NCT04801641

Impact of chronic nabilone self-administration on body weight, metabolic markers, gut microbiota, and neural circuitry in human obesity

Protocol Number: 084/2018

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The signature below denotes confirmation that this research study will be conducted according to all stipulations of the protocol, and according to local legal and regulatory requirements and ICH GCP guidelines.

PI: Bernard Le Foll, MD PhD _____ Date _____

Introduction and Rationale

Obesity is a serious and growing public health issue. Body Mass Index (BMI) is a common diagnostic measure of body fat calculated by dividing an individual's mass (kg) by the square of their height (m2). As per the standards of the World Health Organization (WHO), a BMI greater than or equal to 25 kg/m2 is classified as overweight and a BMI exceeding 30 kg/m2 is categorized as obese. The WHO estimates that in 2014, 39% of adults were overweight and 13% were obese [1]. Obesity elevates the risk of developing numerous ailments including diabetes mellitus and cardiovascular disease, thereby posing a significant economic burden on society [2]. Developing therapeutic strategies effective for the treatment of obesity is therefore of tremendous importance. The endocannabinoid system has previously been investigated as a possible target for anti-obesity drugs. Rimonabant, an inverse agonist of the CB1 receptor developed for managing obesity, was effective in producing weight loss. Unfortunately, the presence of this anorectic drug on the European market was short-lived due to the high incidence of psychiatric side effects [3]. Like Rimonabant, cannabis also targets the CB1 receptor. We have recently shown that the incidence of obesity is reduced in chronic cannabis users [3] and that exposure to the main active ingredient of cannabis (i.e. delta-9 tetrahydrocannabinol or THC) reduces the weight of obese animals [4]. Here, we would like to explore for the first time the direct impact of a drug that is analog to THC called nabilone (used to reduce nausea and vomiting) in human obese subjects. A positive outcome may potentially drive the development of novel pharmacological approaches for the treatment of this disorder.

Background

The Endocannabinoid System and Appetite Regulation: The endocannabinoid system comprises two cannabinoid receptor subtypes, CB1 and CB2, their corresponding endogenous ligands and the processes responsible for their biosynthesis, cellular uptake and metabolism [5-8]. This system has been implicated in the regulation of appetitive behavior [9]. Preclinical research reinforces the role of the endocannabinoid system in metabolic regulation, and the presence of CB1 receptors in the hypothalamus as well as in limbic regions favors the modulation of both homeostatic and hedonic feeding by cannabinoids [9, 10]. These receptors may also regulate energy metabolism via their distribution in the periphery (e.g. gastrointestinal tract, liver, adipose tissue) [10]. In rodents, acute energy intake is increased by CB1 receptor agonists [11], and is inhibited by CB1 receptor inverse agonists or neutral antagonists [12-16]. In agreement with these findings, acute cannabis consumption has been known to stimulate appetite [17], and this has led to the assessment of its use for the treatment of HIV/AIDS-related cachexia [18-20]. It is likely that these feeding-related effects are mediated by cannabis' primary psychoactive component THC, which is a CB1 receptor partial agonist.

The Endocannabinoid System and Body Weight: A few years ago, we recognized that there was no clear data exploring the relationship between cannabis use and body weight in humans. Using data from 2 adult US epidemiological studies, namely the National Epidemiological Survey on Alcohol and Related Conditions (NESARC) and the National Comorbidity Survey-Replication (NCS-R) study, our group probed the incidence of obesity as a function of cannabis use. Surprisingly, the rate of obesity was found to be significantly lower ($p<0.001$) in frequent (>3 times/week) self-reported cannabis users (14/ 17%) as compared to individuals who did not use cannabis in the last 12 months (22/ 25%) (first value in each set is for NESARC and the second for NCS-R) [3]. This effect was retained even post correction for age, sex, and tobacco

consumption in both data sets (Table 1). Indeed, these findings are impressively robust given the large number of respondents in these surveys [3] and have now been duplicated by other groups [21-23]. It would appear then that the risk of being overweight or obese decreases as frequency of cannabis intake increases [21], as we had initially described. More recently, a lower waist circumference has also been reported in current versus former or never users [24]. Interestingly, a meta-analysis with eight independent replication samples showed an inverse association between active cannabis smoking and diabetes mellitus as well [25].

	NESARC (n=41,633)					
Frequency of Cannabis Use	**Crude OR**	**95% CI**		**Adjusted OR**	**95% CI**	
No use in the past 12 months	**1**	Reference		**1**	Reference	
More than once a year (less than once a month)	**0.71**	(0.54	0.92)	**0.82**	(0.63	1.05)
Once a month to 2 days per week	**0.73**	(0.57	0.93)	**0.79**	(0.62	1.01)
3 days per week to every day	**0.59**	(0.44	0.79)	**0.61**	(0.46	0.82)

Table 1: Association between cannabis use and obesity in multivariate analyses using NESARC data (2001-2002). (Extracted from [3]).

Obesity and the brain: There are multiple factors that contribute to obesity (e.g. excessive food intake and lack of physical activity). However, it appears that obesity is also associated with certain neurobiological changes. It has been proposed that there is an enhanced endocannabinoid tone in this disorder [26-28]. Further, over the last decade, a wealth of information has been obtained on the importance of brain dysregulation in obesity, as it can facilitate enhanced desire for food and hyper-response to food-cues [29, 30]. Brain imaging technologies such as Blood Oxygen Level Dependent (BOLD) MRI has enabled exploration of the neural response to food stimuli in humans [31-34]. Indeed, human imaging studies implicate the hypothalamus, limbic, paralimbic, cortical and prefrontal brain systems in the regulation of ingestive behavior [31-33, 35]. These systems comprise brain regions involved in reward, motivation, emotion, memory and executive control; namely the striatum, orbitofrontal cortex, insula, hippocampus, amygdala and prefrontal cortex [33-35]. Importantly, responsivity of this network to food cues is modulated by weight status [34-37]. Obesity is consistently associated with exaggerated or abnormal regional responses to food stimuli [34, 36, 38, 39]. Evidence also shows that food cue-triggered brain activation in obesity is independent of hunger state, positively correlates with increasing BMI and is altered post-weight loss interventions [34, 38, 40, 41]. One MRI investigation showed that a 7-day treatment with the anti-obesity drug Rimonabant, a CB1 antagonist, decreased the neural response to hedonic food stimuli. Indeed, this may be the mechanism by which Rimonabant drives weight reduction [42].

Obesity and the Gut Microbiota: The gut microbiota has been tagged to play a role in obesity [43-46]. Gut microbiota refers to the aggregate of microbial populations inhabiting the gastrointestinal tract. It consists mainly of bacterial species and to a much lesser degree, fungal organisms [47-50]. Inter-individual variation of the gut microbiota has recently been associated with 126 factors, 63 of which are diet-related [51]. The gut flora also modulates host metabolism, influencing adiposity and energy homeostasis, thereby linking gut microbiota to obesity [52-54]. Relative to conventionally-raised (CR) mice, mice reared in the absence of microorganisms (germ-free, GF)

have significantly less body fat even though their nutrient intake is higher than their CR counterparts. Further, when GF mice are populated with gut bacteria from a CR animal, weight gain and adiposity are triggered. Extension of this work suggests that microbiota may mediate diet-induced obesity as GF mice appear to be immune to the effects of a high-fat, high-sugar diet; potentially through mechanisms that increase fatty acid metabolism [43, 44]. Animal research has additionally shown that obesity-related insulin resistance and inflammation are normalized post treatment with broad spectrum antibiotics that reduce gut bacteria in obese mice, favoring a role for gut microbiota in glucose homeostasis and inflammation [55]. With the development of sophisticated assays, it is now possible to identify and quantify the various bacteria present in the microbiota. Indeed, both preclinical obesity models and clinical research studies, suggest that the ratio of Firmicutes to Bacteriodetes, the 2 dominant phyla of the gut, is disrupted in obesity, seemingly driven by higher levels of Firmicutes and lower levels of Bacteriodetes [4, 48, 56].

Obesity and Non-Alcoholic Steatohepatitis

Obese individuals are at an increased risk of developing nonalcoholic fatty liver disease (NAFLD) [99]. The accumulation of fat in the liver can lead to inflammation and nonalcoholic steatohepatitis (NASH). NASH is characterized by hepatic injury, which can progress to fibrosis and cirrhosis. In response to hepatic inflammation and injury, cytokines and chemokines activate signaling cascades that mediate fibrogenesis and cirrhosis [100]. These cyto-chemokines could potentially represent diagnostic biomarkers for NASH. The endocannabinoid system has been implicated in NAFLD and NASH [101]. Epidemiological evidence indicates that cannabis use may be protective against NAFLD [102].

Nabilone (a cannabinoid drug similar to THC)

Nabilone is a pharmaceutical drug product that is a synthetic cannabinoid agonist that is similar to THC. Nabilone is sold under the brand name Cesamet. It is approved for management of nausea and vomiting associated with cancer chemotherapy (i.e. antiemetic agent). Nabilone is available in capsules of 1 mg, 0.5 mg or 0.25 mg. By oral route it is twice as active as THC (see product monograph). Two final doses of nabilone (2 mg or 6 mg per day) will be used. These doses were chosen as they fall within the recommended daily doses of nabilone as per the product monograph (max 6 mg/day); maximum recommended daily dose is included given that the recruited participants are obese and thus will require larger doses of nabilone (a highly lipid-soluble compound) to achieve therapeutic effects.

Research Question

Can nabilone pharmacotherapy promote weight loss in obese individuals?

Aims and Hypotheses

This is a pilot clinical trial to collect preliminary data on the impact of nabilone on various outcomes related to obesity. As there is currently no available data on the impact of nabilone in obese subjects, this group of investigators would like to collect pilot data as a first step. The results of this study will help designing secondarily a fully powered RCT testing the impact of nabilone in obese patients.

Aims:

Primary Aim: To determine the feasibility (e.g. number of completers, nabilone treatment adherence) and tolerability (e.g. number of SAEs per treatment group) of 12 weeks of daily treatment with nabilone in adults with obesity.

Secondary Aim 1: To compare changes in body weight in individuals with obesity receiving daily nabilone treatment versus those receiving the placebo treatment, for 12 weeks.

Secondary Aim 2: To compare changes in abdominal fat in individuals with obesity receiving daily nabilone treatment versus those receiving placebo treatment for 12 weeks.

Secondary Aim 3: To explore potential underlying mechanisms by analyzing the following between the two groups: metabolic biomarkers (glucose, insulin, triglycerides, cholesterol (total, LDL, HDL) and appetite-related peptides (ghrelin, leptin, PYY)), gut microbiota and brain response to visual food/control stimuli.

Secondary Aim 4: To compare changes in biomarkers of nonalcoholic steatohepatits in individuals with obesity receiving daily nabilone treatment versus those receiving the placebo treatment, for 12 weeks.

Hypothesis:

We hypothesize that nabilone will be well tolerated with few SAEs in this population. As one of our secondary outcomes, we will test for the first time the hypothesis that exposure to nabilone versus placebo promotes weight loss in the human obesogenic state. The data collected in this study will be used to design a fully powered RCT to test the hypothesis that nabilone is an effective pharmacotherapeutic strategy to lower body weight in adults with obesity.

Other secondary objectives will be to collect information on metabolic biomarkers, the microbiome, biomarkers of nonalcoholic steatohepatitis, and neural response to food stimuli (in a subset of participants), where we expect to find that nabilone versus placebo attenuates the neural response to hedonic food stimuli.

Study Design

Overview: This is a double blinded randomized controlled pilot trial. Obese participants (n=60) will be randomized to three treatment groups: low dose nabilone (n=20), high dose nabilone (n=20) or placebo (n=20). Subjects will self-administer nabilone or placebo orally, for a 12 week period. To assess our primary feasibility and tolerability outcomes, we will monitor medication adherence, number of completers per treatment group, and medical safety and tolerability for the duration of the trial. At baseline and weekly thereafter, body weight will be measured for the secondary outcome analysis. To obtain exploratory data in regards to the potential mechanisms mediating any observed changes in weight, subjects will also provide blood and fecal samples to quantify blood markers and gut microbiome, respectively, and complete questionnaires at specific time points. Food intake information will also be collected (via Food Frequency Questionnaire (FFQ)). Participants will eat their regular meals and itemize the food using a validated form developed by the Fred Hutchinson Cancer Research Center [57]. Completed forms (records for 3 days (2

weekdays, 1 weekend day)) will be collected at each post-enrolment visit. Participants will also be able to opt in to 2 magnetic resonance imaging scan sessions during the course of the trial. Standardized dietary modifications and/or exercise-related strategies will be provided to complement the pharmacotherapy. We expect to complete this study in 2 years (30 subjects per year).

Participants: Study subjects will be obese adults (males and females) between the ages of 25 and 45. Obesity will be defined as a BMI $\geq$ 30.0 kg/m^2. The maximum weight of the obese participants opting in to the imaging component of the trial is capped at 315 lbs due to the weight capacity limit of the Magnetic Resonance Imaging scanner. Girth will also be measured to ensure fit in the machine (60 cm horizontal, 45 cm vertical). Participants will be assessed to otherwise have good general health with no physical or psychiatric disorders.

Recruitment:

(1) Recruitment will primarily be organized by Dr. Sean Wharton, Medical Director of the Wharton Medical Clinic (WMC). This clinic (with 6 locations in Ontario) caters exclusively to individuals that are obese (BMI>30; population of interest for this study), or significantly overweight (BMI>27) with co-morbidity. Several studies have recruited individuals with obesity from the WMC or used clinic-generated data in publications related to weight or obesity [58, 59]. Logistically, new or returning patients arriving to the WMC, who meet basic inclusion criteria, will be approached for study participation by WMC staff. Patients will not be provided weight loss medications during this time.

(2) In addition to the WMC, participants may recruited through general advertising (online or posters in the Toronto area). Other recruitment approaches include: posting on the CAMH website (CAMH Research Connect), and sharing/distributing brochures with other obesity clinics, hospitals, or community health centres. Written approval (in the form of an email or letter from a relevant clinic administrator or other management) will be sought from any clinics, hospitals, or community health centres prior to distributing the brochures.

Interventions and Procedures:

Participants will be pre-screened for eligibility by telephone or using the REDCap survey (see REDCap section). During pre-screening participants will be deemed eligible to come for Eligibility Assessment if they meet the inclusion criteria, and deemed ineligible to come for Eligibility Assessment if they report any of the exclusion criteria.

Screening Assessment and Consent:

Following the review and signing of the Informed Consent Form, the following measures and assessments will be administered at the screening visit to confirm eligibility will be performed. If the information obtained on all of the measures below is within the specified qualifying range, subjects will be enrolled in the study. The following information will be collected and reviewed during this visit: (1) Medical exam including psychiatric evaluation (with family history), (2) Vital signs (temperature, blood pressure, heart rate, respiration rate), (3) Height/weight (for BMI calculation) and waist circumference, (4) Structured Clinical Interview for DSM V, (5) 12-Lead Electrocardiogram, (6) Blood work (complete blood count, glucose, electrolytes, liver enzymes

(ALT, AST, ALP, GGT), urea, creatinine, etc), (7) Toxicology screen (urine drug cup), (8) Expired breath carbon monoxide level (Pico smokerlyzer), (9) Pregnancy testing (females), (10) Information on alcohol and caffeinated beverage use (Time Line Follow Back (TLFB) for the past one week) [60], (11) Beck Depression Inventory (BDI) [61], (12) Hamilton Depression Rating Scale (HDRS, 21-item version) [62], (13) Questions related to food likes, aversions and/or allergies, (14) Pittsburgh Sleep Quality Index (PSQI) [63], (15) Berlin sleep questionnaire (BSQ) [64], (16) Eating Attitudes Test (Eat-26 [65], and (17) MRI eligibility to assess there are no MRI scanning contraindications. Selective questionnaires will serve as "baseline data" whereby repeat administration during the trial will facilitate comparison post-dosing. These data (BDI, HDRS, PSQI, and/or BSQ) will contribute to the assessment of tolerability of nabilone pharmacotherapy for obesity (e.g. nabilone-induced changes in mood or sleep patterns).

Randomization:
CAMH's pharmacy will establish the randomization code and maintain the blind. Once enrolled, subjects will be randomized into one of three study arms: (1) low dose nabilone (2 mg/day), high dose nabilone (6 mg/day) or (3) placebo. 2 randomization tables will be created with 30 codes in each, one table for females and one for males. Each table will have 2 blocks of 15. In each block, the assignments will be equally distributed between low dose, high dose and placebo (1:1:1).

After each participant is randomized and assigned a code, an emergency unblinding envelop will be created by the pharmacy. Envelops will be sealed with tamper proof tape. The envelope is sent to the night cabinet/ADU at Queen Street.

If an emergency occurs during pharmacy hours, the QI or delegate will contact research pharmacy staff to unblind the code for that participant. In the event of an emergency after hours, the QI or delegate will contact the after hours site manager at Queen Street who will go to the night cabinet/ADU and open the envelop to unblind the code.

Subjects and research team will be blinded. Notably, during the course of the trial (during the final study visit) we will administer a questionnaire asking participants whether they believe they are consuming placebo or active product. i.e. their perception of the design.

Study Visits:
In addition to the screening assessment, there will be a total of 14 study visits. The first post-enrolment visit will involve the pickup of a stool specimen collection kit and the first food frequency questionnaire. For those opting in to the imaging component a brief mock MRI session will be conducted at the screening or first post-enrolment visit to make sure that participants will be comfortable in the space. Thereafter there will be 13 study visits, each ~1 week apart. For those opting in to the imaging component of the trial there will be two MRI scans scheduled within the study visits. *At all of the 13 visits:* The following information will be collected: Height/weight measurements, waist circumference, FFQ from previous week, vitals, AEs/ medical monitoring (SAFTEE scale (validated method for the systematic assessment of side effects in clinical trials) [65] and general inquiries by health professional), alcohol and caffeinated beverage use (TLFB for the past week [60]), toxicology screen (via urine drug cup), urine pregnancy test (females), visual analog scales to measure mood and appetite, BDI, HDRS, PSQI, BSQ and EAT-26. *Visit 1*: For those opting in, subjects will undergo their first MRI scan (baseline) with up to 1.5 hours of

acquisition. Subjects will bring in their first fecal sample for microbiome analysis. Subjects will be dispensed a 1 week supply of drug or placebo. A blood sample will be drawn that could be used to measure levels of nabilone, endocannabinoids and specific metabolic biomarkers (glucose, insulin, cholesterol (total, LDL, HDL), triglycerides, ghrelin, leptin and PYY). The sample will also be used to measure levels of 7 cyto-chemokines (i.e. adiponectin, tumor necrosis factor, CL-2, IL-17, IL-23, IL-37, and MCP) as biomarkers of NASH. *Visits 2 to 12:* A one week supply of product/placebo will be dispensed. *Visits 5 and 9:* Blood samples will be drawn to quantify measures listed in Visit 1. *Visit 12:* For those opting in, subjects will undergo their second MRI scan with up to 1.5 hours of acquisition. This visit will occur while subjects are still under treatment. Ideally, this visit will occur 1 week after visit 11; however, due to scheduling (both participant and MRI availability), a margin of approximately ±3 days will be allowed. Subjects will bring in their 2nd fecal sample. A blood sample will be drawn to quantify measures listed in Visit 1 as well as routine blood measures from the eligibility assessment (complete blood count, glucose, electrolytes, liver/heart enzymes, urea, creatinine, etc). *Visit 13:* This visit will be post treatment completion. Subjects will return their excess capsules, last food intake form, be examined, compensated and discharged.

Primary Endpoint:
Feasibility (number of completers and medication adherence per treatment group) and tolerability (number of SAEs per group)

Secondary Endpoint 1:
Change in body weight over 12 week period to assess efficacy of nabilone as weight loss agent.

Secondary Endpoint 2:
Change in abdominal fat over 12 weeks in those participants opting in to the imaging component of the study.

Secondary Endpoint 3:
Exploration of underlying mechanisms of obesity by comparing changes in metabolic biomarkers, appetite-related peptides, gut microbiota, and response to visual food/control stimuli between groups over 12 week period.

Secondary Endpoint 4:
Change in levels of cyto-chemokines as biomarkers of nonalcoholic steatohepatitis over 12 weeks.

Overview of Study Visits:

		In-Person Screening/ Eligibility		Post-Enrollment Session	1	2	3	4	5	6	7	8	9	10	11	12	13/Discharge
Assessment & Measures	ICF	X	Post-Enrollment														
	Eligibility Assessment	X															
	Mock MRI Session			X													
	Stool Collection Kit Pickup			X											X		
	Stool Sample Drop-Off				X											X	
	Blood Draw	X			X				X				X			X	
	Genetic Sampling Blood Draw				X												
	Baseline MRI Scan				X												
	Final MRI scan															X	
	FFQ			X	X	X	X	X	X	X	X	X	X	X	X	X	
	1 week supply of capsules provided				X	X	X	X	X	X	X	X	X	X	X	X	
	Return Excess Capsules																X
	Submit Final FFQ																X
	Examination & Discharge																X
					To be completed weekly at 13 Post-Enrollment Sessions: Height/weight measurements, waist circumference, FFQ from previous week, vitals, AE log, TLFB (alcohol & caffeine), toxicology, pregnancy test, VAS to measure mood & appetite, BDI, HDRS, PSQI, BSQ, EAT-26												

Imaging Sessions:

If subjects consent for the optional imaging component of the study, they will be scheduled for the two MRI session (Visit 1 and Visit 12) described above. These sessions will include a brain fMRI scan and abdominal imaging. Brain MRI acquisition: Consenting subjects will undergo two BOLD functional MRI scans at the Centre for Addiction and Mental Health. The first scan will be prior to initiation of treatment and the second, after the 11th week (and before the end of the 12th week) of dosing. Subjects will be scanned at the same time of day, after overnight fasting followed by breakfast consumed 4 hours prior to scans. Participants will be instructed what to consume for breakfast during scheduling of their study sessions and will record the contents of their breakfast

in the FFQ. . Each imaging session will last approximately 1.5 hours in length. Following a high-resolution structural scan, BOLD data will be collected. Details of this BOLD paradigm have previously been described [31, 32]. Briefly, the BOLD imaging protocol will consist of multiple functional runs in which food and control picture stimuli will be presented, using a block design. In addition, we will administer an auction task to assess monetary valuation of the food items presented in the images. Subjects will also be asked to respond to questions regarding their mood and appetite (e.g. how hungry are you right now?) on a 10 point visual analog scale. Presentation software will be used for stimulus presentation. Participants will view the same set of images. Compliance with the task will be monitored using in-bore cameras. Following the BOLD sequence, Arterial Spin Labelling will be performed to measure brain perfusion. Abdominal imaging: MRI is considered the gold standard for measuring body composition [66, 67]. We will use an abdominal coil and a state-of-the-art water-triglyceride fat separation method available on our scanner that uses a chemical-shift-based, three-dimensional, volumetric pulse sequence. It enables accurate estimation of relative triglyceride fat fraction maps by correcting for T2* and performing multi-peak fat spectrum modeling [68, 69]. Both subcutaneous and visceral fat will be measured. If time permits, we will also incorporate quantitative T1 mapping, which has been used to improve segmentation [70] and better characterize adipose tissue [71] since qT1 is sensitive to cell swelling, among other things.

Investigational Pulse Sequences: The CAMH MRI Unit is a research site, and one of our mandates is to develop and test new MRI pulse sequences that will advance our understanding of brain function and structure. CAMH has established a research agreement with General Electric (GE) to help achieve these goals. This research agreement allows us to modify the stock pulse sequences to generate higher quality data, and to operate the scanner in "research mode", which gives us the ability to alter certain parameters of the stock pulse sequences. MRI machines are designed to operate with patient safety in mind. Our GE MR750 has three operating levels: Normal Level, First Level and Second Level. All GE scanners in Canada can operate in Normal and First Level mode, and ours is operated at First Level mode for all studies. Second Level mode is available only if a research agreement is in place with GE and REB approval is in place for any changes to safety thresholds beyond First Level. No studies at CAMH are operated at Second Level. When a pulse sequence is prescribed and loaded into memory, the scanner determines whether the parameters selected will exceed the safety limits for First Level. If they do, the scanner will not allow the scan to proceed as prescribed, and the technologist must change the parameters so that the First Level limits are not exceeded. Some of the images we collect use new methods or "investigational" protocols that we have developed. These do not pose any additional risk to the participant – we can only acquire the images if we are within the established safety limits. The additional sequences will not impact the overall duration of time the participant will spend in the scanner. In addition, these sequences are being added as exploratory outcomes and will not affect the study endpoints. Any software associated with investigational sequences will be installed and maintained by the PI of this study.

Diet and Fitness Strategies

Standardized dietary modifications and/or exercise-related strategies will be provided to complement the pharmacotherapy. The Wharton Medical Clinic will assist with this aspect of the study.

Pharmacotherapy: Treatment and Dosing:

Generic nabilone (0.5 mg pms-nabilone) will be purchased through the CAMH Pharmacy and placebo will be prepared by CAMH Pharmacy. The formulation will be capsules (0.5 mg nabilone/capsule), which will be dispensed by the CAMH research pharmacy. One week's supply of capsules will be provided to participants at each weekly visit. Quantity of nabilone/placebo capsules provided to participants will be measured and compared to that at end of each week to monitor participant compliance. Two final doses of nabilone (2 and 6 mg/day) will be used. The nabilone dose in all non-placebo arm subjects will be titrated up to 2 mg/day (1 mg twice a day, in the morning and at bedtime) over a 1 week period: 2 capsules at bedtime for 3 days then addition of 2 capsules in the morning for the following 4 days. Thereafter, low-dose participants will be maintained on the 2 mg/day regimen and high-dose participants will be escalated to 6 mg/day (3 mg, twice a day): addition of 4 capsules at bedtime for 3 days then addition of 4 capsules in the morning for the following 4 days. There will be no down-titration as this is not usually done in nabilone trials.

All subjects will take 12 capsules each day, 6 in the morning and 6 at bedtime: placebo arm will take 6 placebo capsules in the morning and 6 at bedtime, low dose nabilone group will take 2 nabilone and 4 placebo capsules in the morning and 2 nabilone and 4 placebo capsules at bedtime, and high dose nabilone group will take 6 nabilone capsules in the morning and 6 at bedtime. Subjects will be instructed to consume nabilone/placebo capsules daily for a period of 12 weeks.

Genetic Sampling:

Participants will be asked to review an additional consent form that will ask if they are willing to provide an extra blood sample (~20 mL) for a future genetic analysis experiment that could aid in furthering this research. Blood will be drawn during the first weekly visit, during the routine blood draw. Genetic areas of interest will be identified and genotyping will be done using standard molecular biology techniques to analyze the genetic makeup of these areas of interest. Genetic testing will be performed at CAMH and/or with external collaborators. Samples are stored and de-identified without any personal health information. Samples will be destroyed within 20 years of the study start. If the Principal Investigator moves or retires, the samples will be either transferred or destroyed. These samples may be shared with external collaborators for the purposes of pooling data (e.g., ENIGMA). No feedback regarding genotyping results will be given back to the participants. Participants may choose not to participate as this will have no influence on the rest of the study.

Accountability Procedures:

The nabilone capsules (Drug Identification Number 02380900) will be purchased through the CAMH Pharmacy and transported to CAMH pharmacy following the manufacturer requirements (e.g. for handling, transport, and storage) in order to maintain the stability of the investigational product as per the Product Monograph. Storage and handling procedures will be documented by CAMH pharmacy as applicable and will be in accordance with the Product Monograph (i.e. nabilone will be stored at stable room temperature of 15-30°C). The nabilone presents as a white crystalline powder and is formulated into No. 4 hard gelatin capsules with opaque red cap and white body.

The placebo capsules will be packaged the same way as the nabilone capsules and participants will not be able to tell whether they are receiving active or placebo. CAMH pharmacy will maintain drug accountability logs for both active and placebo, in addition to subject dispensing logs as per CAMH pharmacy SOP. Regular reconciliation checks of investigational product and placebo between CAMH pharmacy and study staff will take place during the study. At the end of the study, there will be a final reconciliation (shipped, used, and remaining) and any discrepancies noted will be investigated, resolved, and documented. Unused investigational product will be destroyed and documentation of destruction will be retained in the study files.

Subject Eligibility Criteria

Inclusion criteria:

- Obese adults (BMI > 30.0 kg/m^2).
- Males and females
- Aged between 25 and 45
- For the optional imaging component of the study, a maximum weight (315 lbs) and a maximum girth in line with capacity of the machine (60 cm horizontal and 45 cm vertical; therefore, circumference of scanner is 166.6 cm)
- For women of reproductive potential (WORP) and men whose sexual partners are WORP: use of adequate methods of contraception (effective barrier methods such as male condoms, female condoms, cervical caps, diaphragms, or contraceptive sponges; and highly effective methods of contraception such as oral hormonal contraceptives, intrauterine devices (IUDs), vasectomy, or tubal ligation)
- AST/ALT, bilirubin, and kidney function tests within normal limits at screening.

Exclusion criteria:

- Unstable gastrointestinal, respiratory, endocrinological, cardiovascular or cerebrovascular diseases that would prevent participation in the trial at QI (or its delegate) discretion,
- Unstable major psychiatric disorder(s) (i.e. Axis I Disorders) that would prevent participation in the trial at QI (or its delegate) discretion,
- Current substance use disorders (DSM-V) (excluding tobacco and caffeine),
- History of, or current neurological illnesses, that would prevent participation in the trial,
- Current use or use during the previous month of antipsychotic medications,
- Learning disability, amnesia or other conditions that impede memory and attention,
- Visual impairments that prevent participation in the study,
- Personal or family history of schizophrenia, or psychosis (or psychosis-related) disorders,
- Antibiotic use in the last 4 weeks,
- Previous bariatric surgery,
- Current use or use in the past month of other weight-loss pharmaceuticals,
- Cannabis use in last 6 months,
- Known sensitivity to cannabis or other cannabinoid agents,
- Pregnancy or lactation (females), and
- For the optional imaging component of the study:
    - Presence of metal implants or objects unsafe for MRI such as cardiac pacemakers, metal fragments in the eye, and aneurysm clips in your brain
    - Piercings or jewelry that are unable to be removed

- Tattoos inked with metal dyes

Concomitant medications:

The following medications are contraindicated:

- Antipsychotic medications
- Weight-loss pharmaceuticals
- Cannabis
- Medications that would affect participant safety in trial as assessed by the Qualified Investigator

In addition, antibiotics are a relative contraindication at the discretion of the QI.

Based on the product monograph, there is a potential for interactions between nabilone and diazepam, sodium secobarbital, alcohol, and codeine. Thus, participants will be instructed not to take nabilone with alcohol or any sedative-hypnotics or other psychotomimetic substances.

Screened participants' medications, treatments, and procedures both past and present will be assessed by the Qualified Investigator. Permission to enroll and partake in the study will be up to the discretion of the Qualified Investigator.

Rescue Medication: In the event of adverse side effects to the nabilone, appropriate management will be provided to the participant. No rescue medications will be used.

Consent

All subjects will provide written informed consent prior to study enrolment. i.e. before undergoing any study-related procedures.

Data Collection

Data for this study will be collected via paper forms and/or using REDCap. Any documents from the participant binder including questionnaires completed by the participants and information collected by study personnel using data collection tools will be considered source documents. REDCap may be used as an electronic data capture tool and data collected directly on REDCap will also be considered as source. If REDCap is implemented, all necessary procedures will be followed to ensure compliance with guidelines for regulated studies.

The study results will be stored under a REDCap database in the Krembil Center for Neuroinformatics at CAMH. This will allow us to perform analysis combining multiple modalities (phenotype, genetic, imaging) and to compare with other samples collected either by CAMH investigators or by international collaborators. Anonymized data may be shared with other consortiums of investigators for analysis.

REDCap may be used for electronic data capture. If it is, no personal health information will be entered into REDCap except for a Project that contains only Personal Identifiable Information (PII). The PII collected will consist of name and phone number or email address. The purpose of collecting this PII will be to contact the participant after the screening survey. The participant will access the survey via a provided URL and complete the initial screen. This screen will be piped to a separate project that contains only the PII. No data will be stored with the PII. The screening

survey and PII survey will be linked by piping the participant ID from the screening survey to a unique field code in the PII survey. The use of a REDCap survey will facilitate recruitment because it will provide participants with an efficient means of preliminary screening. It can be problematic to reach by telephone after answering an advertisement because participants often do not answer the phone when the call number is not known. REDCap may be used as Source Documents and CRFs.

Sample Size and Power Calculation

Our proposed 'pilot' sample size is 60 completers (placebo (n=20), low dose nabilone (n=20) and high dose nabilone (n=20)). Combining mice data showing that chronic treatment with both THC [4] and SR141716 (Rimonabant) [72], reduced energy intake and prevented diet-induced obesity (with the response being ~36% greater with Rimonabant), with a 1 year controlled human Rimonabant trial promoting weight reduction [73], we performed a power calculation. A sample of 20 completers (high dose nabilone) was found to be sufficient to achieve 45% power to detect an effect equivalent to a drop of 3.0% in the nabilone arm as compared to a drop of 1.8% in the placebo arm. The power analysis was conducted based on a series of Mont Carlo simulations where synthetic data was generated that mimic the change in the outcome that we expect to find in our trial, and mixed effect models are adjusted to each replication, with the power being defined as the proportion of the replications in which the specified effect was detected. 5000 replications were generated for each sample size. The analysis also assumed a significance level of 0.05, two tailed tests and a correlation between baseline and post of 0.9. We are confident that this pilot study will not only generate an 'effect' but will also yield preliminary safety and feasibility data regarding the use of nabilone for obesity, facilitating a larger trial.

Data Analysis

An initial descriptive and exploratory analysis of the sample will be performed where differences between groups at baseline will be accessed with chi-square tests for categorical variables and One-way ANOVA for continuous variables. Non-parametric tests may be used if the normality assumption is found to be violated. This approach will be taken to test the primary aims of the study (feasibility and tolerability). A mixed effect model, where subject is the random effect and treatment (Nabilone/Placebo), time (baseline, week 1, week 2 …week 12) and treatment by time interaction will enter the model as categorical fixed effect. The hypothesis that nabilone will reduce body weight in adults with obesity will be tested by a contrast that involves the interaction term and compares both treatments regarding their weight at week 12 versus weight at baseline. Intention to Treat analysis will be used and drop-outs will not be removed from the analysis. Mixed Effect models can handle missing values through full maximum likelihood estimation which does not drop any subject but rather uses all available information. Missing values analysis will be conducted by comparing drop-outs with completers in terms of their baseline information. Waist circumference data will be analyzed in a similar fashion. Glucose, insulin, cholesterol, triglycerides, ghrelin, PYY, leptin, endocannabinoids, and/or nabilone between the 3 groups (low/high dose nabilone and placebo), at the 4 time points will be analyzed using a repeated measures ANOVA. Repeated measures ANOVA will also be employed to examine the microbiota data. The criterion for significance for all analyses will be a two-tailed alpha of 0.05. Questionnaire data will be scored as per the validated guidelines of the specific questionnaire (references previously provided). Food intake data will be analyzed using NUTRITIONIST PRO™ DIET ANALYSIS SOFTWARE (Axxya Systems) to assess the intake of macro- and

micronutrients. Depending on the findings, exploratory regression analyses (using general linear models) will be applied to study the relationship between body weight and microbiome, food intake and/or metabolic (markers) data. AEs will be compiled and analyzed as per our previously published clinical trial with Sativex [74]. Sex and age will be used as covariates in the analysis process. SPSS (SPSS Inc., IL, USA) and/or Graphpad Prism will be used to conduct the analyses and for graphing. Interim data analysis may occur.

Imaging data analysis: BOLD fMRI analyses have been described in detail before [31, 32]. Briefly, functional BOLD images will be spatially smoothed with a 6 mm Gaussian filter and motion corrected. A general linear model will then be designed using separate regressors for food and control pictures, consisting of boxcar functions convolved with a standard hemodynamic response function. Regional brain activation will be determined by calculating a contrast of food minus control and generating effect and standard deviation at each brain voxel for each individual. These parametric images will be transformed into Montreal Neurological Institute space [75] and a group analysis conducted using a mixed effects statistical model. Using SPM, individual T-maps will be corrected for multiple comparisons by exclusively listing brain regions containing clusters of voxels with $p < 0.001$ and a volume > 100 ml. This will effectively reduce the risk of false positives to less than 1 in 20 (i.e., $p < 0.05$) for the study. Significant peaks in MNI coordinates will be identified and reported in tables with corresponding t-values. ASL data will be analyzed as per validated methods [76-81]. Change from baseline abdominal fat imaging data will be assessed between nabilone and placebo groups.

Microbiome Profiling: Microbiome profiling will be performed at two time points: (1) before pharmacological intervention and (2) during the later part of the intervention phase. Shotgun metagenomic libraries will be constructed using standard protocols and paired-end sequencing (2x150nt) will be carried out on an Illumina HiSeq at $30x10^6$ reads/ sample. Sequences will be processed through in-house bioinformatics pipelines to remove low quality sequence and human DNA. Filtered reads will be assembled using metaSPades [89] and binned using MetaBat [90]. Bins will be evaluated using CheckM [91] and taxonomy assigned using Kraken [92]. Assembled contigs and bins will be annotated using Prokka [93]. Raw (filtered) reads will also be evaluated for genes and functional predictions and comparisons across sample groups will be computed using HUMAnN [94]. All analyses will occur under the direction of collaborator Dr. Michael Surette, Director of both the McMaster Genomics Facility and the Laboratory of Interdisciplinary Microbiome Research.

Study Monitoring and Access to Study Documents

The PI/ QI will permit trial-related monitoring, audits, REB review, and/or regulatory inspections, by providing direct access to source data/documents. This will be mentioned in the Informed Consent Form. Monitoring will be conducted according to the study monitoring plan.

Ethical Considerations

Prior to study start, the Investigator will address all review comments from the CAMH REB to the satisfaction of the CAMH REB. The Investigator will also obtain approval from Health Canada, then obtain final approval from the CAMH REB as well as meeting all other Health Canada requirements for initiating the study (Clinical Trial Site Information Form).

Risks and Inconveniences

Blood Collection: Blood collection may cause some bruising and discomfort at the site of needle stick, and rarely, a small infection at the skin puncture site. These risks are minimized by using proper techniques.

MRI: Study staff will conduct MRI safety screening to determine whether it is safe for the participant to be scanned. Some people may feel uncomfortable lying still in the confined space of the MRI scanner. A mock MRI session will be conducted to consenting participants. Tingling sensations are felt by some people during certain scans or occasionally, some participants feel dizzy for a few minutes at the end of the MRI study. These are infrequent, but expected sensations. In the case of an incidental finding arising from imaging, the procedures in the MRI Unit Incidental Findings Policy will be followed.

Cannabinoid exposure: Acute and chronic exposure to cannabinoids (recreational or medicinal) has been reported to have adverse effects, including the possibility of addiction [95-98]. However, a Canadian systematic review examining adverse effects of medical cannabinoids found that in 23 randomized controlled trials with oral or oromucosal administration, 96.6% of the adverse events (AEs) were not serious, with dizziness being the most frequently reported in the cannabinoid-exposed arm. Of the serious AEs (SAEs), the most common were relapse of a previous medical condition (e.g. multiple sclerosis), vomiting and urinary tract infection. Notably, the rate of non-serious AEs was higher in the cannabinoid-exposed group than controls but did not significantly differ between groups for SAEs. The range of cannabinoid exposure was 8 hours to 12 months (median: 2 weeks) [98]. As per the nabilone Product Monograph, the most commonly observed adverse reactions to nabilone as reported in the course of clinical trials include drowsiness, vertigo, psychological high (e.g. euphoria), dry mouth, depression, psychomotor impairment, blurred vision, and sensation disturbance. In addition, the following rare AEs have been reported in less than 1% of patients administered nabilone in previous clinical trials: tachycardia, tremors, syncope, nightmares, distortion in the perception of time, confusion, dissociation, dysphoria, psychotic reactions and seizures. Weekly medical monitoring will allow the ongoing assessment of the any emerging effects of the pharmacotherapy. Any medical events will be promptly evaluated by the QI. Further, participants will be provided a physician contact card for any urgent medical inquiries.

Questionnaires: There are no risks associated with the questionnaires, except possibly fatigue. Breaks will be provided as required.

Benefit to participants: Should the nabilone pharmacotherapy be successful for weight loss, obese subjects will directly benefit from participation in the study.

Study Documentation

Investigators will retain a participant identification code list if they need to contact participants after the study. This list will contain the complete name, identification number, address, phone number and email of all participants and will be held confidentially at the investigator's site after completion of the study.

Archiving of Study Documentation
Study data and other essential documents will be retained in a secure setting by the investigators for a period of 15 years as required by current regulations.

Confidentiality
All personal study participant data collected and processed for the purposes of this study will be managed by the investigators and their research staff with adequate precautions to ensure the confidentiality of this data, and in accordance with applicable national and local laws and regulations on personal data protection. The Ethics Committees approving this research, monitors and Health Canada will be granted direct access to all source documents for verification of clinical trial procedures and/or data, without violating the confidentiality of the participants, to the extent permitted by the law and regulations. In any presentation of study results (at meetings or in publications), participant identity will remain confidential.

Participant Safety and Adverse Events
As nabilone is already an approved drug and is available on the Canadian market, this is not a safety study. However, the occurrence of adverse events resulting from study-related pharmacotherapy is a possibility. In this proposed 12 week study, possible side effects will be monitored on a weekly basis in accordance with GCP, and subjects will be advised to seek prompt medical attention for any (serious) adverse effects. Further, the Qualified Investigator (QI) will ensure patient safety. Potential safety concerns and associated solutions will be discussed at regular study meetings. The QI (along with any medical professionals delegated by the QI) will monitor, treat and follow-up on any emerging side/adverse effects. In the event of AEs, the QI will determine severity and expectedness of the event along with any causal association to the study interventions. A physician contact card will also be provided.

Additional Safety Procedures:
1. Participants will be provided with the QI contact information, in case of any unexpected side effects.
2. If necessary, the participants will be transferred home via taxi.

Adverse Events Reporting
AEs will be assessed at each study visit. Study staff will question the subject directly asking: how they are feeling and how have they have been feeling since their last visit. All AEs, whether reported by the subject or observed by study staff/investigators, will be recorded on the AE log along with a brief description, start date/resolution date and any action taken. The AE log will be initialed by the QI, who will make the determination on relationship of the AE to the investigational drug/study procedures. All unexpected AEs determined by the QI to have a causal relationship with the investigational product or as a result of study procedures, will be reported to CAMH REB as part of the annual ethics approval renewal process. Study personnel will manage and report AEs as per CAMH's Standard Operating Procedures.

AEs and SAEs will be reported to the REB consistent with their policy. The Qualified Investigator will report all Serious and Unexpected Adverse Drug Reactions (SUADR) to Health Canada as per Division 5 regulations. All AEs (including SUADRs) will be recorded and described in the AE log.

Type and duration of the follow-up of subjects after adverse event: The Qualified Investigator is responsible for determining the duration of follow-up required for any adverse event dependent on the seriousness of the event. AEs will be followed to resolution if possible.

Termination of the Study

Reasons for withdrawing individual subjects from the study may include one or more of the following:

1) Failure to continue to meet inclusion criteria;
2) Severe side effects related to pharmacotherapy;
3) Major protocol violation;
4) Subject lost to follow-up;
5) Withdrawal of consent;
6) Pregnancy

Notably, any subject may be discontinued from the study at the discretion of the Qualified Investigator if it is deemed to be in the best interest of the subject.

Sixty participants will be enrolled. Dropouts or withdrawn participants will not be replaced. Enrolment will continue until the recruitment target is met or the trial is stopped. Participants withdrawing from the study will be contacted by the study research team requesting to follow up with any unresolved adverse events. Once withdrawn from the study, no further study data will be collected. However, every effort will be made to obtain permission to document the reason for withdrawal. Data collected until the time of withdrawal/dropout may be used in analyses.

Follow-up for subjects withdrawn from investigational product treatment/trial treatment: The Qualified Investigator will determine the duration of follow-up required for subjects withdrawn from investigational product treatment/trial treatment (e.g. if the reason is any adverse event dependent on the seriousness of the event).

Criteria for the Termination of the Trial

The study will be terminated upon completion of the recruitment targets. The study may also be terminated due to adverse events or at the discretion of the QI/Sponsor.

Funding

This study is funded by the Physician Services Incorporated Foundation and a Canadian Institutes of Health Research (CIHR) Catalyst Grant.

Participant Compensation

1 Assessment visit: $35
1 Pick-up Visit: $10
9 Routine Visits: $180 ($20/ visit)
4 Routine Visits with blood sampling $140 ($35 per visit)
2 Imaging Sessions: $230 ($115 per visit)
1 Genetic Sampling blood draw: $25
Should participants have to come in for any extra time they will be compensated $15/hour.

TOTAL: $620
(Taxi vouchers or tokens will be provided when/if needed).

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