed due to lack of self-reported diagnosis. Two records were removed due to lack of demographic information. Forty records were removed due to a diagnosis other than PD or MS, leaving a total sample of 595 records. The completeness rate was 77.3% with 538 records in the final dataset filling out 100% of the survey.

Demographic information is shown in Table 1. The sample was made up of 76.3% PD and 23.7% MS. The average age of the PD group was greater than the MS group ($T = 15.948$, $P < 0.001$). The MS group had a lower proportion of men ($\chi^2 = 24.606$, $P < 0.01$). Body mass index, obesity status, and education status did not differ between the PD and MS groups (BMI, $T = 0.420$, $P = 0.675$; Obesity Status, $\chi^2 = 0.084$, $P = 0.772$; Education Status, $\chi^2 = 2.338$, $P = 0.126$).

Cannabis Users and Non-User Demographics

Demographic comparisons between current cannabis users and non-users are shown in Table 2. Non-users are defined as any individual who is not currently using cannabis, and includes individuals who have tried cannabis in the past. The sex and education status of current cannabis users and non-users was similar (sex, $\chi^2 = 0.034$, $P = 0.854$; education status, $\chi^2 = 1.519$, $P = 0.218$), but the current cannabis users were younger, had lower BMI, and were less likely to be classified as obese (age, $F = 4.464$, $P = 0.035$; BMI, $F = 6.070$, $P = 0.014$; obesity status, $\chi^2 = 7.173$, $P = 0.007$).

Cannabis Use Characteristics

Cannabis use characteristics are shown in Table 3. Seventy percent of the sample reported having used cannabis at least once within their lifetime, and 44% reported currently using cannabis. Of the current cannabis users, 74% stated their use was for medicinal purposes, but only 42% reported possessing a medical cannabis card. Respondents with MS were more likely to have used cannabis previously and be current cannabis users (Past, $\chi^2 = 14.322$, $P < 0.001$; Current, $\chi^2 = 38.683$, $P < 0.001$). Usage purposes, possession of a medical card, and method of cannabis usage were not different between the PD and MS respondents (Purpose, $\chi^2 = 0.282$, $P = 0.595$; Card, $\chi^2 = 2.491$, $P = 0.120$, Method, $\chi^2 = 0.373$, $P = 0.830$). However, MS respondents were more likely to report the reduction of prescription medications with cannabis use ($\chi^2 = 22.878$, $P < 0.001$), were more likely to report using cannabis for at least 1 year ($\chi^2 = 6.186$, $P = 0.013$), are using cannabis on more days per week ($T = 3.332$, $P = 0.001$), and reported cannabis being more effective at relieving their symptoms ($T = 3.121$, $P = 0.002$) than the respondents with PD. When non-users were asked if they would consider using cannabis if scientifically shown to be beneficial, 97.9% responded “yes”.

Self-reported Scales

No interactions between Cannabis Use × Disease Diagnosis were detected for any of the GNDS, NHP, FSS, ABC, or IPAQ values ($P > 0.05$), signifying that differences between the cannabis users and non-users were not due to a specific disease diagnosis.

Table 4 contains the average values for the aggregate GNDS score, GNDS subscales, NHP scales, FSS, ABC, and the IPAQ. Current cannabis users had lower

scores, signifying less disability, on the GNDS (F = 7.481, *P*= 0.006), and specifically within the Memory (F = 4.717, *P* = 0.030), Mood (F = 9.328, *P* = 0.002), and Fatigue (F = 6.870, *P* = 0.009) subscales. No differences were detected in any of the NHP domains (F < 1.637, P > 0.201). Current cannabis users also reported a lower impact of fatigue, as shown by lower FSS scores (F = 4.219, *P* = 0.040). No differences were detected between the current cannabis users and non-users in time spent (min/week) in: moderate to vigorous physical activities (F = 0.520, *P* = 0.471), walking (F = 1.036, *P* = 0.309), sitting (F = 0.001, *P* = 0.987) or balance confidence (ABC, F = 0.049, *P* = 0.825). Although not reaching significance (F = 3.702, *P* = 0.055) there may be an interaction between cannabis use status and balance in the MS group, resulting in people with MS using cannabis reporting lower balance confidence.

Discussion

To our knowledge this is the first study which investigated the patterns of cannabis use amongst people with PD and MS and compared measures of disability between cannabis users and non-users. Our data suggests that a large proportion (44%) of respondents with PD and MS are currently using cannabis. Our results also show that current cannabis users self-report lower levels of disability compared to non-users. Specifically we observed this in scales representing memory, mood, and fatigue. It is also important to note that current cannabis users did not report higher/worsened symptoms in any scale or measure, although there was a borderline significant interaction between balance confidence, cannabis use status, and an MS diagnosis.

This interaction suggests that cannabis use may negatively affect balance in people with MS.

Effectiveness of Cannabis

The current cannabis users in our sample reported that cannabis was quite effective. Eighty-five percent reported cannabis' effectiveness as moderate or above in relieving their symptoms, 4 or greater on a 0-7 Likert scale. Unfortunately, one of the limitations of our study is that it was not possible to identify the exact symptoms our respondents were treating with cannabis. An interesting finding from our data is that people with MS reported a greater effectiveness of cannabis compared to the PD group. This may also be supported by the finding that respondents with MS using cannabis were more likely to report reducing the use of prescription medications since beginning cannabis use, and may be contributing to a greater perceived effectiveness by people with MS. This finding is in-line with an examination of prescription drug use by Bradford and Bradford (2016). In their investigation, they reported significant reductions in daily doses filled for prescription drugs per physician in states with medical cannabis laws, especially in the realm of pain medications.

Possible Effects of Cannabis

Acute cannabis intoxication is known to negatively affect cognitive processing but these impairments often resolve themselves after a period of abstinence (Fried et al. 2005). Due to these known effects it was interesting to see that the current cannabis users in our sample reported better scores within the memory and mood subscales of the GNDS. It is known that cannabis can impair working memory (Han et al. 2012, Schoeler and Chattacharyya 2013) and is linked to depressive symptoms, although the

link between cannabis use and depression may be weaker than previously thought (Feingold et al. 2017). Individuals who have cognitive dysfunctions and mood disorders may refrain from cannabis use in fear of exacerbating these symptoms, and this may have led to our results. The placebo effect can also not be ruled out, as people may expect their mood to improve with cannabis use. Further research is needed to determine the effects of cannabis on these parameters in individuals with PD and MS and these domains should have increased priority of monitoring if a person begins using cannabis.

Weight gain is often thought to occur with cannabis use, and is one of the reasons its use is often suggested. In our discussions with people interested in the effects of cannabis this negative effect is often brought up. Cannabis use can lead to increased caloric intake (Foltin et al. 1986). It has been shown that cannabis consumption can contribute to obesity when initiated during adolescence (Ross et al. 2016), but in a large study of adults in the United States, Le Strat and Le Foll (2011) reported a lower prevalence of obesity in cannabis users compared to non-users. Combined with our results, it does not appear that significant weight gain should be of concern for patients contemplating cannabis use. Whether cannabis use is protective of obesity in PD and MS cannot be determined from our sample, and long term monitoring of obesity and metabolic syndrome parameters should be monitored in patients using cannabis as cannabis is known to affect the metabolism of several tissues (Cavuoto et al. 2007, Kola et al. 2005).

Our results show that the current cannabis users and non-users are spending the same amount of time performing Moderate-to-vigorous physical activity, walking, and

time spent sitting. Acute cannabis use is shown to induce a transient amotivational state in non-users, but regular cannabis use may prevent this from occurring (Lawn et al. 2016). Cannabis has also been shown to negatively affect motor performance (Ramaekers et al. 2006), which could lead to lower physical activity levels. These negative effects do not seem to be manifested within our sample; although effects of acute intoxication from cannabis products cannot be ignored. While this data on physical activity is interesting, it needs to be further explored utilizing objective measures to determine the interactions of cannabis and physical activity participation in the PD and MS populations.

Differences in use between PD and MS

In our sample a greater proportion of people with MS report using cannabis. Most cannabis laws specifically state pain and muscle spasms related to MS are appropriate conditions in which to allow cannabis use. Respondents with MS tended to be younger and more likely to have used cannabis in the past. This may contribute to the increased prevalence of cannabis use and the greater usage of cannabis throughout the week in the respondents with MS. Future studies should begin to identify specific symptoms that people with PD and MS are using cannabis for and which symptoms, other than pain and spasticity, are most effectively treated using cannabis.

Limitations of the study

One of the major limitations of our study, and most others, is how we define cannabis. It is well-known that cannabis products can have a wide range of concentrations in regards to the two most studied cannabinoids: Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). The current body of literature on

the negative effects of cannabis is mostly focused on the psychoactive ingredient THC. Several investigations have shown that CBD can ameliorate the negative aspects of THC (Schoeler and Bhattacharyya 2013, Hollister and Gillespie 1975, Wright et al. 2013), as well as having beneficial effects in its own right (Espejo-Porras et al. 2013, Crippa et al. 2016). The current lack of detailed knowledge, i.e. external validity, about the products individuals are using, as well as which products medical professionals should recommend, creates a quagmire for both medical professionals and patients alike.

As with most surveys, biases in: selection, self-report, recall, social-desirability, and generalizability of the sample are all prominent limitations. Our data was captured in the form of an open web-based survey and allowed anyone with access to the internet to participate. While acceptance of cannabis use is rising we cannot discount the fact that because the title of the survey included “cannabis” many individuals may not have participated due to an inherent aversion to anything dealing with this topic. This may have led to the increased proportion of current cannabis users in survey compared to others (Finseth et al. 2015, Banwell et al. 2016, Venderova et al. 2004, Ware et al. 2005). Although, a recent report shows that the proportion of older adults using cannabis is increasing at a much higher rate than previously expected (Kaskie et al. 2017). It is possible that our convenience sample more closely reflects this trend than the previous studies referenced, but caution must be advised in the generalizability of our results. We also found that current users believe cannabis to be highly effective, which may be influenced by selection and self-report biases of the sample. For example, it is unlikely that individuals who believe cannabis provided no benefit would

continue using it. While these limitations exist, measures to counter-balance them have been taken. These measures include a relatively large sample size and following guidelines established for reporting web-based surveys (Eysenbach 2004).

It is also important to note that this sample is largely limited to people who access the internet and are somewhat familiar with the use of online tools. This may reflect that our sample has a higher cognitive ability than the PD and MS populations as a whole. While our data add significantly to our current knowledge of cannabis' effects, results from this survey should be used to inform controlled research, rather than reach definitive conclusions about cannabis' efficacy. Randomized control trials with high external validity are needed for medical professionals and patients to make informed decisions about cannabis use.

Important Gaps in Knowledge

Neuroimaging modalities including, magnetic resonance imaging and positron emission tomography are an integral part of disease diagnosis and monitoring. Yet it is largely unknown how cannabis use alters human brain connectivity, function, and structure. To date there is no conclusive neuroimaging evidence showing that cannabis alters brain structure in healthy adults (Weiland et al. 2015), although several studies have shown functional differences between cannabis users and non-users (Chang and Chronicle 2007, Volkow et al. 1996). Romero et al. (2015) reported that in people with MS brain volume reductions were associated with cognitive impairment, and in people with MS using cannabis the association between volume loss and cognition was stronger. Due to the a cross-sectional nature of Romero et al. (2015) the authors are unable to determine whether cannabis use caused a greater reduction in brain volume,

but it is important to note that current cognitive dysfunction may be a contraindication of cannabis use as it may exacerbate cognitive impairments. How/if cannabis affects brain structure in neurological conditions remains unknown, and longitudinal cause/effect neuroimaging studies are needed to determine these associations.

Conclusions[2]

In spite of the limitations of this study, we observe that a large proportion of individuals with PD and MS are currently using cannabis as a medical treatment. Our results show cannabis users are reporting lower levels of disability, most notably in domains of memory, mood, and fatigue. It also appears that a large proportion of users are self-medicating with cannabis, as indicated by the fact that only 42% of the current cannabis users reported possessing a medical cannabis card.

As our survey shows, a significant number of people with PD and MS are already using cannabis in the absence of empirical data for or against cannabis use. In addition, given the fact that the removal of legal barriers may lead to a significantly increased number of cannabis users, the challenge faced by the medical profession in the coming years is to play catch-up and help patients make an informed decision on whether to use cannabis.

[2] **Author Contributions**

J.H.K collected data, analyzed and interpreted the data, and wrote the manuscript. K.L., analyzed and helped interpret the data. N.B.K., F.P., B.W.F., J.M.H., W.R.S., help interpret the data. T.R. directed the study and helped interpret the data. All authors contributed critical feedback to the manuscript.

Table 2.1. Sample Demographics

PD: Parkinson's disease; MS: multiple sclerosis; BMI: body mass index; SD: standard deviation.
* $P < 0.05$; ** $P < 0.01$; ns – not significant

	Total	PD	MS	T-Test / χ^2 results
Age, years [mean(SD)]	57.3(12.4)	61.1 (9.5)	45.1 (12.8)	**
Sex (%)				
Men	52.3	57.9	34.0	**
Women	47.7	42.1	66.0	
BMI [mean(SD)]	26.3 (5.5)	26.4 (5.3)	26.1 (6.1)	ns
Classified as Obese (%)	20.0	20.3	19.1	ns
4-year degree or higher (%)	56.6	58.4	51.1	ns

Table 2.2. Demographic comparisons between cannabis users and non-users

Main effect of Cannabis Use Status was identified for Age and BMI. No interactions were detected between Cannabis Use Status and Diagnosis (P > 0.457)
PD: Parkinson's disease; MS: multiple sclerosis; BMI: body mass index; SD: standard deviation.
* P < 0.05; ** P < 0.01; ns – not significant

	Total		PD		MS		ANOVA / χ^2 results
	Non	Use	Non	Use	Non	Use	
Age [mean(SD)]	59.7 (11.1)	54.3 13.2)	61.7 (9.5)	60.0 (9.2)	47.0 (11.8)	44.3 (12.3)	*
Sex							
Men (%)	52.0	52.7	56.3	60.6	25.5	38.7	
Women (%)	48.0	47.3	43.7	39.4	74.5	61.3	
BMI [mean(SD)]	26.8 (5.5)	25.7 (5.4)	26.7 (5.4)	25.8 (5.2)	27.3 (6.4)	25.6 (5.9)	*
Classified as Obese (%)	24.0	15.1	23.4	15.2	27.7	15.1	**
4-year degree or higher (%)	58.6	53.5	57.7	58.8	63.8	44.1	ns

Table 2.3. Cannabis Use Characteristics by disease diagnosis

Past and current use is reported as a percentage of the total sample. All other variables are reported as a percentage of the current users.
PD: Parkinson's disease; MS: multiple sclerosis; BMI: body mass index; SD: standard deviation; Rx: Prescription
*P < 0.05; **P < 0.01; ns – not significant

	Total	PD	MS	T-Test / χ^2 results
Past Use (%)	70.3	66.3	83.0	**
Current Use (%)	43.7	36.6	66.4	**
Medicinal Use (%)	73.7	72.3	76.1	ns
Possess Medical Card (%)	42.1	38.4	48.4	ns
Reduced Rx since started cannabis (%)	59.1	47.8	78.5	**
Smoke Only (%)	38.1	40.9	33.3	ns
Edibles Only (%)	6.3	6.3	6.5	ns
Smoked + Edibles (%)	19.4	19.5	19.4	ns
Using longer than 12 months (%)	75.0	69.8	83.9	*
Days/Week [mean(SD)]	5.0 (2.3)	4.6 (2.4)	5.6 (2.1)	**
Effectiveness [mean(SD)]	6.4 (1.8)	6.2 (1.8)	6.9 (1.6)	**

Table 2.4. Self-reported levels of neurological disability

Data reported as mean (SD). Guy's Neurological Disability Scale (GNDS) is scored from 0 to 34, and the Nottingham Health Profile (NHP) is scored from 0-100, higher values represent greater disability. EL = energy level, P = pain, ER = emotional reaction, S = Sleep, SI = social isolation, PA = physical abilities. FSS = Fatigue Severity Scale range is 0-9 with higher values representing a greater impact of fatigue, ABC = Activities of Balance Confidence range is 0-10 with lower scores representing less confidence in maintaining balance, MVPA = moderate and vigorous physical activities
No interactions between Current Cannabis Use Status x Diagnosis were identified.
* $P < 0.05$ main effect of Current Cannabis Use Status
$ $P < 0.05$ main effect of Diagnosis

	Total [Mean(SD)]		PD [Mean(SD)]		MS [Mean(SD)]		ANOVA / χ^2 results
	Non	Use	Non	Use	Non	Use	
GNDS Total	24.4 (6.1)	23.1 (6.4)	24.2 (6.0)	22.7 (6.4)	25.7 (6.5)	23.8 (6.2)	*
GNDS Memory	1.3 (1.0)	1.2 (1.1)	1.3 (1.0)	1.1 (1.0)	1.7 (0.9)	1.4 (1.1)	* $
GNDS Mood	1.5 (1.5)	1.3 (1.4)	1.5 (1.4)	1.2 (1.3)	2.1 (1.5)	1.5 (1.6)	* $
GNDS Vision	1.2 (1.3)	1.2 (1.3)	1.2 (1.2)	1.0 (1.3)	1.4 (1.5)	1.3 (1.3)	ns
GNDS Speech	0.8 (0.9)	0.7 (0.9)	0.9 (0.9)	0.9 (0.9)	0.6 (0.8)	0.4 (0.7)	ns
GNDS Swallow	0.8 (1.0)	0.7 (0.9)	0.8 (1.0)	0.7 (1.0)	0.7 (1.0)	0.6 (0.9)	ns
GNDS Arm / Hand	10.0 (1.2)	9.8 (1.1)	9.9 (1.2)	9.8 (1.1)	10.1 (1.1)	9.9 (1.0)	ns
GNDS Mobility	2.2 (1.3)	2.1 (1.4)	2.2 (1.3)	2.1 (1.4)	1.8 (1.4)	2.0 (1.3)	ns
GNDS Bladder	1.5 (1.4)	1.4 (1.4)	1.4 (1.4)	1.2 (1.4)	1.6 (1.4)	1.7 (1.3)	$
GNDS Bowel	1.2 (1.2)	0.9 (1.2)	1.2 (1.2)	1.1 (1.3)	0.9 (1.1)	0.6 (1.0)	ns
GNDS Fatigue	2.5 (1.5)	2.4 (1.7)	2.4 (1.5)	2.2 (1.5)	3.4 (1.1)	2.7 (1.8)	* $
GNDS Sex	1.4 (0.5)	1.5 (0.5)	1.4 (0.5)	1.5 (0.5)	1.5 (0.5)	1.6 (0.5)	ns
NHP EL	46.8 (39.5)	45.2 (39.1)	44.6 (39.3)	37.8 (36.6)	60.9 (38.3)	57.9 (40.1)	$
NHP P	30.1 (32.1)	31.8 (35.9)	29.5 (31.3)	27.9 (34.3)	33.5 (37.2)	38.4 (37.9)	$
NHP ER	27.4	23.9	26.9	20.7	30.8	29.3	$

	(29.2)	(27.6)	(28.8)	(26.1)	(31.3)	(29.2)	
NHP S	39.9 (31.7)	37.0 (30.5)	39.5 (31.6)	36.4 (30.5)	42.4 (32.7)	38.0 (30.6)	ns
NHP SI	25.6 (29.7)	23.7 (29.3)	25.1 (29.1)	20.0 (26.7)	29.2 (33.9)	30.3 (32.6)	$
NHP PA	28.7 (23.1)	25.5 (22.5)	28.6 (22.7)	22.4 (20.1)	29.3 (26.0)	31.0 (25.5)	ns
FSS	4.8 (1.7)	4.7 (1.8)	4.7 (1.7)	4.4 (1.7)	5.7 (1.1)	5.3 (1.7)	*
ABC	7.4 (2.7)	7.5 (2.7)	7.4 (2.7)	7.9 (2.5)	7.5 (2.8)	6.8 (3.1)	ns
MVPA (min / week)	730 (1056)	808 (1140)	744 (1068)	894 (1142)	639 (981)	659 (1128)	ns
Walking (min / week)	326 (468)	374 (585)	332 (460)	392 (534)	286 (519)	344 (662)	ns
Sitting (min / week)	1848 (788)	1858 (825)	1831 (782)	1764 (792)	1957 (827)	2027 (860)	ns

CHAPTER III – MANUSCRIPT II

CANNABIS USE, DISABILITY, AND PHYSICAL ACTIVITY PARTICIPATION IN PEOPLE WITH MULTIPLE SCLEROSIS

Summary

Cognitive and physical disabilities are hallmark symptoms of Multiple Sclerosis. Previous investigations into the effects of cannabis on MS related spasticity have shown improvement s in mobility with short term cannabis supplementation. Currently it is unknown how long term, more than 6 months, cannabis use affects physical function and mobility in people with MS. We compared measures of mobility, physical activity, and cognitive function of 13 current cannabis users to an established historical data set of people with MS. All users tested positive for the presence of Δ9-Tetrahydrocanibinol (THC). Our comparisons failed to find any differences in physical performance or physical activity participation between the current cannabis users and non-users. However, current users reported greater fatigue severity and performed worse on the test of cognitive function. These results persisted when age and sex were taken into consideration. Due to the cross-sectional nature of this study we are unable to determine if cannabis is responsible for the greater fatigue and cognitive dysfunction in this population, but these domains should be closely monitored in people with MS currently using cannabis.

Introduction

Many uncertainties remain around the use of cannabis as a medicine in the United States. Currently 28 states and the District of Columbia have authorized medicinal cannabis for a variety of diseases and multiple sclerosis (MS) is often a qualifying disease. MS is an inflammatory disease of the central nervous system characterized by the demyelination and degeneration of neurons, often leading to long-term physical and cognitive disability. Mobility is the hallmark of disability classification in MS, and reduced walking abilities can lead to reduced physical activity participation and a lower quality of life in these patients (Krüger et al. 2017). Several previous investigations into cannabis use and MS have reported beneficial effects on mobility (Zajicek et al 2003, 2005; Vaney et al. 2004). Unfortunately, these studies were for relatively short durations, so the effects of long term cannabis use on mobility in PwMS are unknown.

The cannabinoid receptors are the primary targets affected by compound in the *Cannabis sativa* plant. A large portion of these receptors are located within outflow nuclei of the basal ganglia suggesting a role in motor control (Herkenham 1992). Previously, studies in healthy regular cannabis users have shown reduced motor performance relative to non-users (Pillay et al. 2008, King et al. 2011) and serum Δ9-Tetrahydrocannabinol (THC) levels, the compound that leads to the cannabis “high”, are associated with physical impairments (Ramaekers et al. 2006). These negative effects on physical function would suggest that cannabis use in MS may not be beneficial in the long term. This discrepancy makes it very important to determine how long term

cannabis use affects motor performance in MS, as disability measures are mostly measured by ambulation status.

Previously our research group measured a variety of physical function tests in PwMS. This data set includes performance on the: MS functional composite (MSFC), handgrip strength, the timed up-and-go (TUG), and physical activity participation. In the current investigation we wanted to determine if long-term cannabis use has negative effects on these parameters. Based on the findings from healthy individuals, our a priori hypothesis is that PwMS who have been using cannabis for an extended period of time would perform worse on these tests. To test this hypothesis we recruited PwMS currently using cannabis for 6 or more months and compared their results to our previously collected dataset.

Materials and Methods

Ethical Statement

All procedures were approved by the Colorado State University Institutional Review Board and all participants signed informed consent before participating in any aspects of the protocol.

Participants

Twenty-two participants were recruited for this study. After providing signed informed consent, a urinalysis was performed to determine cannabis use status (iScreen IS1THC dipstick, Alere Toxicology, Waltham MA, USA). Participants then completed a battery of tests that were performed in an earlier study (Ketelhut et al. 2017) to quantify ability level. Objective tests included the MSFC, handgrip strength,

and the TUG. Questionnaires were used to evaluate the participants' perceptions of disability using the Patient Determined Disease Steps (PDDS, Hohol et al. 1995) and the fatigue severity scale (FSS, Krupp et al 1989).

The MSFC consists of 3 tests: a 25 foot walk test (WT), the 9-hole peg test (9HPT), and the Paced Auditory Serial Addition Test (PASAT). Participants were asked to walk 25 feet as quickly and safely as possible. This was performed 2 times with the lowest score being used for analysis. During the 9HPT participants were instructed to pick up 9 plastic pegs, 1 at a time, and then place the pegs into a 3x3 grid. Once all pegs were inserted they were immediately instructed to remove them, 1 by 1, and return them to the dish. This was done twice with each hand, beginning with the dominant hand. The time to complete was measured with a handheld stop watch and the quickest time was used as their score. The PASAT is a test of cognitive function where participants are asked to add two single digit numbers voiced on a computer. One digit was spoken every three seconds and the amount answered correctly was recorded. Further explanation of the MSFC can be found in Cutter et al. (1999) and Fischer et al. (1999).

Handgrip strength was measured using a hydraulic hand dynamometer (Lafayette Instruments, Lafayette IN, USA). Participants performed the test while in a seated position with their elbow at 90 degrees with their arm held against their torso (Mathiowetz et al. 1985). The test began with a count down and participants were then instructed to squeeze as hard as they could for 3 seconds, maintain force output for 3 seconds, and then to relax (Rudroff et al. 2014). Three to 5 trials were performed starting with the dominant hand then alternating to the other. The highest force output

recorded while maintaining proper position was used for analysis. The last objective assessment, the TUG, required the participants to rise from a seated position, walk 3 meters, turn around, and return to the starting seated position (Schoene et al. 2013). The time taken to complete the task was measured with a handheld stopwatch, with the lowest time being used in the analysis.

Current cannabis users were also given an ActiGraph GT3X+ (ActiGraph Corp. Pensacola FL, USA) to monitor their physical activity levels for 7 days. Participants were instructed to wear the monitor on their right hip at all times except while performing water based activities and while they slept. The monitors were initialized using the low-frequency extension feature and a sampling rate of 30Hz. Cut points to determine moderate to vigorous physical activity (MVPA), light, and sedentary time were adopted from Sandroff et al. (2012, 2014). Data were downloaded with 15 sec epochs and accelerometer counts in the vertical axis were analyzed. Wear time was validated with the following criteria: wear time minimum of 10h/day and 4 valid days consisting of 1 weekend day (Toriano RP et al. 2008).

Once all participants had completed the study the data was compared to the previously collected dataset used in Ketelhut et al. (2017). Physical activity data in this data set was collected and analyzed in the same way as it was in the current study. This previous data set consisted of 30 PwMS who were known non-cannabis users at the time of data collection.

Statistical Analysis

All data are reported as Mean (Standard Deviation) unless otherwise noted. Continuous variables were compared between the cannabis users and the existing data

set using unpaired Student's T-Test and PDDS distribution was compared using Mann-Whitney U Test. Comparisons were made between the entire dataset (Cannabis users, N=13; non-users, N=39) and another analysis matched non-users (N=25) from the data set to the users, similar to Ghaffar and Feinstein (2008). Historical records were matched to the current cannabis records based on age (±5 years) and sex. All analyses were perform using IBM SPSS Statistics for Windows version 24 (IBM Corp., Armonk NY, USA) with alpha set at < 0.05.

Results

Analysis 1, whole data set

Physical activity data was not used for 5 participants (4 from previous data set and 1 from the cannabis users) due to not meeting wear time requirements or ActiGraph errors. Demographic and functional data was still used from these participants. The age of the sample was 53.9(13.1) with an MS duration of 14.1 (9.8) years. The current cannabis users were identified by a positive urinalysis for the presence of THC. Of the 13 current cannabis users, 11 have been using cannabis for more than 12 months, while 2 users have been using for 6-12 months. The users reported using cannabis 6.6 (0.8) days per week and 2.2 (1.4) times per day. 3 individuals reported using products that were CBD dominant, CBD:THC > 5:1, the 10 remaining used THC dominant products (THC:CBD > 1:1. The current cannabis users did not differ from the non-users in any demographic variables: Ht. Wt., BMI, Age, or Dx Duration ($P > 0.20$). Physical performance was also similar between the groups ($P > 0.12$), although, cannabis users reported greater levels of fatigue ($P = 0.03$) and performed worse on the PASAT ($P =$

0.01) compared to the non-users. Physical activity participation was also similar between the groups ($P > 0.17$). All results and p-values are located in Table 1.

[Table 1]

Analysis 2, matched

Twenty-five records from the database were within 5 years of age and the same sex as the 13 cannabis users. No additional variables differed from the previous analysis when participants were matched for age (±5 years) and sex, although the difference in FSS ($P < 0.01$) and PASAT (0.03) increased slightly. When matched with individuals of the similar age and sex (users N=12, non-users N= 23) physical activity was not different between the groups ($P > 0.16$). Data is displayed in Table 2.

[Table 2]

Discussion

Contrary to our original hypothesis, current cannabis users and non-users with MS performed similarly on tests of physical ability. Although cannabis users reported greater levels of fatigue and performed worse on the test of cognitive function compared to non-users. Previous examinations into cannabis use and cognitive function have shown similar findings.

Cognitive Function

Cognitive dysfunction affects an estimated 40-60% of PwMS (Rao et al. 1991, Lyon-Caen et al. 1986). Honarmand et al. (2011) performed cognitive testing of 50 PwMS, with half being classified as current cannabis users. In that study the average duration of cannabis use was 26.6 year and with the range of use being 1-41 years. They

determined that cannabis users performed more poorly for tests of information processing speed, working memory, and executive functions. These results persisted when effects of age, sex, education, premorbid intelligence, disability, and disease course were taken into account. Our findings agree with this previous report, although the composition of cannabis products used in Hornamand et al. (2011) was not reported.

Vaney et al. (2004) performed a randomized, double-blind, placebo-controlled cross-over trial in PwMS and used the MSFC as an outcome measure. In their study they found that PASAT scores were unaffected by 14 days of cannabis supplementation. Several important differences between Vaney et al. (2004) and our current study exist, the first being the duration of cannabis use. Participants in the current study have been using cannabis for a significant period of time, while Vaney et al. (2004) was only a 2 week intervention. Another important factor for the discrepancy between our findings could be due to the varying ratios of THC:CBD. In the trial a controlled ratio of THC:CBD of 2.8:1 was used while most individuals in our study used much greater ratios of THC:CBD. Wright et al. (2013) reported that CBD can ameliorate some of the negative cognitive effects of THC in monkeys, but the interactions in man have not been fully elucidated.

Cannabis and Physical Ability

Physically the cannabis users and non-users were very similar. Results from the MS Functional Composite, TUG, and Handgrip were not different. Previous interventional studies shown small improvements in physical ability with a pharmaceutical based cannabis extracts. Zajicek et al. (2003) measured walking speed

during a 10 m walk. After cannabis supplementation speeds increased 3 fold compared to the non-treatment groups. However it is unknown if the improvement in walking were directly related to the lower spasticity, which was the primary outcome investigated, or if the cannabis supplementation improved walking ability through a separate mechanism. One of the limitations of this current study is that it is cross sectional. Due to this we cannot say if physical abilities are improved by cannabis use or not. Currently we can infer that long-term cannabis use is unlikely to be detrimental to physical abilities in PwMS due to the fact that both groups performed similarly except during times of acute intoxication.

Cannabis and Fatigue

In some popular media stories about cannabis, one beneficial effect that is touted is increased energy level from certain strains of cannabis. A recent survey performed by our research group also showed that cannabis users with Parkinson's disease and MS reported lower levels of fatigue. These findings did not translate to the current project, and in fact opposite results were identified. Fatigue is a multi-faceted symptom with origins throughout the nervous system and peripheral systems (Rudroff et al. 2017). Cannabinoid receptors are found throughout the body and could play a role in the manifestation of fatigue.

THC and CBD are generally considered to be the most prominent of the cannabinoids but can have opposite and complicated interactions on the cannabinoid receptors. Most of the products that participants reported using were THC dominant, which is known to cause the "high" recreational users often seek. This component can induce lethargy and drowsiness (Cao et al. 2016). The THC dominance of the products

used may be partially responsible for the higher FSS scores measured in this study. Long term negative effects of THC on motivation may have been previously overstated but it will be important to continue to monitor how cannabis use effects fatigue in PwMS, as fatigue is often one of the most disabling symptoms PwMS have (Bashki 2003).

Limitations

The main limitation of this study is that it was a cross-sectional design. This type of investigation does not allow for the effects of cannabis use to be studied. During interviews with the participants many expressed their inability to perform a variety of tasks when not using cannabis. To improve the design of future studies performance measures should be tested while on and off drug. A washout period of roughly 30 days could be used and effects of cannabis use could then be measured by differences in performance between the two conditions within an individual. This would also remove many confounding variables that exist. Interventional studies are desperately needed to gather information to help patients and care providers make informed decisions about cannabis use. Another limitation of the study is the small sample size. While this sample is larger than some of the previous cannabis studies in PwMS, the heterogeneous nature of MS makes it difficult to apply results to the population as a whole. Along the lines of the small sample size the only measures of disability in this study is the PDDS, which is a patient reported outcome. While it is very similar to the Expanded Disability Status Scale, which is a physician performed test, both mainly rely on walking ability. They do not take into account more subtle disease parameters such as lesion volumes or location, nervous system morphometric measures such as cortical thickness or white matter integrity, or neuroenergetics. MS is known to affect these measures, and

cannabis may be providing benefits, or consequences, to brain health that do not readily manifest in the clinical tests performed in this examination.

Conclusions

From the current analysis we show that PwMS currently using cannabis and those who are not perform similar in a variety of functional tasks, ranging from measures of mobility to arm and hand function. While these results suggest that cannabis may not be harmful to physical performance, cognitive function was lower in the cannabis users compared to the non-users. Periods of abstinence may be able to reverse some of the negative effects of cannabis use (Chang et al. 2006, Jacobus et al. 2012), however it may be difficult to incorporate abstinence periods in a clinical population. Regular testing of cognitive function should be performed in people thinking about starting cannabis use and possible benefits and consequences should be weighed carefully by care providers and patients.

Table 3.1. Demographic and functional test values for the user and non-user groups

Dom = Dominant, ND = Non-dominant, WT = Walk Test, 9HPT = 9 Hole Pet Test, MVPA = Moderate and Vigorous Physical Activity, LPA = Light Physical Activity, PA = Physical Activity

	N (non / user)	Non	Users	*P* - value
Sex (M / F)	39 /13	10 / 29	4 / 9	
Age	39 / 13	54.9 (12.8)	51.0 (14.2)	0.356
Height (m)	39 / 13	1.7 (0.1)	1.7 (0.1)	0.459
Wt. (kg)	39 / 13	74.8 (19.5)	77.8 (17.9)	0.631
BMI	39 / 13	25.8 (5.1)	28.1 (6.5)	0.201
MS Duration	39 / 13	15.0 (9.1)	11.5 (11.6)	0.273
PDDS	39 / 13	2, 0-6	2, 0-6	0.957
Handgrip (Dom)	39 / 13	33.4 (9.6)	29.3 (7.4)	0.171
Handgrip (ND)	39 / 13	31.1 (10.4)	28.1 (8.3)	0.342
25ft WT (sec)	39 / 13	6.3 (3.3)	5.6 (1.7)	0.440
9HPT (sec, Dom)	39 / 13	21.9 (6.8)	23.3. (7.6)	0.252
9HPT (sec, ND)	39 / 13	22.7 (4.5)	24.6 (6.6)	0.252
PASAT	39 / 13	42.1 (12.1)	32.4 (9.9)	0.012
TUG (sec)	39 / 13	9.7 (7.3)	9.2 (3.4)	0.816
MVPA (min/day)	26 / 12	31 (22)	28 (19)	0.722
LPA (min / day)	26 / 12	202 (40)	224 (57)	0.170
Total PA (min / day)	26 / 12	232 (52)	252 (68)	0.331
Sedentary	26 / 12	672 (58)	595 (118)	0.350

Table 3.2. Matched analysis of Demographic and functional test values for users and non-users.

Dom = Dominant, ND = Non-dominant, WT = Walk Test, 9HPT = 9 Hole Pet Test, MVPA = Moderate and Vigorous Physical Activity, LPA = Light Physical Activity, PA = Physical Activity

	N (non / user)	Non	Users	*P* - value
Sex (M / F)	25 / 13	7 / 18	4 / 9	
Age	25 / 13	55.1 (12.8)	51.0 (14.2)	0.374
Height (m)	25 / 13	1.7 (0.1)	1.7 (0.1)	0.406
Wt. (kg)	25 / 13	75.9 (18.6)	77.8 (17.9)	0.764
BMI	25 / 13	26.1 (5.3)	28.1 (6.5)	0.314
MS Duration	25 / 13	15.1 (9.6)	11.5 (11.6)	0.323
PDDS	25 / 13	2, 0-6	2, 0-6	0.927
Handgrip (Dom)	25 / 13	34.2 (9.6)	29.3 (7.4)	0.120
Handgrip (ND)	25 / 13	32.4 (9.4)	28.1 (8.3)	0.174
25ft WT (sec)	25 / 13	5.9 (2.2)	5.6 (1.7)	0.599
9HPT (sec, Dom)	25 / 13	22.9 (5.9)	23.3 (7.6)	0.851
9HPT (sec, ND)	25 / 13	22.5 (3.9)	24.6 (6.6)	0.234
PASAT	25 / 13	42.0 (13.2)	32.4 (9.9)	0.027
TUG (sec)	25 / 13	9.0 (3.9)	9.2 (3.4)	0.838
MVPA (min/day)	23 / 12	32 (22)	28 (19)	0.574
LPA (min / day)	23 / 12	200 (41)	224 (57)	0.158
Total PA (min / day)	23 / 12	232 (55)	252 (58)	0.357
Sedentary	23 / 12	617 (59)	595 (118)	0.458

CHAPTER IV – MANUSCRIPT III

BRAIN GLUCOSE UPTAKE AND ASSOCIATIONS WITH DISABILITY IN PEOPLE WITH MULTIPLE SCLEROSIS: DOES CANNABIS USE PLAY A ROLE?

Summary

Investigations into resting brain function in healthy individuals, as measured by Positron Emission Tomography (PET) and the glucose analogue ^{18}F-Fluorodeoxyglucose (FDG) have shown that regular cannabis users had lower glucose uptake (GU) in regional cerebral areas. It has been suggested that this lower GU may account for acute cognitive deficits seen during cannabis intoxication. Lower GU has also been observed in people with multiple sclerosis (PwMS), and lower GU has been associated with disease symptoms such as fatigue and reduced walking ability. The aim of this study was to examine resting GU of the brain in PwMS currently using cannabis (N=8) and non-users (N=8). Across subjects, greater GU in regional brain areas (cerebellum, frontal- parietal- occipital- temporal lobes, brain stem) was associated with less disability; specifically: fatigue, disability status, and pain. Although most disability measures were similar between the groups, cannabis users had greater GU areas throughout the fontal and temporal lobes. Cannabis users scored worse during the addition test representing cognitive function but this was not correlated with GU. While cannabis use may have beneficial effects on disability, its effects on cognitive function should be monitored closely.

Introduction

Cannabis use in healthy and clinical populations continues to rise in the United States. A major hurdle to widespread medical acceptance or rejection, besides the Schedule I classification set by the United States Drug Enforcement Agency that

severely restricts research, is that benefits and consequences of long term cannabis use in clinical populations have not been established. Even without widespread acceptance or legalization, a large portion of people with MS (PwMS) have reported using cannabis. It is estimated that 20-66% of PwMS are currently using cannabis (Banwell et al., 2016, Kindred et al., 2017)

Previously, several short-term interventional studies, 6 months or less, have investigated the effects of cannabis supplementation on spasticity and serum inflammatory markers in PwMS (Killestein et al., 2002; Zajicek et al., 2003, 2005; Katona et al., 2015; Zettle et al., 2016). In general, some beneficial effects were seen in spasticity and walking ability (Killestein et al., 2002, Zajicek et al., 2003, 2005). Psychological examinations in PwMS using cannabis have shown that users perform worse on cognitive tasks and may have higher comorbid psychological disorders (Ghaffar et al., 2008; Honarmand et al., 2011), although the duration of cannabis use is not reported. Neither of these studies were interventional, so causation of these negative effects cannot be assessed. Currently, there is a lack of published literature regarding long duration with regular cannabis users with PwMS.

Common methods used to access disease progression / status in MS are measures of brain activity. Glucose is the main energy substrate used by the nervous system; therefore glucose uptake (GU) can be used as measure of nervous system activity (Tashiro et al., 2008). Positron emission tomography (PET) is a metabolic imaging technique that uses the decay of positron-emitting elements to quantify tracer uptake. One of the most commonly used tracers is [^{18}F]-Fluorodeoxyglucose (FDG), which is a glucose analogue. FDG uptake in the nervous system can be used as a

biomarker of disability in PwMS and previous FDG-PET studies in PwMS have linked lower brain glucose utilization to poorer measures of disability (Roelcke et al., 1997, Kindred et al., 2015a).

Roelcke et al. (1997) showed that during rest PwMS had a lower metabolic rate of glucose within the brain when compared to healthy controls. Furthermore, they showed that within the MS group participants with higher levels of fatigue had a lower metabolic rate within in the frontal cortex and basal ganglia compared to individuals with lower levels of fatigue. Kindred et al. (2015a) reported lower cerebral FDG uptake in PwMS compared to healthy controls during treadmill walking. In this study the PwMS had a lower self-selected walking speed, and uptake within several brain regions correlated with outcomes in the healthy adults, but not the MS group. It was stated that this difference could represent an uncoupling of brain activity in PwMS and walking, possibly reflecting the plasticity of the brain to maintain function in this clinical population.

Utilizing FDG-PET in the study of cannabis, Volkow et al. (1996) investigated FDG uptake in healthy individuals who were regular users and non-users. At baseline the users had lower uptake in the cerebellum and after acute ingestion of cannabis glucose uptake was increased in the prefrontal cortex and cerebellum of both groups (Volkow et al., 1996). Similar findings have also been seen using other imaging modalities, e.g. MRI. Filby et al. (2017) reported altered resting global and regional cerebral blood flow, oxygen extraction, and the cerebral metabolic rate of oxygen in regular cannabis users compared to non-users. Block et al. (2000) also showed lower

cerebellar blood flow using the [^{15}O]-H_2O PET tracer. However, it is unknown, whether cannabis use affects the central nervous system glucose uptake in PwMS.

The goal of this project was to measure and compare resting FDG-uptake of the brain in PwMS who are currently using cannabis with non-users. We hypothesized that FDG uptake would be lower in the cannabis users compared to the non-users, and that this lower level would correlate with worse outcomes in subjective and objective measures of disability.

Materials and Methods

Ethical Statement

All procedures were approved by the Colorado State University Institutional Review Board and by the Colorado Multiple Institutional Review Board. All participants signed informed consent before participating in any aspects of the protocol.

Participants

Sixteen participants, 8 cannabis users and 8 non-users with similar ages and sex, from an earlier study (Study 2) were invited to undergo FDG-PET/ CT imaging to measure resting brain glucose uptake. These participants were chosen due to having similar age and sex. Induvials of each group were chosen with the closest ages to each other. Previously the participants performed multiple evaluations to assess physical and mental abilities. Participants completed the following questionnaires: Activities of Balance Confidence (ABC, Powell and Myers 1995), Beck's Depression Inventory (BDI, Beck and Beamesderfer 1974), Fatigue Severity Scale (FSS, Krupp et al. 1989), MOS Pain Effects Scale (PES, Stewart and Ware 1992), Numerical Rating Scale measure of

spasticity (NRS, Farrar et al 2008), and the MS Quality of Life – 54 (MSQOL, Vickrey et al 1995). Objective measurements included: the 25 foot walk test, 9 hole peg test, and paced auditory serial addition test (PASAT), handgrip strength, the timed up-and-go (TUG), and an instrumented version of the modified clinical test of sensory impairment on balance (mCTSIB, Boulgarides et al. 2003).

The 25 foot walk test, 9-hole peg test, and the PASAT are the component tests of the Multiple Sclerosis Functional Composite. This battery was administered in accordance with the instructions published by the National MS Society (Fischer et al. 2001) and as has been done previously in our laboratory (Ketelhut et al. 2017). The quickest times the participants could walk 25 feet and put 9 plastic pegs in a 3x3 grid and remove them were used for the analysis. The PASAT was scored as the number of correct answers, max of 60, given by the participant during the 3 min test. Handgrip strength was measured using a hydraulic hand dynamometer (Lafayette Instruments, Lafayette IN, USA). The maximal force output while maintaining proper position (Mathiowetz et al. 1985) was used as their strength measurement. The mCTSIB was performed on a BTrackS balance plate (Balance Tracking System, San Diego CA, USA), which tracked center of pressure movement over a 30 sec period of time. Participants were given 3 opportunities to complete each of the 4 conditions: eyes open, eyes closed, eyes open on a foam pad, and eyes closed on a foam pad. If a participant was unable to complete a condition they were scored a zero. Participants were also given a log to track their cannabis use. After a period of 7 days, participants returned the logs and any available product labels listing the tetrahydrocannabinol (THC) and cannabidiol (CBD) contents of the cannabis products used to the investigators. Weekly

alcohol use was also recorded to be ensure image differences were not related to alcohol intake, as it has been reported that alcohol can be a confounding variable.

Participants arrived at the Colorado Clinical and Translational Research Imaging Center (CTRIC) after at least a 4 hour fast, and users were asked to refrain from cannabis use for 8 hours prior to their visit. After consent was signed participants provided a urine sample and then rested in a seated position for at least 5 min. A urinalysis was conducted to test for the presence of THC in all participants (iScreen IS1THC dipstick, Alere Toxicology, Waltham MA, USA). All users tested positive for the presence of THC in their urine. Blood glucose levels were checked to ensure an appropriate fasting glucose level was present (< 200 mg/dL). Once appropriate blood glucose levels were confirmed a trained radiological technician inserted a catheter into an antecubital vein. Approximately 9 mci of FDG was then measured and injected. After injection the catheter was removed and participants were instructed to rest quietly for 35 min. Once the rest period was over participants were taken from the rest area and positioned on the PET/CT camera's bed. While lying down participants crossed their arms across their chests and straps were used to hold their arms and head in positon.

Image Acquisition

Imaging was performed on a Phillips Hybrid Gemini TF 64 camera (Philips Healthcare, Cleveland OH, USA). An initial regional CT attenuation scan was performed and after 45 min of tracer uptake a one bed PET scan was initiated. The CT scan was acquired with the following parameters: 120kV, 100mAs, and 2mm slice thickness. Ten minutes of PET list mode data of the brain was collected and reconstructed using RAMLA reconstruction method to generate attenuation corrected images of 2mm slice

thickness. The CT image map was used to reconstruct the corrected images. After the images where reconstructed they were burned to a compact disc in DiCom format and transferred to the Integrative Neurophysiology Laboratory at Colorado State University for further analysis.

Image Analysis

DiCom formatted images where imported into Analyze 11.0 (Mayo Clinic, Rochester, MN, USA). CT corrected PET images were then converted into Standardized Uptake Value (SUV) images (Kindred et al 2015b) and exported as Analyze 7.5 files. SUV images were (1) spatially normalized to Montreal Neurological Institute space and (2) smoothed to an FDG template within SPM12 (The Wellcome Trust Centre for Neuroimaging, London, UK), similar to previous works (Tuulari et al 2013).

An unpaired t-test was then performed within SPM12 to identify areas of differing uptake between the cannabis users and non-cannabis users with a relative threshold masking set at 0.8. T-contrasts of "-1 1" and "1 -1" were tested with a p-value set to 0.05 and an extent threshold (k_e) = 0 (voxels). Mean SUV values for brain regions were extracted from the SUV images using the Marsbar toolbox for SPM (Brett et al 2002). Regions extracted included: anterior and posterior cerebellar lobes, frontal lobe, temporal lobe, occipital lobe, parietal lobe, medulla, midbrain, and pons. Similar regions have been used in a previous study (Volkow et al. 1996).

Statistical Analysis

All data are reported as Mean (Standard Deviation) unless otherwise noted. Whole brain statistical analysis was performed within SPM. Group comparisons

between the cannabis users and non-users for imaging, demographic and functional variables were performed using unpaired student's t-tests. Pearson's correlations were used to determine if glucose uptake was associated with any of the disability measures. ROI data was visually inspected to identify possible outliers and/or non-physiological values. Cohen's D measures of effect size were calculated for the ROI. All analysis was performed with IBM SPSS Statistics for Windows version 24 (IBM Corp., Armonk NY, USA) with alpha set to 0.05.

Results

The average age of the sample (N=16) was 50.2(13.6), with an average disease duration of 11.7 (8.3) years and median disability level of 2 (range 0-6). Seven out of the 8 cannabis users had been using for more than 12 months, and one user had been using for 6 months. The users reported ingesting cannabis an average of 2.3(1.5) times per day and 6.9(0.4) days per week. Cannabis usage was mostly via edibles and smoking, although one user reported using dabs. Most labels collected from the products had > 1:1 ratio of THC:CBD, with many products containing no CBD, although 3 users primarily used CBD based products. The comparisons between the users and non-users for demographic, questionnaires, and functional variables were mostly similar, except for handgrip strength of the dominant hand ($P = 0.02$), PASAT scores ($P = 0.02$) and their perceived change of health over the last year ($P = 0.03$), which the cannabis users had worse values. All values and significance levels are located in Table 1.

[Table 1]

SPM analysis revealed multiple areas throughout the brain, primarily within the frontal and parietal lobes, in which cannabis users had greater uptake (Figure 1). There were no areas in which the cannabis users had lower uptake. Table 2 displays the specific regions. These results persisted when alcohol consumption was used as a covariate during SPM analysis. Mean SUVs for the groups and effect sizes for the 9 ROI are located in Table 3.Correlations between ROI SUV and measured variables were found for disease duration, disability level, FSS, PES, NRS, and multiple subdomains of the MSQOL-54 ($P < 0.05$). None of the variables that differed between the groups, e.g. dominant handgrip, PASAT, and MSQOL-54 change in health sub score correlated with ROI SUV. The correlation analyses for performance variables and brain regions are located in Table 4 and Table 5.

[Figure 1, Table 2, Table 3, Table 4, Table 5]

Discussion

We are the first to report the resting glucose uptake values for PwMS regularly using cannabis. The results showed that cannabis users had greater uptake in several areas throughout the brain, primarily within the frontal and parietal lobes. In regards to ability levels between the groups, no differences were seen except in handgrip strength and cognitive function. Cannabis users had lower values on the handgrip strength tests and the PASAT compared to the non-users. However, these differences could not be explained by resting brain activity in any region.

Glucose Uptake, Disability, and Pain

Previous studies (Roelcke et al 1997, Kindred et al. 2015a) suggest that lower glucose uptake may be an important biomarker in the tracking and progression of MS severity and symptoms. Our results seem to agree with these studies as disability status and pain assessments both correlated negatively with glucose uptake within the sample. One of the prominent reasons for cannabis use is for the management of pain (Chong et al. 2006, Banwell et al. 2016). The effectiveness of cannabis usage in this domain is evident in states that have legalized medicinal cannabis. In these states legalization has led to a reduced number of opiate prescriptions (Bradford and Bradford 2016, Bradford and Bradford 2017). The PES and MSQOL-54 Pain subscale correlated with regional SUV throughout the brain. Cannabis may beneficially impact nervous system glucose uptake in PwMS, leading to improvements in pain management.

Cognitive function and cannabis use

Cognitive function is often affected in many PwMS, with estimates ranging from 40-60% (Rao et al 1991, Lyon-Caen et al 1986). Cannabis use has also been associated with cognitive impairments in healthy individuals and PwMS (Kalant 2004, Ramaekers et al 2006, Ghaffar et al 2008, Romero et al 2015). In PwMS specifically, Romero et al (2015) shows that cognitive scores are correlated with the lower brain volume present in PwMS using cannabis. Our methods did not allow us to reasonably quantify brain volume, as CT images were performed at low intensities for attenuation correction not anatomical identification, but activity was greater in several regions in our sample. Previous works have recently questioned cannabis's effects on morphometric measures, and may have been overstated by previous research groups. Weiland et al (2015) examined the associations between cannabis use and brain volume. In that

study, MRIs were compared between 79 non-users and 79 cannabis users. They showed that no differences existed between the groups in subcortical structures when controlling for the effects of alcohol use, gender, age, and other variables. They suggest that that long-term cannabis use is unlikely to have lasting deleterious effects on brain morphology, unlike other drugs such as alcohol. How activity, volume, and cannabis use are connected in PwMS needs further study.

Depression and cannabis use

In a previous project performed by our group using an anonymous online survey we saw that cannabis users reported less disability related to mood (Kindred et al. 2017). This result did not translate to our current study. In the present study mood was assess by the BDI and values were similar between the groups. This was also reflected in the emotional well-being subscale of the MSQOL. Previous studies have shown an association with depressive symptoms and cannabis use (Grant 1995, Chen et al. 2002), although this link may not be as strong as originally suggested when confounding variables are taken into account (Danielsson et al. 2016, Feingold et al. 2017). A study of major depressive disorder using FDG-PET found moderate correlations between the Hammond Depression Rating Scale and two areas of the frontal cortex. We failed to find any correlation between the BDI and the SUV of any ROI in this study, although our study had a smaller sample size and may account for this lack of finding.

Fatigue and cannabis use

Roelcke et al. (1997) showed that PwMS who had greater levels of fatigue had widespread lower glucose uptake within the frontal cortex compared to PwMS with less

fatigue. Fatigue was assessed using the same instrument, the FSS. In the current study fatigue levels were similar between the cannabis users and non-users and associations to the frontal lobe were also seen, although correlations were also seen in the cerebellum and brain stem. In a recent survey, project 1 (Kindred et al 2017), cannabis users had lower FSS scores, although in project 2 we see that our cannabis users reported higher scores when compared to age/sex matched individuals from a previous investigation (Ketelhut et al. 2017). As fatigue is often one of the most disabling symptoms of MS (Bakshi 2003) it will be important to continue to try and determine causes of fatigue and how cannabis use may or may not affect this symptom.

Limitations

The most prominent limitation of this study is the cross-sectional design. Due to this fact we are unable to directly test the effect of cannabis use on resting brain activity. It still unknown whether cannabis use increases or decreases glucose utilization in people with MS, but in this study individuals using cannabis for prolonged periods of time had greater uptake when compared to non-users. Long-term intervention based studies are required to truly determine if cannabis use has a positive or negative effect on nervous system bioenergetics. Another limitation is the variety of cannabis products being used. Most of the product labels collected during the study reported much greater amounts of THC compared to CBD. It is generally thought that CBD would have the most therapeutic value due to its non-psychoactive properties (Iuvone et al 2009, Wright et al 2013). In this study all users tested positive for THC via urinalysis, but circulating blood levels of THC, its derivatives, and CBD may provide additional insight into nervous system health and cannabis use. Due to the variety of products clinical

populations may be using these results cannot be generalized to all cannabis types/users.

Another limitation of this study is the small sample size. Calculated effect sizes were small to moderate (0.29-0.58) and it is possible that with a greater sample size larger regions of significance may be identified. This may indicate a beneficial effect of cannabis use, either in preserving nervous system function or possibly preserving nervous system volume. Certainly neither of these conclusions can be supported by the current findings but studies of much larger scale may be able to shed more light on the causal effects of regular cannabis use on brain activity in PwMS.

Conclusions

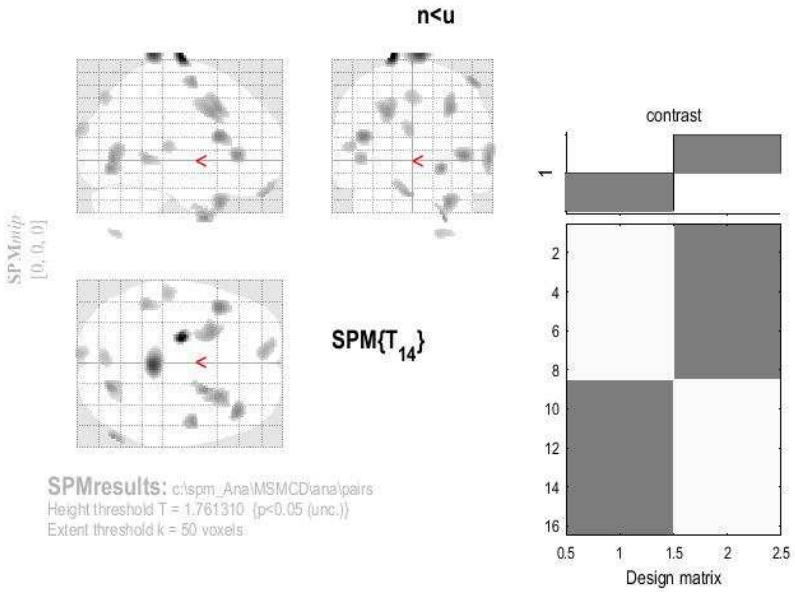
Cannabis use and acceptance as a medicinal product is at record highs in the United States (Geiger 2017, Swift 2017), and its use is only expected to continue to increase. In this study we found that PwMS who currently use cannabis had greater nervous system activity at rest in small regions throughout the brain, as measured by FDG uptake, to those who do not use cannabis. There are several possible explanations for this increased uptake. One possible reason could be attributed to the proposed neuroprotective effects of cannabis. One theory related to neurodegenerative diseases is that as axons are damaged the mitochondria in those areas can become stressed (Kalman and Leist 2003, Su et al. 2013, Campbell et al. 2014). This results in mitochondrial dysfunction and a reduction in glucose metabolism within the axon. This in turn results in the accumulation of lactate. This lactate can then be forced into the blood stream, down a concentration gradient. This neurodegeneration could then be pseudo detected by increased blood lactate concentrations. Previously, it has been

shown that PwMS can have 3x the resting blood lactate concentrations compared to healthy controls (Amorini et al. 2014). Lactate has also been shown to reduce nervous system glucose uptake. Smith et al. (2003) measured resting glucose uptake while infusing lactate and showed that as lactate concentrations increased glucose uptake decreased. Kemppainen et al. (2005) also looked at glucose uptake during cycling and found that as intensity increased, blood lactate increased, and brain glucose uptake decreased. It is possible that cannabis may provide a neuroprotective effect and reduce blood lactate concentrations or cannabis may alter neuroenergetics in some way that glucose uptake is increased. Unfortunately no serum lactate measures were performed in this investigations so this reasoning is still hypothetical but easily tested in future studies.

FDG PET imaging has been used by several labs investigating glucose uptake in animal models of MS (Radu et al. 2007, Buck et al. 2012,Faria et al. 2014). In these studies FDG was used to quantify areas of inflammation or glial cell activity. It was shown that FDG can be used to identify active lesions or sites of neuroinflammation. It is possible that increased FDG uptake in the cannabis users is due to increased neuroinflammation within the identified regions. While this is certainly possible it is unlikely to be the case as no participants reported recent relapses. The groups also tested similarly for physical function, which would seem to indicate that the increased uptake seen in the cannabis users was not leading to decreases in performance, which would be expected during a relapse.

We also found that measurements of disability were similar between the users and non-users, except for the tests of handgrip strength and cognitive function. Due to

the cross-sectional nature of this study we cannot conclude whether cannabis use led to the higher uptake in the users group. Most participants were also using THC dominant strains of cannabis and these results may not apply to individuals using CBD dominant strains. Further testing is needed in this area. Although most measures were the same between the two groups, cognitive function was lower in the cannabis users group. Previous studies have shown the detrimental effects of cannabis on cognitive function (Fried et al 2005, Ghaffar et al 2008, Honarmand et al 2011). This domain should certainly be monitored by care providers when an individual is using cannabis to ensure cognitive function is not being adversely affected. To conclude, long-term cannabis does not appear to have negative effects on brain glucose uptake, and may lead to beneficial effects on brain bioenergetics, but still should be used under medical supervision with an eye on possible negative cognitive effects.



SPMresults: c:\spm_Ana\MSMCD\ana\pairs
Height threshold T = 1.761310 {p<0.05 (unc.)}
Extent threshold k = 50 voxels

Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm mm mm		
p	*c*	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	k_E	p_{uncorr}	$p_{FWE\text{-}corr}$	$q_{FDR\text{-}corr}$	*T*	$(Z_=)$	p_{uncorr}			
0.000	16	0.994	0.997	74	0.949	0.580	0.996	3.54	2.94	0.002	-18	-16	84
		0.992	0.997	183	0.909	0.820	0.996	3.00	2.59	0.005	4	-38	84
		0.992	0.997	156	0.917	0.951	0.996	2.48	2.22	0.013	-44	18	18
		0.992	0.997	157	0.917	0.962	0.996	2.40	2.17	0.015	40	32	2
		0.992	0.997	191	0.906	0.967	0.996	2.36	2.13	0.017	24	6	-48
						0.969	0.996	2.34	2.12	0.017	28	18	-36
						0.991	0.996	1.97	1.82	0.034	20	2	-40
		0.993	0.997	120	0.930	0.969	0.996	2.33	2.11	0.017	62	-72	-2
						0.982	0.996	2.17	1.98	0.024	64	-68	6
		0.993	0.997	86	0.944	0.970	0.996	2.33	2.11	0.018	22	-74	-8
		0.992	0.997	188	0.907	0.971	0.996	2.32	2.10	0.018	30	16	40
		0.994	0.997	60	0.955	0.971	0.996	2.31	2.10	0.018	50	-6	62
		0.991	0.997	290	0.878	0.973	0.996	2.30	2.08	0.019	-24	12	38
		0.993	0.997	101	0.938	0.983	0.996	2.15	1.97	0.025	-44	-44	0
		0.994	0.997	69	0.951	0.984	0.996	2.14	1.96	0.025	-6	54	-30
						0.988	0.996	2.04	1.88	0.030	-12	62	-22
		0.994	0.997	63	0.954	0.988	0.996	2.05	1.89	0.029	-6	-100	24
		0.994	0.997	72	0.950	0.989	0.996	2.02	1.87	0.031	-54	-26	10
		0.993	0.997	92	0.941	0.990	0.996	2.00	1.85	0.032	-2	-80	50
		0.994	0.997	61	0.955	0.992	0.996	1.94	1.80	0.036	20	-70	-62

table shows 3 local maxima more than 8.0mm apart

Height threshold: T = 1.76, p = 0.049 (0.995)
Extent threshold: k = 50 voxels, p = 0.960 (0.994)
Expected voxels per cluster, <k> = 8205.525
Expected number of clusters, <c> = 5.12
FWEp: 5.664, FDRp: Inf, FWEc: Inf, FDRc: Inf

Degrees of freedom = [1.0, 14.0]
FWHM = 42.8 45.0 40.7 mm mm mm; 21.4 22.5 20.4 {voxels}
Volume: 1890392 = 236299 voxels = 22.0 resels
Voxel size: 2.0 2.0 2.0 mm mm mm; (resel = 9826.52 voxels)

Figure 4.1. Areas of higher FDG uptake in the cannabis users relative to the non-users

SPM glass brain showing areas of greater uptake in the cannabis users (N=8) compared to the non-users (N=8). Significance and Montreal Neurological Institute coordinates are also displayed.

Table 4.1. Demographics and performance test values

PT = 9 hole peg test, Dom = Dominant, Non = Non-Dominant, PASAT =Paced Auditory Serial Addition Test, TUG = Timed up and go, mCTSIB = modified Clinical Test of Sensory Impairment on Balance, ABC = Activities of Balance Confidence, BDI = Beck's Depression Inventory, FSS = Fatigue Severity Scale, PES = Pain Effects Scale, NRS = Numerical Rating Scale of Spasticity, MSQOL = Multiple Sclerosis Quality of Life – 54, PH = Physical Health, RLPP = Role Limitations due to Physical Problems, RLEP = Role Limitations due to Emotional Problems, EWB = Emotional Well Being, HP = Health Perceptions, SF = Social Function, CF = Cognitive Function, HD = Health Distress, SexFunc = Sexual Function, CH = Changes in Health, SSF = Satisfaction with Sexual Function, QOL = Overall Quality of Life

	Non-users	Users	*P-value*
Sex (M/F)	2 / 6	2 / 6	
Age	50.8 (13.2)	49.6 (15.0)	0.875
Disease Duration	14.9 (8.3)	8.4 (7.4)	0.121
Ht. (m)	1.7 (0.1)	1.7 (0.1)	0.186
Wt. (kg)	79.0 (25.5)	75.7 (20.0)	0.521
Handgrip (Dom, kg)	36.9 (9.2)	27.1 (3.8)	0.015 *
Handgrip (Non, kg)	32.5 (10.8)	24.5 (3.1)	0.064
25Ft Walk Test (sec)	6.2 (4.8)	5.8 (2.1)	0.855
PT (Dom)	23.1 (6.6)	24.2 (9.4)	0.798
PT (Non)	22.7 (5.1)	24.7 (7.8)	0.570
PASAT	43.5 (8.8)	31.8 (8.6)	0.018 *
TUG	12.1 (14.3)	9.7 (4.1)	0.644
mCTSIB-EO	48.2 (9.9)	59.1 (16.1)	0.143
ABC	78.5 (18.6)	69.5 (26.1)	0.437
BDI	12.6 (7.1)	14.1 (6.1)	0.657
FSS	4.9 (2.3)	5.3 (1.3)	0.690
PES	13.9 (6.8)	16.1 (6.4)	0.506
NRS	3.6 (4.0)	1.9 (1.8)	0.282
MSQOL-PH	55.6 (30.2)	56.3 (25.3)	0.965
MSQOL-RLPP	40.6 (39.9)	50.0 (46.3)	0.671
MSQOL-RLEP	100.0 (0.0)	100.0 (0.0)	1.000
MSQOL-Pain	65.4 (26.3)	51.7 (22.0)	0.276
MSQOL-EWB	76.9 (28.4)	73.8 (24.0)	0.816
MSQOL-Energy	49.5 (20.9)	39.5 (18.7)	0.331
MSQOL-HP	57.5 (21.5)	55.6 (15.2)	0.844
MSQOL-SF	60.4 (24.7)	60.4 (24.3)	0.997
MSQOL-CF	58.8 (25.5)	48.1 (23.1)	0.397
MSQOL-HD	56.3 (30.3)	56.3 (27.1)	1.000
MSQOL-SexFunc	52.1 (33.3)	76.1 (31.0)	0.158
MSQOL-CH	75.0 (23.1)	43.8 (29.1)	0.032 *
MSQOL-SSF	56.3 (34.7)	53.1 (16.0)	0.821
MSQOL-QOL	67.9 (19.6)	62.1 (20.6)	0.574

Table 4.2. Regions within the brain where cannabis users have greater uptake compared to non-users extracted from SPM analysis.

Regions with significant maxima identified on SPM where cannabis users have greater uptake compared to non-users. Region labels identified using MNI coordinates from SPM output in the Harvard-Oxford brain map available in MRICron. R = right, L = Left

Region Name	Center of Mass	Area (mm^3)
L Precentral Gyrus	-19, -15, 80	592
R Postcentral Gyrus	2, -37, 82	1464
L Inferior Frontal Gyrus – Pars Opercularis	-43, 18, 17	1248
R Inferior Frontal Gyrus – Pars Triangularis	41, 33, 3	1256
R Temporal Pole	29, 14, -37	1528
R Lateral Occipital Cortex – Inferior Division	61, -70, 2	960
R Intracalcarine Cortex	22, -73, -9	688
R Middle Frontal Gyrus	29, 14, 43	1504
R Precentral Gyrus	49, -7, 62	480
L Middle Frontal Gyrus	-23, 9, 41	2320
L Middle Temporal Gyrus, Posterior Division	-46, -43, 0	808
L Frontal Pole	-9, 56, -25	552
L Planum Temporale	-55, -26, 10	576
L Precuneous Cortex	-2, -79, 49	736

Table 4.3. Average Standardized Uptake Values for identified Regions of Interest and calculated effect sizes

Ant = anterior, Post = Posterior

	Non-users (N=8)	Users (N=8)	*P-value*	Cohen's D
Cerebellum (Ant)	6.6 (1.0)	7.2 (1.5)	0.386	0.45
Cerebellum (Post)	6.7 (0.9)	7.2 (1.6)	0.426	0.41
Frontal lobe	7.4 (1.4)	8.0 (1.5)	0.365	0.47
Occipital lobe	8.4 (1.6)	9.3 (2.0)	0.305	0.53
Parietal lobe	8.1 (1.5)	9.0 (1.9)	0.318	0.52
Temporal lobe	6.9 (1.3)	7.7 (1.4)	0.266	0.58
Medulla	5.8 (1.0)	6.1 (1.4)	0.570	0.29
Midbrain	6.4 (1.1)	6.8 (1.2)	0.513	0.34
Pons	5.0 (0.8)	5.4 (1.1)	0.318	0.37

Table 4.4. Pearson's correlations between the performance tests and Region of Interest Standardized Uptake Values across all participants (N=16).

CA = Cerebellum Anterior, CP = Cerebellum Posterior, FL = Frontal Lobe, OL = Ocipital Lobe, PL = Parietal Lobe, TL = Temporal Lobe, PDDS = Patient Determined Disease Steps, PT = 9 Hole Peg Test, Dom = Dominant, PASAT = Paced Auditory Serial Addition Test, TUG = Timed Up-and-Go, mCTSIB-EO = modified Clinical Test of Sensory Impairment on Balance – Eyes Open, ABC = Activities of Balance Confidence, FSS = Fatigue Severity Scale, PES = Pain Effects Scale, NRS = Numeric Rating Scale of Spasticity
* = $P < 0.05$
** = $P < 0.01$

	CA	CP	FL	OL	PL	TL	Pons	Midbrain	Medulla
PDDS	-.514 *	-0.488	-0.348	-0.386	-0.311	-0.35	-.512 *	-0.485	-.538 *
25Ft Walk Test	-0.263	-0.203	-0.084	-0.163	-0.082	-0.124	-0.326	-0.27	-0.303
PT (Dom)	-0.437	-0.44	-0.319	-0.331	-0.283	-0.317	-0.434	-0.422	-0.433
PT (Non)	-0.38	-0.365	-0.267	-0.302	-0.288	-0.266	-0.384	-0.369	-0.378
PASAT	0.013	-0.002	0.048	0.009	-0.002	-0.009	0.129	0.102	0.102
TUG	-0.22	-0.169	-0.023	-0.097	-0.03	-0.059	-0.29	-0.229	-0.251
mCTSIB-EO	-0.19	-0.221	-0.117	-0.114	-0.071	-0.082	-0.207	-0.253	-0.187
ABC	0.428	0.418	0.318	0.359	0.284	0.303	0.408	0.376	0.441
BDI	-0.372	-0.363	-0.399	-0.436	-0.38	-0.36	-0.365	-0.362	-0.367
FSS	-.498*	-0.456	-.504*	-0.477	-0.436	-0.46	-.503*	-.575*	-.512*
PES	-.677**	-.665**	-.557*	-.620*	-.574*	-.557*	-.721**	-.657**	-.714**
NRS	-.513*	-0.457	-0.473	-.528*	-0.483	-.508*	-0.46	-0.466	-.531*

Table 4.5. Pearson's Correlations between the Multiple Sclerosis Quality of Life Inventory and Region of Interest Standardized Uptake Values across all participants (N=16)

CA = Cerebellum Anterior, CP = Cerebellum Posterior, FL = Frontal Lobe, OL = Ocipital Lobe, PL = Parietal Lobe, TL = Temporal Lobe, PH = Physical Health, RLPP = Role Limitations Due to Physical Problems, RLEP = Role Limitations Due to Emotional Problems, EWB = Emotional Well-being, HP = Health Perceptions, Social Function, CF = Cognitive Function, HD = Health Distress, SexF = Sexual Function, CH = Changes in Health, SSF = Satisfaction with Sexual Function, QOL = Quality of Life
* = $P < 0.05$

	CA	CP	FL	OL	PL	TL	Pons	Midbrain	Medulla
PH	.537 *	.510 *	0.452	0.458	0.409	0.429	.506 *	.538 *	.532 *
RLPP	.577 *	.593 *	0.458	0.488	0.434	0.444	.552 *	.509 *	.557 *
RLEP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pain	0.496	.540 *	0.377	0.41	0.37	0.371	0.495	0.45	.507 *
EWB	0.009	0.015	0.155	0.127	0.112	0.121	0.045	0.097	-0.038
Energy	0.131	0.131	0.138	0.129	0.091	0.083	0.138	0.1	0.144
HP	0.291	0.269	0.262	0.238	0.223	0.221	0.351	0.378	0.285
SF	0.367	0.37	0.33	0.342	0.306	0.276	0.338	0.389	0.308
CF	-0.087	-0.06	0.084	0.045	0.045	0.014	-0.033	-0.052	-0.072
HD	.517 *	.499 *	.524 *	.545 *	.498 *	0.492	.523 *	.528 *	0.487
SexF	-0.101	-0.17	-0.1	-0.101	-0.123	-0.103	-0.084	-0.035	-0.13
CH	0.378	0.435	0.367	0.348	0.334	0.345	0.333	0.359	0.388
SSF	-0.462	-0.449	-0.39	-0.422	-0.451	-0.41	-0.483	-0.391	-.515 *
QOL	0.253	0.26	0.333	0.35	0.328	0.289	0.253	0.302	0.199

CHAPTER V – OVERALL CONCLUSION

In the current set of studies we compared measure of disability in people with MS using cannabis and not using cannabis. Our general consensus is that cannabis use does not negatively affect physical function or brain glucose uptake, although it may play a role in cognitive dysfunction. The largest limitation of these projects is that they are all cross-sectional, so causation of differences cannot be identified. Future interventional studies must be conducted to identify which domains of disability are positively or negatively affected by cannabis use. Future projects would ideally have longitudinal and/or interventional designs with standardized cannabis products need to be standardized. Studies have shown that cannabidiol (CBD) may ameliorate some of the negative cognitive effects of the main psychoactive component of the *Cannabis sativa* plant, Δ9-Tetrahydrocannbinol (THC). If the high THC contents of the products most of the participants were using contributed to the cognitive dysfunction seen in these results, increasing CBD content may help lessen or prevent the increased cognitive dysfunction in people with MS.

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