The Influence of Cannabis Inhalation During Exercise on Cardiovascular Health and Function: Exploring the Optimal Balance of Cannabinoids and Mode of Administration to Decrease Risk and Maximize Benefits

PROTOCOL IDENTIFIER:	UOG-HPL-CAN **(**REB# 18-11-013)
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Table of Contents

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP). The Principal Investigator-Sponsor will assure that no deviation from, or changes to the protocol will take place without prior agreement from Health Canada, or the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants.

The protocol, informed consent form(s), recruitment materials, and all participant materials have been submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	The Influence of Cannabis Inhalation During Exercise on Cardiovascular Health and Function: Exploring the Balance of Cannabinoids and Mode of Administration to Decrease Risk and Maximize Benefits
Study Description:	The current study will examine the acute cardiovascular response to cannabis consumption at rest, and when combined with exercise. It will examine the influence of inhalation method, and cannabinoid concentration.
Objectives:	Primary Objective: Determine the effects of cannabis consumption at rest and during exercise on the human cardiovascular system Secondary Objective: ıInvestigate the influence of consumption methods (smoking vs. vaporizing) and cannabinoid balances (i.e. THC vs. CBD). Determine the effects of cannabis consumption on exercise performance
Endpoints:	Primary Endpoints: Carotid-Femoral Pulse Wave Velocity, Brachial Artery Flow-Mediated Dilation, Cardiac Function (dynamics), Muscle Sympathetic Nerve Activity Secondary Endpoints: Blood Pressure, Heart Rate, Work Completed, Rate Pressure Product, Rating of Perceived Exertion
Study Population:	20, healthy male and female recreational cannabis users aged 19-45yr will be recruited for the current study
Description of Sites/Facilities Enrolling Participants:	All activities will be performed at the Human Performance and Health Research Laboratory in the Department of Human Health and Nutritional Sciences, College of Biological Sciences, at the University of Guelph. All research will be conducted in Guelph, Ontario, Canada. Participants will healthy volunteers.

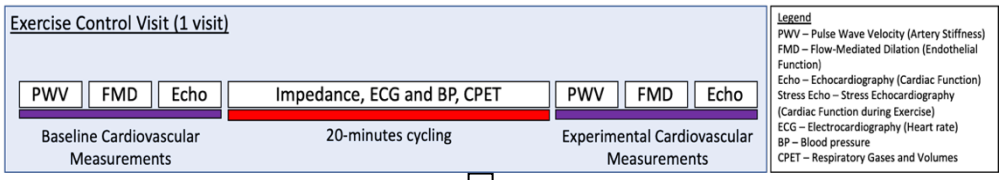
Description of Study Intervention:	Participants will consume 100mg of cannabis via smoking or vaporizing. Cannabis consumed will possess THC and CBD ratios of either 13.6±2.7%:<1%, or <1%:14.5±2.9%.
Study Duration:	It is estimated that the current study will require a 12 month period for completion, beginning from the onset of study enrollment, to the completion of data analysis
Participant Duration:	Depending on degree of involvement, participant duration of this study will be either 6 weeks, or 10 weeks.

1.2 SCHEMA

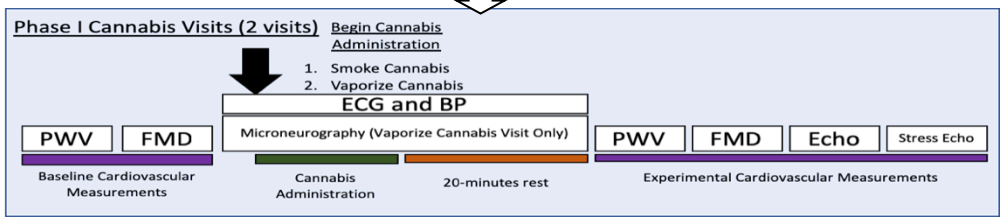
Visit 0 Introductory Visit

Obtain informed consent, screen for participant eligibility, obtain measures of height, weight, ECG, blood pressure, and heart rate. Measure maximal aerobic capacity via VO_2 max test.

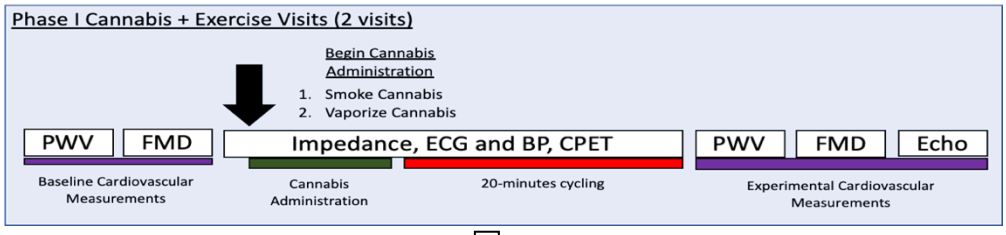
Visit 1 Exercise Control Visit



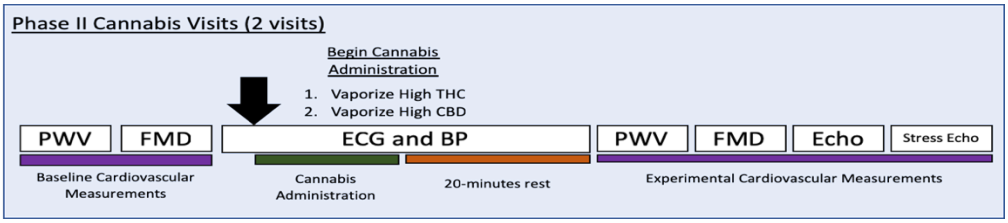
Visit 2-5 Phase I Cannabis Visits



Visits 2-5 Phase I Cannabis + Exercise Visits



Visit 6-9 Phase II Cannabis Visits



Visit 6-9 Phase II Cannabis + Exercise Visits

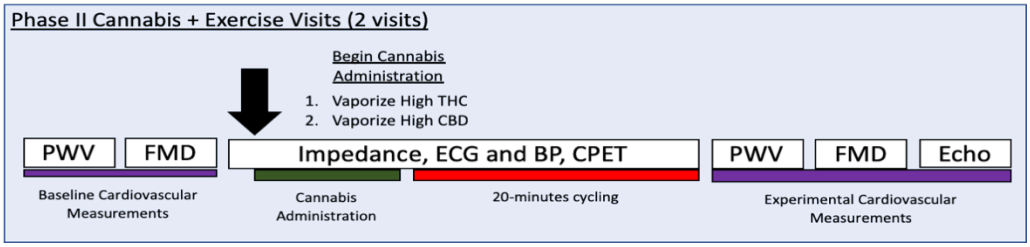


Figure 1. *Schematic breakdown of study structure, and schedule of measures.*

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedure	Introductory Visit (Visit 0), Day 0	Exercise Control Visit (Visit 1), Day 7±1 day	Phase I Cannabis Visit (Visit 2), Day 14±1 day	Phase I Cannabis + Exercise Visit (Visit 3), Day 21±1 day	Phase I Cannabis Visit (Visit 4), Day 28±1 day	Phase I Cannabis + Exercise Visit (Visit 5), Day 35±1 day	Phase II Cannabis Visit (Visit 6), Day 42±1 day	Phase II Cannabis + Exercise Visit (Visit 7), Day 49±1 day	Phase II Cannabis Visit (Visit 8), Day 56±1 day	Phase II Cannabis + Exercise Visit (Visit 9), Day 63±1 day
Informed Consent	X									
Eligibility Questionnaire	X									
Physical Activity Readiness Questionnaire	X									
Height	X									
Weight	X	X	X	X	X	X	X	X	X	X
Blood Pressure and Heart Rate	X	X	X	X	X	X	X	X	X	X
Urine Test	X	X	X	X	X	X	X	X	X	X
Inquiry of Changes to Health and Prescription Status		X	X	X	X	X	X	X	X	X
VO_2 Max Test	X									
Pulse Wave Velocity		X	X	X	X	X	X	X	X	X
Brachial Artery FMD		X	X	X	X	X	X	X	X	X
Echocardiography		X	X	X	X	X	X	X	X	X
20 Minute Maximal Cycling Exercise		X		X		X		X		X
Vaporization of High THC Cannabis			X	X				X	X	
Smoking of High THC Cannabis					X	X				
Vaporization of High CBD Cannabis							X			X
Indirect Calorimetry/CPET	X	X		X		X		X		X
Microneurography (MSNA)			X							
Stress Echocardiography			X		X		X		X	
Measurement of Continuous Blood Pressure		X	X	X	X	X	X	X	X	X
ECG	X	X	X	X	X	X	X	X	X	X
Impedance Cardiography		X		X		X		X		X
Drug Effects Questionnaire			X	X	X	X	X	X	X	X
This study will use a randomized crossover design. Phase I of this study will occur prior to the initiation of phase II. If a participant enters the study in phase II, but did not participate in phase I, they will be required to complete the introductory and exercise control visits. Visits 2-5 and 6-9 will occur in a randomized order, which will be individualized for each participant.										

2 INTRODUCTION

2.1 STUDY RATIONALE

Cannabis is used by a large number of Canadians, with a lifetime prevalence of use of 42.5%, and 12.2% of Canadians reporting having used cannabis in the past year[1]. With the recent change in federal legislation legalizing recreational use, cannabis consumption is likely to increase. Given its previously illicit status globally, there is a significant lack of research examining the effects of recreational cannabis use on cardiovascular function. This lack of knowledge on the effects on human physiology is a gap that must be filled given the possible increase in widespread use among Canadians. Studies performed in animal models suggest a number of adverse cardiovascular effects of cannabis consumption, including reduced endothelial function, increased arterial stiffness, and sympathetic nerve activity[2–4]. Each of these outcomes have been implicated as risk factors for cardiovascular disease[5–7]. One area that has been especially unexamined is the unique physiological effects of cannabis of varied cannabinoid compositions, specifically high and low concentrations of delta-9-

tetrahydracannabinol (THC) and cannabidiol (CBD). Additionally, minimal research has examined the different effects of two popular methods of consumption, smoking and vaporizing. Preliminary evidence has suggested that vaporizing removes some of the harmful components associated with smoking, such as the intake of particulate and carbon monoxide[8,9]. Research has also identified numerous adverse cardiovascular effects of smoking THC-containing cannabis, in addition to other harmful pro-inflammatory and pro-oxidant effects[4]. In contrast, CBD has been identified as possessing anti-inflammatory[10] and anti-oxidant effects[11,12]. Understanding consumption of cannabis of varied cannabinoid content through different methods of intake on the cardiovascular system is necessary to properly understand the health risks of recreational cannabis use, and how such risks can be mitigated. Unsurprisingly, no research has been conducted on whether or not these cardiovascular effects persist under the additional cardiovascular stress of exercise. This question becomes of relevance considering recent evidence that suggests that concurrent cannabis use with exercise is increasingly common[13]. Furthermore, exercise is a well-established transient stress that can induce temporary arterial stiffening[14], impaired endothelial function[15], and increased myocardial workload[16]. The independent effects of cannabis and exercise have never been comprehensively tested in combination and, thus, the current study seeks to identify the cardiovascular response to this combined stimulus.

2.2 BACKGROUND

Plant species of the *Cannabis* genus have been used by humans for millennia, with the earliest use possibly occurring as early as 8000BC[17]. Despite its long history of use by humans, both the physiological effects and the therapeutic potential associated with cannabis consumption remain incompletely understood. A substantial proportion of clinical research focusing on cannabis has attempted to evaluate its efficacy for treating non-communicable diseases such as multiple sclerosis and epilepsy, as well as for treatment of pain[18–20]. Due in part to this work, cannabis can now be physician prescribed to patients in a number of countries globally[21]. While a large body of clinical research has aided in unveiling the utility of cannabis in improving a range of outcomes, a comprehensive understanding of the physiological effects of the plant is lacking.

It is well established that consumption of cannabis can induce a state of euphoria, commonly referred to as a subjective “high”. The primary mechanism responsible for these effects is the interaction between delta-9-tetrahydrocannabinol (THC), commonly the most abundant cannabinoid in cannabis[22]; and the endogenous cannabinoid receptor 1 (CB1)[23]. When cannabis is consumed, concentrations of dopamine are increased in the brain[24], leading to a “high”. A large proportion of recreational cannabis use can be attributed to the psychoactive effects associated with cannabis consumption. Despite cannabis being best known for containing THC and consequentially possessing neurological effects, species of the plant contain over 100 different cannabinoids[22], and cannabinoid receptors are expressed by numerous types of cells throughout the body[25].

Previous research has associated cannabis use with the occurrence of stroke[26] and myocardial infarction[27]. In light of this finding, subsequent research has identified that CB1, and the second putative endogenous cannabinoid receptor 2 (CB2), are expressed throughout the different components of the cardiovascular system[28,29]. To date there is a lack of knowledge regarding both the long-term cardiovascular consequences of cannabis consumption, and the acute cardiovascular response. The impact of cannabinoid exposure on the cardiovascular system has been tested in numerous in vitro and in vivo models with equivocal results; in some cases,

suggesting detrimental effects and others suggesting therapeutic potential. In murine models, regular cannabinoid treatment has been demonstrated to attenuate the development of atherosclerosis[30] and provide cardioprotection[31]. Conversely, acute cannabinoid exposure, acute secondhand cannabis smoke, and habitual cannabis smoking have been associated with decrements in cardiac function[28], impaired vascular endothelial cell function[3], and vascular aging[32], respectively. These contrasting findings clearly demonstrate the complexity of the cardiovascular consequences of acute and habitual cannabis.

Given the lack of a comprehensive understanding of short- and long-term cannabis use on cardiovascular health, the need for such an investigation is pertinent to public health. Understanding the cardiovascular consequences of long-term use would allow policy makers to continue to develop informed health recommendations and warnings regarding recreational cannabis use. The necessity of this knowledge is accentuated by the recent legalization of recreational use, and a likely increase in cannabis consumption amongst the general public.

The current trial seeks to observe the acute cardiovascular response to cannabis consumption using cardiovascular measures that have been identified as predictive of long-term cardiovascular health. These outcomes will provide insight into the potential long-term impact of recreational cannabis consumption on cardiovascular health.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Largely owing to elicit status, a comprehensive risk profile of cannabis consumption has not been developed. There does, however, exist a number of immediate and long-range risks. It has been established that consumption of cannabis containing THC results in impairments in cognition and memory[33,34]. In addition to these impairments, users may experience panic attacks[35]. The most severe psychological risk of consuming cannabis is the development of a mild to severe psychotic episode[36], which occurs on rare occasions. The diverse effects of cannabis on the brain have resulted in the recommendation that cannabis should not be consumed prior to operating motor vehicles. Impairment may typically last between 3-4 hours[37], with some evidence indicating that in impairment can persist for 6-8 hours[38].

In addition to immediate psychological risks, cannabis consumption is associated with a number of physical risks. Smoking cannabis can result in a transient sinus tachycardia[39]. Reports have associated cannabis use with acute cardiovascular events[27] and stroke[26], although a causative relationship has not been established. Despite these physical risks, cannabis is largely considered safe[40]. In rare instances, cannabis consumption can induce hyperemesis[41].

Cannabis use may also present immediate economic risks to some individuals. Due to the long-standing elicit status and the only recent legalization of recreational use, many employers still prohibit cannabis use by employees.

Long-range risks of cannabis use are less understood than acute risks. Physical and psychological risks have been identified, however, certain long-range risks have not been directly attributed to cannabis itself, due to the existence of certain behaviors or requisite activities associated with use. Physical long-range risks include reductions in pulmonary function[42–44], respiratory infection[45], and alterations in brain structure[46]. Long-term cannabis use has been associated with an increased rate of development of schizophrenia[47,48], mood and

anxiety disorders[49], and bipolar disorder[50]. Early habitual recreational cannabis use is a strong risk factor for development of cannabis use disorder[51]. Although not fully understood, cannabis use in adolescents has been associated with lower educational attainment[52].

2.3.2 KNOWN POTENTIAL BENEFITS

The benefits of cannabis and cannabinoids have largely been derived from clinical and pre-clinical studies examining cannabis and cannabinoids for their efficacy in treating symptoms of chronic disease. Acutely, cannabis and cannabinoids have been reported to suppress nausea and vomiting[53], with particular efficacy in patients receiving chemotherapy[54]. Cannabis has also been shown to be effective in stimulating appetite[55], leading to the approval of Dronabinol (THC) prescription for the treatment of HIV-related anorexia by the U.S. Food and Drug Administration[56]. Particular interest in the ability of cannabis to treat neurological disorders exists, as its therapeutic potential has been examined in patients with epilepsy[57], chronic neuropathic pain[58–60], and multiple sclerosis[61–63]. In patients with non-cancer pain, evidence exists to suggest that cannabis may be efficacious in reducing symptoms[64]. Cannabis may also be an effective treatment strategy for improving spasticity in multiple sclerosis[62]. Cannabis containing CBD may also possess anti-epileptic effects in children and young adults with treatment-resistant epilepsy[65].

It is important to highlight that the therapeutic benefits of cannabis have largely been explored in animal models and clinical populations. Of interest to non-clinical populations, cannabis and certain cannabinoids may have beneficial effects on mood[66,67]. This benefit, however, is founded upon a limited evidence base, as the strongest evidence that exists to support mood-elevating effects of cannabis have been demonstrated in a population of patients suffering from major depression secondary to chronic disease[59,68–70]. Whether or not this benefit exists with recreational use remains to be examined. In addition CBD has been shown to be anxiolytic[71,72]. Similar to mood elevating effects, anxiolytic effects have not been demonstrated in a non-clinical population, though it has been demonstrated using animal models[73].

Of particular interest to the present study is the anecdotal and reported benefits of cannabis use during prolonged exercise. Recently, it has been reported that cannabinoids are the most widely used performance enhancing substance by ultra-endurance athletes[74]. This is the first published report that supports the widespread use of cannabis during ultra-endurance exercise; a previously anecdotal fact. The perceived benefit of cannabinoids in ultra-endurance exercise may be explained by the potential anti-nausea and anxiolytic effects that have been demonstrated in clinical populations. Intriguingly, studies examining the effects of cannabis smoking on work capacity provide contradictory results, suggesting that cannabis reduces exercise capacity[2] and reduces the time to onset of angina during exercise[75]. In interpreting these contradictory reports, it should be noted that the effects of cannabis consumption on exercise performance have not been rigorously examined[76], thereby justifying one of the aims of the present study.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The large number of pre-clinical studies and clinical trials that have examined medicinal cannabis use in the treatment of chronic disease have resulted in a large body of literature; detailing many potential benefits and risks of cannabis use. Largely owing to interest in the therapeutic potential of cannabis across a multitude of disease populations, far less effort has been directed toward understanding the risk-benefit profile of recreational cannabis use in a

non-clinical population. As of October 2018, recreational cannabis use was legalized in Canada for individuals aged 19 and older. Given the already prominent use of cannabis[1] among Canadians, and a potential increase in use associated with legalization, the need for an understanding of the risk-benefit profile of cannabis under recreational conditions is pertinent. Furthermore, the recently published reports of the prominence of cannabis use in endurance sport[74] warrants the need for a greater understanding of the interaction between cannabis and exercise.

When assessing the risk-benefit profile of recreational cannabis use, it must be considered that the majority of recreational users are seeking the euphoric effects associated with the subjective "high". Beyond this largely subjective effect that may be considered a benefit only by some, the most beneficial effects to a healthy individual using cannabis recreationally might be considered the effects on mood and anxiety; effects about which the existence remains uncertain. A further confounding factor that must be considered is the method of consumption employed with recreational use. Recreational cannabis use is commonly performed by smoking, vaporizing, and by ingestion. Smoking is reported to be the most common method of consumption, a process that involves inhalation of carcinogens and combusted material[77]. Given the harmful nature of this behavior that commonly occurs concomitantly with cannabis use, characterizing the risk benefit profile of recreational use is challenging at this time.

Despite lacking a robust list of potential benefits of recreational use in healthy individuals, the justification for an investigation into the acute physiological effects of consumption is counter balanced by the rarity of acute adverse effects entirely attributable to cannabis use in these individuals. Cannabis induced hyperemesis and cannabis induced psychotic episodes are rare[78]. While other more consequential risks of cannabis use such as acute coronary syndrome and stroke have been suggested[4], no investigation has identified a causal relationship. These reported acute cardiovascular risks are especially intriguing given the increasing popularity of use by athletes in and out of competition[13]. Exercise is a potent transient cardiovascular stress, and many questions surrounding the effects of the combined stimulus of exercise and cannabis remain. Despite reports of acute risks of cannabis consumption, it should be noted that presently a much larger evidence-base suggests that recreational cannabis use is associated with long rather than short term health consequences.

Although the acute risks of cannabis use appear to be rare, current knowledge suggests that they may be inherent to use and therefore should be minimized, but cannot be completely avoided. An effective approach to ensuring participant safety in the context of cannabis use requires pre-eminent planning in the case of known acute risks, and selection of a population that does not possess an elevated risk of experiencing the most commonly reported adverse effects. The present study will monitor participants following cannabis consumption to ensure participant safety. Participants will be monitored until the effects of cannabis have subsided. Further, a dosage has been selected that is less potent and smaller than what is available for typical recreational use. Choice of dosage was selected as a dose that is likely to exert physiologically meaningful effects, but not impose elevated risk to participants. Participants in the present study will be experienced users, who will not be unfamiliar with the effects of consumption. Additionally, participants possessing commonly known risk factors that may predispose to acute risk will be excluded.

The present study will examine primary endpoints that have been correlated with long term cardiovascular health. Therefore, the results will provide substantial insight into the potential long-term health risks of recreational cannabis use without exposing participants to repeated long-term exposure to cannabis. Another advantage of the present study will be the examination

of these predictive outcomes under differing conditions in which cannabis is commonly used recreationally; namely, with different cannabinoid compositions and different consumption modalities. The present study will provide the most comprehensive examination of the cardiovascular response to recreational cannabis use to date under multiple ecologically valid conditions. It will also provide novel insights into the interaction between cannabis use and exercise.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective of the current study is to determine the acute effects of cannabis consumption at rest and during exercise on the human cardiovascular system, with particular focus on the influence of method of consumption (smoking vs. vaporizing), and cannabinoid composition (THC vs. CBD).	The primary endpoints will be measures of arterial stiffness, endothelial function, muscle sympathetic nerve activity, and cardiac function. Arterial stiffness and endothelial function will be assessed via carotid-femoral pulse wave velocity and brachial artery flow-mediated dilation. Muscle sympathetic nerve activity (MSNA), or mironeurography, will be characterized using indices of sympathetic outflow, such as burst incidence and burst frequency. Cardiac function will be assessed using echocardiography. Primary endpoints will be measures of systolic function, diastolic function, and tissue mechanics. These measures include but are not limited to ejection fraction, E/A ratio, myocardial twist, and myocardial strain.	Carotid-femoral pulse wave velocity has been chosen as a primary endpoint as it has been shown that cigarette smoking, which is not dissimilar to cannabis smoking in many ways, results in arterial stiffening both acutely and chronically. Furthermore, the relationship between pulse wave velocity and risk of cardiovascular events has been well documented. Previous research has identified that a 1SD increase in pulse wave velocity is correlated with a 16-20% increase in risk for cardiovascular events[79]. Similar to pulse wave velocity, brachial artery flow-mediated dilation has been shown to have predictive value in determining risk of cardiovascular events in healthy subjects[80]. Microneurography (MSNA) will allow recording of the electrical activity of sympathetic nerves. This technique provides insight into the autonomic control of the cardiovascular system. This technique will further allow exploration of the mechanisms underlying other primary outcomes. Burst incidence and burst frequency are commonly reported measures of muscle sympathetic nerve activity.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		Ejection fraction, E/A ratio, cardiac twist, and cardiac torsion have all been chosen as these are standard measures for evaluating each respective aspect of cardiac function. Each of these measures are clinically relevant and commonly reported in current cardiac literature.
Secondary		
The secondary objective of the present study is to determine how consumption of cannabis prior to exercise affects performance.	Secondary endpoints will be used to evaluate physiological responses to exercise and will include objective measures of work capacity. These endpoints, which will be performed using in-lab cycle ergometry include, average power output, distance travelled, rating of perceived exertion, oxygen consumption, cardiac output, heart rate, blood pressure, and rate-pressure product.	Each secondary endpoint has been selected in order to examine subjective, and physiological responses to exercise. Cardiac output, heart rate, oxygen consumption, and rate pressure-product have all been selected in order to examine how cannabis consumption may alter the physiological response to exercise. The response in each of these variables is well characterized during normal exercise. Average power output and distance travelled have been selected as objective measures of performance, and will be examined to explore the relationship between any physiological perturbations associated with cannabis, and actual work capacity. Rating of perceived exertion will be measured to explore the psychological effects of cannabis consumption experienced during exercise.
Tertiary/Exploratory		
N/A	N/A	N/A

4 STUDY DESIGN

4.1 OVERALL DESIGN

The study will use a randomized cross-over design. It will be structured by division into two phases. Both phases will examine the acute cardiovascular response to cannabis consumption,

both at rest and during exercise. *Phase I* will examine if and how this response differs whether cannabis is smoked or vaporized. *Phase II* will examine how this response is influenced by cannabinoid concentration within inhaled cannabis. Phase II of the current study will use a single-blind design, with participants being unaware of the relative cannabinoid concentrations of the cannabis they consume. Cannabis will be inhaled using methods of smoking (phase I only) or vaporization (phase I and II). Each experimental study visit (study visits 2-9), 100mg of cannabis will be consumed via one of the two aforementioned methods of inhalation, followed by a subsequent rest period, or by 20 minutes of exercise. Primary endpoints will be examined pre- and post-consumption each visit (with the exception of muscle sympathetic nerve activity, which will only be performed on a single study visit), while secondary endpoints will be recorded during exercise.

Each phase of the study will require participants to complete an introductory and control visit, as well as 4 experimental study visits, where cannabis inhaled under alternating conditions (phase I: smoking vs. vaporizing high THC cannabis, phase II: high CBD cannabis vs. high THC cannabis) followed by exercise, or a time-matched rest period. The order in which these visits will occur will be randomized, and individualized for each participant. A within-subjects design will be utilized, so that each participant completes each condition. High THC cannabis used will be the cannabis strain “Rex”, while the high CBD strain will be “Kalea”. The dose size and strength will not be altered throughout the course of the study.

Continuous blood pressure, heart rate, and electrocardiogram monitoring will occur prior to, and during cannabis administration (prior to exercise) on all visits where cannabis is consumed. This procedure will be followed throughout the entirety of the 40-minute cannabis consumption period. If either systolic or diastolic blood pressure exceeds 160mmHg or 90mmHg at rest, respectively, exercise will not be performed, and the study visit will not continue. If heart rate exceeds 100bpm or falls below 40bpm at rest, exercise will not be performed, and the study visit will not continue.

All research will be performed within the department of Human Health and Nutritional Sciences at the University of Guelph.

There are no planned interim analyses planned for the current trial.

A sub-group analysis will be performed, with participants divided by sex, to determine the existence of any sex differences in primary and secondary endpoints.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

A within subject design has been chosen for this study due to potential variability in primary endpoints between individuals. Specifically, variation in pulse wave velocity and flow-mediated dilation. Published reference values for pulse wave velocity in our selected age group may range between 3.92m/s and 8.26m/s[81]. This substantial range requires a within-subjects design to allow for meaningful assessment of the effects of cannabis on arterial stiffness. Similar to pulse wave velocity, flow-mediated dilation may vary substantially between and within subjects, with values as high as 20% reported in healthy individuals[80]. This design is further warranted due to the choice of secondary endpoints. Exercise performance, and the accompanying physiological responses, are highly dependent on numerous factors that are likely to vary between individuals. Such factors include weight, fitness level, and familiarity with cycling. This further warrants the need for a within subject design, as these variables would have to be

matched if using parallel groups. Even if groups were matched, there is a strong likelihood that significant variability would exist in performance metrics.

The proposed study does not employ a placebo-control or a double-blind design. This is a consequence of the challenge owed to masking the device used to inhale cannabis in phase I. Vaporization and smoking are distinct methods of cannabis inhalation, and as such different devices must be used, making blinding difficult. A single-blind design will be utilized in phase II, in that participants will not be aware of the strain of cannabis they are consuming. Although collection will not be double-blind, investigators will be blind to condition during data analysis.

Randomization of the order of experimental visits in each phase will occur to combat improvement in performance as a result of better of pacing strategies, or improvements in fitness. Randomization will be performed using a randomization software (www.random.org). The order in which visits will occur will be individualized to each participant.

4.3 JUSTIFICATION FOR DOSE

For all cannabis administration a standard 100mg dose of dried cannabis flower will be consumed by smoking or vaporization. Depending on the experimental condition, cannabis will be either Rex (13.6±2.7% THC, <0.5%CBD dry weight) or Kalea (<1% THC, 14.5±2.9% CBD dry weight). This dose will not be altered throughout the course of the study and will be given to all participants.

A 100mg dose of dried cannabis flower was selected based upon published recommendations[82] suggesting that maximum daily cannabis use for individuals prescribed medical cannabis should not exceed 400mg daily, and should be consumed over 4 doses each day. The selection of this singular dose was also made with consideration to the context of the cannabis use in the selected population. The current study will examine recreational cannabis users, who have consumed cannabis in the past year whose use has not exceeded an average of twice per week. Given that recreational users are being examined, the quantity of commercially available products for recreational use was used to inform the dosage. In Ontario, cannabis is sold nearly exclusively through the Ontario government's online platform (https://ocs.ca). This online platform features a large range of pre-rolled cannabis products that contain 0.33-1g of dried cannabis flower with THC and CBD concentrations up to 28% dry weight and 13% dry weight, respectively[83,84]. Although this 100mg dose of Rex and Kalea may be seemingly small, previous evidence indicates that a dose of this size is sufficient to elicit cardiovascular responses to smoking and vaporization of cannabis[85].

A recent summary of the safety of CBD indicate that a dosage of 15-160mg of CBD have been used in studies with no ill effects[86]. Additionally, the dose of ~15mg of CBD was chosen to closely parallel the CBD content of inhaled products commercially available through the Ontario government. There is currently no evidence examining the effect of CBD on primary endpoints of the present study. It is therefore difficult to quantify how CBD impacts primary outcomes Thus, selecting dose based on safety and ecological validity was used as the primary justification.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed either phase I or phase II of the study including the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA:

To be eligible to participate in each phase study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 19-45yr
4. In good general health as evidenced by medical history and free of chronic disease
5. Must be experienced with cannabis use. This requires recreational cannabis use of a minimum of once per week in the past 30 days as confirmed by urine test
6. Experience consuming cannabis recreationally by vaping dried cannabis

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. **Deemed unfit to exercise by the PARQ+.**
   The goal of the present study is to examine the acute cardiovascular response to cannabis consumption at rest and during exercise. As such participants must be able to safely complete the pre-determined exercise protocol.
2. **Current or past diagnoses of substance abuse disorder**
3. **Failure of recreational substance urine screening test**
   - **Substances to be tested for include: Cocaine, Crack, Morphine, Heroin, Fentanyl, Amphetamines, Ecstasy, Methamphetamine, Spice and AB-Pinaca (synthetic cannabis), Methcathinones, LSD, and Ketamine.**
4. **Current, past, or strong family history of psychosis, or has previously experienced a cannabis-related psychotic episode**
5. **Current or past diagnoses of cannabis use disorder**
6. **Current or past diagnoses of any mood or anxiety disorder**
   Exclusion criteria 2-5 will be implemented to ensure that no individuals are enrolled in the study who could carry an elevated risk of experiencing a severe cannabis related adverse event (such as cannabis induced hyperemesis, or a psychotic episode). Each of these exclusion criteria represent contraindications for cannabis prescription.
7. **Identified ECG abnormalities**
8. **Systolic blood pressure exceeding 160mmHg**
9. **Diastolic blood pressure exceeding 90mmHg**
10. **Resting heart rate exceeding 100bpm, or lower than 40bpm**
11. **Diagnosed with respiratory disease**
12. **Diagnosed with cardiovascular disease**
13. **Diagnosed with liver disease**

14. **Diagnosed with kidney disease**
    Individuals with cardiovascular, respiratory, liver, or kidney diseases will be excluded from this study as primary and secondary endpoints may be confounded by disease states.
15. **Is Pregnant or planning to be pregnant**
    It is not currently established how cannabis consumption by a pregnant woman impacts a developing fetus. As such, females who are planning to be, or are pregnant will be excluded as a precautionary measure.
    a. Female subjects of childbearing potential and male subjects when sexually active with a female of childbearing potential must agree to use contraception for the duration of the trial.
        i. For female subjects of childbearing potential, contraception methods include:
            1. A highly effective method of contraception (such as oral hormonal contraceptives, IUD, IUS, bilateral tubal ligation);
            2. Double barrier method defined as the use of a male condom with cervical cap/diaphragm with spermicide;
            3. Male partner with a confirmed successful vasectomy; OR
            4. Abstinence defined as refraining from heterosexual intercourse consistent with the preferred and usual lifestyle of the subject.
        ii. For male subjects when sexually active with a female of childbearing potential, contraception methods include
            1. Vasectomy (confirmed successful);
            2. Condom with spermicide; OR
            3. Abstinence defined as refraining from heterosexual intercourse consistent with the preferred and usual lifestyle of the subject.

16. **Currently taking prescription medication (excluding contraceptive medication)**
17. **Is a cigarette smoker**
    It is well established that cigarette smoking negatively impacts numerous endpoints evaluated in the current study. As such no cigarette smokers will be included.

5.3 LIFESTYLE CONSIDERATIONS

The day of each experimental visit, participants are asked to:

- Refrain from cannabis consumption for the 48 hours prior to the beginning of each visit. (Confirmed by urine THC test, positive test result will result in rescheduling)
- Refrain from consumption of alcohol for the 24 hours prior to the beginning of each visit.
- Refrain from exercising vigorously for 24 hours prior to the beginning of each visit.
- Refrain from caffeine consumption for 8 hours prior to the beginning of each visit.
- Refrain from vitamin supplementation for 8 hours prior to the beginning of each visit.
- Fast for 6 hours prior to the beginning of each visit
- Avoid operating a motor vehicle for the entirety of the date of a study visit where cannabis is consumed
- Arrange to have a trusted individual escort them to their residence following completion of their study visit.

5.4 SCREEN FAILURES

If a participant who consents to participate in this study but is deemed ineligible via a screening assessment (PARQ+, Eligibility Questionnaire) will not have identifying information recorded. Only the reason for ineligibility will be recorded. Responses to the PARQ+ or eligibility questionnaire will be destroyed. Individuals who do not meet the criteria for participation in this trial will not be rescreened.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

We seek to recruit 20 individuals per phase, 10 of which will be female and 10 of which will be male. Participants will be recruited from the community in Guelph, Ontario. In line with our IRB approved methods, recruitment posters will be posted at the University of Guelph and around the city. A social media posting will be sent via Twitter from the official Human Performance and Health Research Laboratory account. Additionally, the study poster will be presented to students in undergraduate lectures at the University of Guelph. Participants may also be recruited by word of mouth. Each participant who completes a session of microneurography will be compensated $100 CAD in line with typical institutional procedures.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The two cannabis strains examined in the current study are Rex and Kalea (See accompanying Investigator's Brochure). These cannabis strains will be given in the form of dried cannabis flower. Each of these cannabis strains are produced by MedReLeaf. Both Rex and Kalea are harvested from *Cannabis sativa*. These strains have been selected as they differ substantially in their concentration of the two most abundant cannabinoids THC and CBD. Rex will contain 13.6±2.7% THC and <1% CBD by dry weight; Kalea will contain <1% THC and 14.5±2.9% CBD by dry weight. For more detail about the investigational products please see the accompanying Investigator's Brochures.

6.1.2 DOSING AND ADMINISTRATION

All cannabis consumed in relation to the current study will be done so during a study visit, within a laboratory setting in a specifically designed cannabis administration chamber. Participants will not be required to consume cannabis for the purpose of the study outside of their visits to the laboratory.

In each study visit a standard dosage of 100mg of Rex or Kalea will be administered to participants, who will consume via inhalation of smoke or vapor. Participants will be seated in a chamber designated for cannabis consumption. One investigator will retrieve a pre-weighed 100mg dose of the appropriate dried cannabis flower, depending on the phase of study, and the study visit being completed. The investigator will load the 100mg cannabis dose into either a glass smoking apparatus or a "Mighty Medic" vaporizer. The vaporizer or smoking apparatus will be given to the participant within the cannabis chamber.

Cannabis will be inhaled according to a standardized inhalation procedure. This procedure involves 5 seconds of inhalation, 10 seconds wherein smoke or vapor is held in, and 45

seconds of rest. This procedure will be repeated until all cannabis has been exhausted. After receiving the full dose of cannabis, an investigator will guide the participant out of the chamber.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Aurora Cannabis Inc. will provide the exact amounts of Rex and Kalea needed for the completion of the study by 20 individuals. As the required sample size has been determined to be 20 individuals, with each participant consuming 100mg of cannabis on four separate occasions, per phase of study, 12 grams of Rex and 4 grams of Kalea will be required. An additional 3 grams of Rex and an extra gram of Kalea will be purchased to account for the potential of participant drop-out. Cannabis will be stored in a locked safe in a room with restricted access. Only members of the investigatory team will have access to the room where the cannabis is stored, and the room will not be accessed alone by any researcher. The safe will be secured to the wall of the room. Each entry to the room for the access of cannabis will be recorded in a log, where time of exit and entry, and purpose of entry will be recorded. Additionally, all cannabis will be immediately inventoried upon receipt, and a constant record will be kept of how much cannabis is removed at any time. Cannabis will be organized into pre-weighed 100mg samples, and will be labelled by sample number. Any time cannabis is removed, the responsible individual will be required to record what sample was removed. Weekly checks of cannabis inventory will be performed. Any excess cannabis that remains at the end of the data collection period will be destroyed. Cannabis will be ground and mixed with equal parts soil before being discarded.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Please see the investigator's brochure for information related to formulation, appearance, packaging, and labeling. Each brochure was prepared by MedReLeaf, a subsidiary of Aurora Cannabis Inc.

6.2.3 PRODUCT STORAGE AND STABILITY

Each strain of cannabis will be stored locked safe in a room with restricted access. Samples will be stored in 100mg preparations.

6.2.4 PREPARATION

Prior to receiving any cannabis, the investigatory team will label and prepare child resistant jars that will contain individual 100mg dosages. Each container will be labelled with a sample number for tracking purposes. These containers will be kept in the safe. Upon receiving a shipment of cannabis, an investigator will transport the cannabis to the room for storage. They will then pre-weigh 100mg samples using a digital scale, and place a 100mg dose of dried cannabis flower into each container. A master list of which strain each sample number corresponds to will be kept.

At the point in each study visit where the participant is prepared for cannabis consumption, two members of the research team will retrieve the appropriate cannabis sample from the safe. The sample will be loaded into the appropriate inhalation device within the restricted access room.

The smoking apparatus will be prepared by placing the entirety of one dose into the bowl portion of the glass smoking apparatus. The device will then be given to the participant to begin the inhalation protocol.

The Mighty Medic vaporizer will be loaded according to the manufacturer's instructions. The cooling unit will be rotated 90° in the counterclockwise direction. One dose will then be placed in the now exposed filling chamber. The cooling unit will be moved to the initial position, and the vaporizer will be set to a temperature of 190°C and given to the participant to begin the inhalation protocol.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

As the current study utilizes a randomized crossover (within subject) design, no randomization to independent groups is necessary. Instead, the order in which experimental study visits take place will be randomized, and individualized to each participant. Upon enrollment in the study, the individualized order of study visits will be determined using a random number generating software (*https://www.random.org*). Each study visit will be assigned a number from 1-4. Random numbers will be generated, and the order of study visits will correspond to the order in which the numbers were generated by the software.

Phase I of this study will not be blinded owing to challenges in masking the device used to inhale cannabis. *Phase II* will be single blinded, with the strain of cannabis consumed unbeknownst to the participant. Participants will not be unblinded to the study conditions at any point. All investigators will however be blind to condition during data analysis.

Primary and secondary endpoints used in this study are robust in preventing observer bias. Each primary endpoint will be determined using computer software, while secondary endpoints will also be determined by the use of automatic devices that provide data that is not modifiable by investigators. During any analysis, the investigator will be blinded to condition

6.4 STUDY INTERVENTION COMPLIANCE

At all times during cannabis consumption, a minimum of two members of the research team will be present to ensure compliance to the inhalation protocol. They will guide participants through the protocol and ensure that they comply with the protocol timing . If participants intentionally deviate from protocol (holding breath, taking secondary breaths) they will be instructed to refrain during future cycles. If deviation continues data will not be collected during that study visit, and the protocol will be stopped. Acceptable deviations may occur, such as participants coughing. In the instance of coughing, the protocol will be paused until the participant is ready to resume. While monitoring, researchers will evaluate when all cannabis has been exhausted. At this point the inhalation protocol will be ceased.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported are concomitant prescription medications, over-the-counter medications and supplements.

6.5.1 RESCUE MEDICINE

The study site will not supply rescue medication as dried cannabis flower is not being examined in the context of treatment of disease or symptom relief.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

If a participant experiences a cannabis-related severe adverse at any point during participation in the study, or new information regarding their health becomes available to them that would contraindicate cannabis use, they will be immediately discontinued from further trials. Investigators will inquire whether there have been any changes in health or prescription medication status each study visit. Severe cannabis related adverse events will be classified using the adverse event form, but will also by default include any instance of cannabis induced hyperemesis or any degree of psychosis. If a participant voluntarily withdraws, they will not be required to provide a reason, and no further data collection will occur. However, if a participant decides to withdraw following consumption of cannabis during a study visit, they will be required to remain in the laboratory for 4 hours following the moment of consumption, so that their safety may be monitored. Any adverse event that occurs will be recorded using an adverse event form.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. This includes during the inhalation procedure.

If a participant wishes to withdraw during or after the inhalation of cannabis they will be required to remain in the laboratory for the requisite recovery period of 4 hours. Each hour subjective effects, heart rate, and blood pressure will be measured.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- If any severe adverse event (AE), or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant reports meeting an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to complete study procedures

The reason for participant discontinuation or withdrawal from the study will be recorded. Participants who discontinue the study will be replaced to ensure that the required pre-determined statistical power is met.

7.3 LOST TO FOLLOW-UP

A participant will be considered withdrawn if he or she fails to return for a scheduled visit and is unable to be contacted by the study staff.

The following actions must be taken if a participant fails to return to the laboratory for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within one day, and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed withdrawn, the investigator or designee will make an effort to regain contact with the participant. These contact attempts will be documented.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Participants will be screened for eligibility using two separate screening procedures during the introductory visit. The first screening procedure will be an investigator generated eligibility questionnaire. This questionnaire will evaluate whether the participant fits inclusion criteria and does not meet any exclusion criteria. The second screening tool will be the PARQ+, a validated questionnaire designed to determine whether participants are fit to safely exercise. The PARQ+ may determine that a participant requires clearance from their physician. If they do not obtain a signed doctor's note, they will not be eligible to be participate. All screening procedures will occur during the introductory visit, and within one week of the exercise control visit. Additionally, at the start of each study visit participants will be asked if there have been any changes to their health status, or prescription medication status.

Efficacy assessments of primary objectives are as follows:

- Arterial stiffness will be assessed before and after cannabis consumption with and without subsequent exercise under each condition, during each study visit. Carotid-femoral pulse wave velocity will be used to determine arterial stiffness. Carotid-femoral pulse wave velocity will be measured by placing a tonometer probe at the site of the carotid and femoral pulse. Pulse waveforms will be juxtaposed against a lead-II electrocardiogram to determine pulse wave velocity.
- Endothelial function will be characterized before and after cannabis consumption with and without exercise under each condition during each study visit. Endothelial function will be measured using brachial artery reactive hyperemic flow-mediated dilation. The brachial artery will be imaged using a General Electric Vivid-i ultrasound system. After 1 minute of imaging, a tourniquet placed on the antecubital fossa will be inflated to 220mmHg for a period of 5 minutes will imaging continues. After this period 3 minutes of ultrasound imaging will be recorded to assess the vasodilatory response to occlusion. The relative (%) change in arterial diameter following release of the tourniquet will be the primary endpoint.
- Muscle sympathetic nerve activity will only be performed before and after vaporizing of high THC cannabis. Microneurography will be performed by inserting a tungsten microelectrode into the peroneal nerve. Upon identification of a sympathetic nerve

bundle, baseline activity will be recorded for a maximum of 10 minutes. Participants will then follow the standard smoking protocol, while sympathetic activity is recorded during the rest period.
- Cardiac function will be assessed using echocardiography. Echocardiography will be performed prior to exercise during the exercise control visit, and will be performed after consumption of cannabis on each study visit. Ejection fraction, E/A ratio, myocardial twist, and myocardial strain have all been chosen as these are standard measures for evaluating respective aspects of cardiac function. The heart will be viewed via the parasternal and apical imaging windows, and will be imaged using the Vivid-I ultrasound system.

Efficacy assessments of secondary objectives are as follows:
- Blood pressure, heart rate, and myocardial oxygen demand will be determined using a Finapres device which measures beat-to-beat heart rate and blood pressure using a finger cuff. The product of these two endpoints will represent the rate-pressure product, an indicator of myocardial oxygen demand. These outcomes will be assessed in the 20-minute period following cannabis consumption during each study visit. Therefore, recordings will take place at rest and during exercise depending on the given study visit.
- Indirect calorimetry will be performed during all exercise bouts, and will be used to evaluate oxygen consumption and other cardio-respiratory measures such as respiratory exchange ratio, minute ventilation, and caloric expenditure. Participants will be instrumented prior to each exercise bout, and measurement will only take place during the exercise bout.
- Participants will also be asked to provide ratings of perceived exertion every 2 minutes during each exercise bout. Ratings will be collected using the 6-20 Borg scale of perceived exertion.
- Exercise performance will be quantified by numerous endpoints such as average power output, and total work completed in the allotted exercise period (20 minutes). These outcomes will be assessed using the manufacturer software provided with the cycle ergometer that will be used in all exercise bouts.

No results of primary or secondary endpoints will be shared with participants until the completion of the data collection period. If participants wish to see results from any of the primary or secondary endpoints, all results will be provided in aggregate.

8.2 SAFETY AND OTHER ASSESSMENTS

The two screening procedures (Eligibility Questionnaire, PARQ+) will be implemented in order to ensure that only participants who are fit to exercise, and who possess no characteristics that contraindicate medicinal cannabis use will be included in the study. As previously mentioned, each of these questionnaires will attempt to identify specific characteristics that suggest that study inclusion would not be recommended for a given individual.

Participant safety will be monitored throughout each study visit via investigator supervision of study procedures, monitoring of vital signs, electrocardiogram, imaging assessments, and questionnaires. Additionally, adverse events will be recorded throughout each study visit in detail, should they occur.

Upon arrival to the laboratory participants will have their seated blood pressure measured prior to each incidence of cannabis consumption or exercise. Participants must fall in the

normotensive range in order for continuation of study procedures. Blood pressure and heart rate will be recorded throughout all study procedures following cannabis consumption. Additionally, participant's heart rate will be monitored via electrocardiogram. Digital electrocardiographic measures will not be analyzed following study visits; however, it will be monitored during each study visit. In the event of electrocardiographic abnormalities, study procedures will be halted. If electrocardiogram abnormalities are detected and medical attention is necessary, emergency services will be immediately contacted. The principal investigator will be responsible for monitoring for electrocardiogram abnormalities. The principal investigator is trained to do so through his certification as a Certified Exercise Physiologist, accredited by the Canadian Society for Exercise Physiology.

In the assessment of certain primary endpoints, ultrasound imaging of the brachial artery and the heart will occur. If any vascular or cardiac abnormalities become evident to investigators, then emergency services will be contacted, if necessary. As the investigators performing these measures are not physicians, they will not attempt any further specialized imaging, or provide a diagnosis to the participant.

Throughout each smoking protocol, a member of the investigatory team will supervise participants to ensure maximal adherence. If the participant engages in behaviors that are likely to augment the cannabis dosage, such as prolonged breath holding, they will be instructed to cease such behaviors. This investigator supervision will ensure that participants are receiving the desired dose of cannabis.

After completion of study procedures, participants will be required to remain in the laboratory for a period of 4 hours beginning from the end of the cannabis inhalation procedure. This period of monitored recovery has been implemented in an effort to minimize the subjective effects of cannabis experienced by participants prior to their release from the laboratory. In addition, participants will be required to complete the drug effects questionnaire each hour following the completion of study procedures. This questionnaire will evaluate the extent to which participants are experiencing the subjective effects of cannabis. If participants indicate that they are still experiencing subjective effects at the end of the 4-hour recovery period via the DEQ, they will be required to remain until effects have subsided. Blood pressure and heart rate will also be monitored throughout the recovery period. Measurements will be taken each hour with the administration of the DEQ.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event will be defined according to the ICH-GCP as: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting)[87].

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event will be defined according to the ICH-GCP as: Any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,

or

- is a congenital anomaly/birth defect (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

Severity of adverse events will be classified from mild to life-threatening. All classifications are defined as:

- Mild: Awareness of sign or symptom, but easily tolerated
- Moderate: Discomfort enough to cause interference with normal daily activities
- Severe: Inability to perform normal daily activities
- Life Threatening: Immediate risk of death from the reaction as it occurred

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by a member of the investigatory team who examines and evaluates the participant based on temporal relationship and his/her judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect. The following scale of relatedness will be used:

- Not related: An adverse event which is not related to the study
- Unlikely: An adverse event for which an alternative explanation is more likely
- Possible: An adverse event which might be due to the study. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
- Probable: An adverse event which might be due to the study. The relationship in time is suggestive. An alternate explanation is less likely.
- Definite: An adverse event which cannot be reasonably explained by alternative explanation. The relationship in time is very suggestive.

8.3.3.3 EXPECTEDNESS

Dr. Jamie Burr will be responsible for determining whether an adverse event is expected or unexpected. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information provided in the Investigator's Brochure.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event or serious adverse event may come to the attention of study personnel during study visits and interviews of a study participant. Participants will be told in the initial study visit that they should report any adverse events they are experiencing after completing any study procedures.

All adverse events including local and systemic reactions not meeting the criteria for serious adverse events will be captured on the adverse event form. Information to be collected includes event description, time of onset, assessment of severity, relationship to study product (assessed by an investigator), and time of resolution/stabilization of the event. All adverse events occurring while in the study must be documented appropriately regardless of relationship. All adverse events will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an adverse event. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an adverse event.

8.3.5 ADVERSE EVENT REPORTING

Each adverse event is to be assessed to determine if it meets the criteria for serious adverse event. If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate.

All adverse events will be reported on the adverse event form. Adverse events should be reported using concise medical terminology on the form for collection of serious adverse event information. Dr. Jamie Burr will be responsible for completing and signing off all adverse event reports. Adverse drug reactions will be reported to the IRB and Health Canada within 15 days.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate. Health Canada and IRB requirements for reporting of serious adverse events will be followed.

If a serious adverse event occurs, the PI-Sponsor is to be notified within 24 hours of awareness of the event by any investigator. In particular, if the serious adverse event is fatal or life-threatening, notification to the PI-Sponsor must be made immediately, irrespective of the extent of available adverse event information. In the event that the investigator does not become aware of the occurrence of a serious adverse event immediately, the investigator is to report the event to the IRB and Health Canada within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

For all serious adverse events, the investigator is obligated to pursue and obtain any relevant information. In addition, an investigator may be requested to obtain specific additional follow-up information in an expedited fashion. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided.

Serious Adverse events will be noted and recorded on adverse event recording sheets according to ICH GCP. The standards for reporting serious adverse events, as defined in the ICH guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Harmonized Guideline) will be strictly followed by study investigators.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Participants in this study will not be informed of any adverse events experienced by other participants. If the investigatory team discovers any incidental findings that suggest the participant seek medical attention, they will be informed immediately, and their participation in the study will be suspended until they have seen a physician. Only once they have obtained written permission from their physician, can they continue participation in the study.

8.3.8 EVENTS OF SPECIAL INTEREST

There are no events of special interest that are likely to occur other than those discussed in previous sections.

8.3.9 REPORTING OF PREGNANCY

Upon screening, participants will be required to complete an eligibility questionnaire, which will enquire if the participant is pregnant or planning to be pregnant. If participants indicate they are or are planning to be pregnant they will not be enrolled in the study. All female participants will be required to provide a urine sample in order to complete a urine pregnancy test. If the next study visit occurs more than 30 days after the initial visit, the test will be repeated. If a participant becomes pregnant during study participation they will be withdrawn from the study. Pregnancy will be reported as the reason for withdrawal.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

An unanticipated problem will be defined as any incident, experience, or outcome that meets ALL of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the REB-approved research protocol and informed consent document, or the Investigator Brochure; and (b) the characteristics of the research participant population being studied; and
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the [investigational product(s)] or procedures involved in the research); and
- Suggests that the research places research participants or others at a greater risk of harm (including physical, psychological, economic, ethical or social harm) than was previously known or recognized.

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8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events will be reported to the IRB within 2 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures) within 7 days of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be made aware of any unanticipated problems that have occurred that may indicate that the risk/benefit ratio of study participation has changed.

9 STATISTICAL CONSIDERATIONS .

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):
  1. Pulse Wave Velocity will be increased following exercise, smoking high THC cannabis, and vaporizing high THC cannabis relative to resting baseline; effects of cannabis consumption and exercise are likely to be additive. Vaporizing high CBD cannabis will not increase pulse wave velocity.
  2. Percent flow mediated dilation will be decreased following exercise and smoking high THC cannabis; effects are likely to be additive. Vaporizing both high THC and high CBD cannabis will not decrease percent flow mediated dilation.
  3. Ejection fraction will be decreased following smoking and vaporizing high THC cannabis. Exercise and vaporization of high CBD cannabis will not be associated with reductions in ejection fraction.
  4. E/A Ratio will be decreased following high THC cannabis smoking only.
  5. Myocardial strain will be decreased following high THC cannabis smoking and vaporizing only.
  6. Myocardial twist will be decreased following high THC cannabis smoking and vaporizing only.
  7. Burst Frequency will be increased following high THC cannabis only.
  8. Burst Incidence will be increased following high THC cannabis only.

- Secondary Efficacy Endpoint(s):
  1. Systolic Blood Pressure will be increased following cannabis consumption and will be decreased following all bouts of exercise.
  2. Diastolic blood pressure will be unchanged under all conditions
  3. Heart rate will be increased following cannabis consumption and exercise.
  4. Stroke volume will be increased with exercise, and will be reduced following cannabis consumptions. These competing effects will result in an attenuation of increases in stroke volume when exercise is preceded by cannabis consumption.
  5. Cardiac output will be unchanged under all conditions
  6. Work completed will be reduced when preceded by cannabis consumption of all forms
  7. Average power output will be reduced when preceded by cannabis consumption of all forms
  8. Oxygen consumption will be reduced when exercise is preceded by consumption of high THC cannabis
  9. Rating of Perceived Exertion will be increased following consumption of high THC cannabis
  10. Rate-pressure product will be increased during exercise, when the bout is preceded by cannabis consumption.

9.2 SAMPLE SIZE DETERMINATION

In accordance with our power calculation, 15 participants are required to have a power of at least 0.8 with an alpha of 0.05, for expected changes in measures of endothelial function (pilot work, change in FMD in response to exercise pre = 4.8 ± 2.5 %, post = 2.7 ± 1.7%) (FMD in rats in response to cannabis smoke, pre = 7.5 ± 2.5%, post = 2.4 ± 1.4 %)3, artery stiffness (pilot work, change in PWV pre = 7.4 ± 1.3 m/s, post (at rest) = 6.0 ± 1.3 m/s) and cardiac function (pilot work, peak longitudinal strain: pre = -18.3 ± 1.2 %, post = -17.0 ± 1.9 %), therefore we will recruit 20 participants in each phase of the study to account for potential dropout.

9.3 POPULATIONS FOR ANALYSES

Only participants who complete all study procedures will be included for analysis of efficacy endpoints. All participants who consume cannabis at any time will be included in the analysis of safety endpoints.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Our study will be divided into two phases each of which will be analyzed independently. All tests of significance will be two-tailed, and statistical significance will be based upon an alpha error probability of 5% and a power for 80%. The data will be evaluated using both parametric and non-parametric tests, as applicable. If the data are found to be not normally distributed, the raw data will be transformed using an appropriate transformation model in order to perform the parametric assessments. Non-parametric tests will be performed on raw data. The data will be assessed as continuous and will be presented as means with standard deviations. A one-way analysis of covariance will be used to assess the treatment

effects (Phase I: exercise, vaporizing THC, smoking THC, vaporizing + exercise THC, smoking THC + Exercise, Phase II: exercise, vaporizing THC, vaporizing CBD, vaporizing + exercise THC, Vaporizing CBD + Exercise) on the primary endpoints. Covariates will be introduced into the models in order to assess the impact of certain potential factors on outcome, including fitness, blood pressure, resting heart rate, and any other factors that are observed during the study that appear to potentially influence the outcome.

Our secondary endpoints will be assessed using a repeated measures one-way ANOVA and paired t-tests. If there are significant interactions differences between pairs of means will be determined with a Bonferroni post-hoc test.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

All primary endpoints will be measured as continuous variables and will be repeated after each intervention performed on a given study visit. A one-way ANCOVA will be used to assess treatment effects. Covariates will include basic cardiovascular and haemodynamic variables that may confound primary endpoints. These covariates include heart rate, blood pressure, and pulse wave velocity. Results will be presented as adjusted means with standard deviations. Primary endpoints will only be analyzed for participants who complete the entirety of at least one phase of the proposed study. Missing data will be imputed using mean imputation from all other measurements collected from the given participant. Outliers will be included and excluded in independent analysis, both of which will be reported.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Analysis of secondary endpoints will be independent of analysis of primary endpoints. All secondary endpoints will be measured as continuous variables and will be reported as adjusted means with standard deviations. Repeated measures one-way ANOVA and paired Student's *t*-tests will be used for analyses. If there are significant interactions differences between pairs of means will be determined with a Bonferroni *post-hoc* test. Missing data will be imputed using mean imputation from all other measurements collected from the given participant. Outliers will be included and excluded in independent analysis, both of which will be reported.

9.4.4 SAFETY ANALYSES

Safety analysis will be performed using summary statistics during treatment interventions. Adverse events will be coded according to the Medical Dictionary for Regulatory Activities and will have severity, and relationship of adverse event to study intervention presented by System Organ Class and preferred term groupings. Each instance of an adverse event will be presented as a discrete event. Duration of events, severity, relationship to intervention, expectation, and outcome will be presented. Adverse events that lead to premature discontinuation will be presented in a table, along with all treatment-emergent serious adverse events.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline characteristics of participants will be presented in a table. No descriptive statistics other than mean and standard deviation will be presented as the proposed study uses a within-subjects design.

9.4.6 PLANNED INTERIM ANALYSES

No interim analysis is planned for the proposed study. *Phase I* of this study will be completed in advance of *phase II*, and as such efficacy and safety analyses for *phase I* may occur before the completion of *phase II*. The results of *phase I* will not be used to modify the desired sample size or planned analyses of *phase II*.

9.4.7 SUB-GROUP ANALYSES

Sub-analysis of each primary and secondary endpoint will be performed based upon sex. This analysis will be performed identically to all efficacy and safety analyses with the only adjustment being the exclusion of the sex not of interest.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA.

Data collected from each participant will be recorded by measure and study visit. Each participant will be assigned a study ID.

9.4.9 EXPLORATORY ANALYSES

There are no planned exploratory analyses in the current study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

A consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The consent document is submitted with this protocol.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. The consent form has been Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The participant will be provided the consent document in advance of their first study visit. During the first laboratory visit, an investigator will explain the study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw

from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator-Sponsor will promptly inform study participants and the Institutional Review Board, and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators. This confidentiality is extended to cover all individual test results collected during this study, in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the principal investigator-sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Upon enrollment into the study, each participant will be assigned a participant ID. A master list of all ID's will be kept separate from all study results. Data collected will be labelled only with participant ID's. Upon completion of data collection, the master list linking identifying information to participant IDs will be destroyed. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored within the laboratory of Dr. Burr and will only be labelled with participant ID's. Data stored will not include the participant's contact or

identifying information. Digital study data used by research staff will be secured and password protected.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

No biological specimens will be obtained in this study.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator-Sponsor	**Qualified Investigator**
Dr. Jamie Burr PhD	Dr. Margo Mountjoy MD, PhD
University of Guelph	University of Guelph
50 Stone Road E	50 Stone Road E
(519) 824-4120x52591	(519) 885-5426x21122
burrj@uoguelph.ca	mmsportdoc@uoguelph.ca

10.1.6 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Dr. Kerry Ritchie will act as the monitor for this clinical trial. She will be located on-site, and will conduct a minimum of three monitoring visits. These visits will include an initiation visit, an interim monitoring visit, and a close out visit. The initial visit will involve the PI and the investigatory team in addition to the monitor. In this meeting the protocol, investigational product, SOP's, ICH-GCP, Health Canada regulations, AE reporting, protocol deviation, and stakeholder responsibilities will be discussed to ensure all parties understand their roles in the study. During the interim visit, certain items will be reviewed for data verification for all participants, while other items will be viewed for only a selection of participants. Monitoring reports will be distributed to the entire investigatory team following the interim visit. The close out visit will involve a review of all documentation for accuracy and completeness.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

Quality management of study conduct, data collection, documentation, and completion will be performed.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice

(ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring, and inspection by local and regulatory authorities. Any deficiencies or quality control issues identified by the monitor will be corrected by the investigatory team.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data will be recorded in encrypted digital files for the purpose of analysis. All hard copy data recorded will be archived within the lab of Dr. Jamie Burr. Regular monitoring will ensure that all hard copy data will be correct in both hard copies and electronic records. Participant's inclusion in the study will be recorded via archiving of the consent form and all hard copy data. All investigators will be held responsible for proper data handling procedures. A master list of participant IDs will be maintained throughout the course of data collection and analysis. After this period, the master list will be destroyed and all individual data will be completely de-identified.

10.1.8.2 STUDY RECORDS RETENTION

Study documents and de-identified individual data will be retained for 25 years after the completion of the data collection period. This time period of retention is in accordance with the requirements of the IRB. No records will be destroyed without the written consent of the IRB. It is the responsibility of the investigator-sponsor destroy these documents when they are no longer required to be retained.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or International Conference on Harmonization Good Clinical Practice (ICH GCP). The noncompliance may be either on the part of the participant or the investigators. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and record deviations within 2 working days of identification of the protocol deviation, or within 2 working days of the scheduled protocol-required activity. All deviations must be addressed in study documents.

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy. It requires scientists to submit final peer-reviewed journal manuscripts to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 25 years after the completion of the primary endpoint by contacting Dr. Jamie Burr.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the cannabis industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the University of Guelph has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CRF	Case Report Form
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MSDS	Material Safety Data Sheet
NIH	National Institutes of Health
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale

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