**Effect of acute exercise on endocannabinoid and mood responses in adults with cancer: A pilot study**

**Short title:** *Exercise, endocannabinoid and mood responses in cancer patients*

**Sponsor: Chris O’Brien Lifehouse**

# **Project Team Roles and Responsibilities**

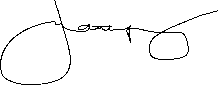
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Role in the project: Recruitment, manuscript and protocol preparation, publication and dissemination

|  |  |
| --- | --- |
| **Protocol Title** | Effect of acute exercise on endocannabinoid (eCB) and mood responses in adults with cancer: A pilot study |
| **Protocol version** | 1.0 |
| **Objectives** | Primary objective:  To compare the effect of a single bout of exercise on eCB and affective (mood) responses in adults with cancer currently receiving chemotherapy treatment versus their healthy peers.  Secondary Objective:  To examine the impact of relationships between eCB and mood parameter responses to exercise in adults currently receiving cancer treatment and their healthy peers. |
| **Study design** | A controlled trial comparing the effect of a single bout of exercise on eCB and mood responses in adults with cancer currently receiving chemotherapy treatment versus age-matched, healthy peers will be implemented. |
| **Planned sample size** | As it is a pilot feasibility study, the decision regarding sample size is pragmatic. We aim to recruit 20 participants for this study. Where applicable, sample size estimates to inform future trials will be computed *post hoc.* |
| **Selection criteria** | *Cancer patients:* Adults (>18 years) with cancer currently receiving chemotherapy treatment; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, medical approval of the treating oncologist, sufficient English to provide consent and complete study procedures.  *Healthy controls:* Adults (>18 years); age and BMI-matched to the cancer patients; no history of systemic cancer treatment; free of medically diagnosed cardiac, metabolic, pulmonary, neurological and/or uncontrolled risk factors; sufficient English to provide consent and complete study procedures. |
| **Study Procedure** | Twenty participants (10 cancer patients and 10 healthy controls) will be recruited to engage in a single testing session involving baseline data collection, a resting condition (30 min) and an exercise condition (30 min). Outcome measures including ten visual analog scales to evaluate changes in affective states (mood) acutely (i.e. (i) pain, (ii) nausea, (iii) anger, (iv) sadness, (v) happiness, (vi) energy, (vii) fatigue, (viii) anxiety, (ix) euphoria, and (x) depression), and eCB and related biogenic lipids (i.e. AEA, 2-AG, AA, PEA, OEA and SEA) will be evaluated before and after each condition to evaluate changes over time and between groups. |
| **Statistical considerations** | eCBs and mood will be assessed using a repeated measures ANOVA with time x group x condition as within-subject variables. Regression models will be used to evaluate relationships between eCB and mood variables of interest. Statistical significance will be set at p < 0.05, and effect sizes and confidence intervals will also be reported. |
| **Time Period of Data Collection** | Start date: March 1, 2022  End date: February 28, 2023 |
| **Duration of the Study** | 1 year |
| **Funding (if applicable)** | The SurFebruary Cancer Research Fund, $40,000 |
| **Sponsor (if applicable)** | Chris O’Brien Lifehouse Living Room |

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| **Protocol**  **Version Number** | **Date** | **Summary of Changes** |
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**Abbreviations/Acronyms**

2-AG: 2-arachidonoylglycerol

AA: arachidonic acid

AE: adverse event

AEA: anandamide

BNDF: brain-derived neurotrophic factor

BMI: body mass index

CB1: cannabinoid receptor type 1

CB2: cannabinoid receptors type 2

COBLH: Chris O’Brien Lifehouse

eCB: endocannabinoids

ECS: endocannabinoid system

ESAS: Edmonton Symptom Assessment Scale

HADS: Hospital Anxiety Depression Scale

OEA: oleoylethanolamide

PEA: palmitoylethanolamide

POMS: Profile of Mood States

QoL: quality of life

SAE: serious adverse event

SEA: stearoylethanolamide

SpO2: oxygen saturation

THC: Δ9-tetrahydrocannabinol

VAS: visual analog scale(s)

# **Background** **Information/Rationale**

Exercise has been investigated as a therapeutic adjunct to conventional cancer treatments since the early 1980’s (1). Clinical trials conducted over the past 40 years have consistently shown that exercise is safe, can counteract cancer treatment-related side effects (e.g. fatigue) and elicit many other physiological, functional and psychological adaptations including improved quality of life (QoL) (2). Despite these findings and widespread support for the integration of exercise services into cancer care, the vast majority of cancer patients and survivors do not meet minimum physical activity guidelines and remain significantly less active than their age-matched peers (3). Cancer survivors report many self-reported, psychosomatic barriers to exercise which most commonly include fatigue, pain, low motivation and lack of enjoyment (4). There is a need to Identify barriers and facilitators to improve patient desire to exercise which in turn may improve patient outcomes.

In healthy adults, it is widely acknowledged that participation in a single exercise session can acutely improve mood and induce pleasurable sensations. Peak experiences have often been described as a sense or state of euphoria, colloquially known as ‘the runner’s high’ (5). Terms ascribed to the experience have included: ‘happiness’, ‘elation’, ‘joy’, ‘inner harmony’, ‘boundless energy’ and ‘ecstasy’ (6). Reductions in anxiety and pain are also commonly noted (6). Despite these well documented benefits of acute exercise on mood, the biochemical pathways mediating such responses are not known. Historically, the ‘runner’s high’ was believed to be induced by the release of opiate peptides called endorphins given their effect on pleasure and pain-reducing (analgesic) biochemical pathways and research showing elevations in circulating endorphins post exercise (7). However, the central role of endorphins is not supported by the evidence, and it is becoming increasingly recognized that changes in affective experiences (mood, pleasure sensations and pain relief) depend upon other neurochemicals, membrane lipids, gases (nitric oxide), and gene expression factors (7).

The endocannabinoid system (ECS) is an underlying system contributing to homeostasis in many of our body’s physiological systems, cognitive processes and emotions including but not limited to mood, memory, appetite, energy, pain and neuro-immune modulation (13). These symptoms are commonly experienced by cancer patients receiving chemotherapy, often experienced together as symptom clusters. The relationship of these common cancer and cancer treatment related symptoms may be linked to the dysregulation of the ECS. Endocannabinoids (eCBs) are endogenous, lipid-based retrograde neurotransmitters that exert a range of biochemical effects mediated by binding to two primary G-protein coupled receptors, including cannabinoid receptor type 1 (CB1) and type 2 (CB2) (8). CB1 receptors are located in the central nervous system and mediate the analgesic and psychotropic effects of endo- and exocannabinoids (i.e. Δ9-tetrahydrocannabinol (THC), the major psychoactive cannabinoid component of *Cannabis* plants) (8). CB2 receptors are primarily found outside the brain in immune system tissues and mediate the anti-inflammatory effects of endo- and exocannabinoids (8). Collectively, the CB1 and CB2 receptors, known endogenous cannabinoids (e.g. anandamide (AEA), 2-arachidonoylglycerol (2-AG)) and related biogenic lipids (i.e. arachidonic acid (AA), palmitoylethanolamide (PEA), oleoylethanolamide (OEA) and stearoylethanolamide (SEA)) comprise the endocannabinoid system (ECS). Recent studies have shown that endocannabinoids play a role in inhibiting or counteracting tumour progression (9, 10), and therefore the ECS is being more rigorously investigated as a target for anticancer therapies, potentially including medicinal cannabis (11, 12). By contrast, dysfunction of the ECS has been associated with several chronic diseases, including multiple sclerosis, inflammation, epilepsy, schizophrenia, glaucoma, cardiovascular disease, obesity and various cancers (12). In cancer, dysregulation of the ECS including changes in endocannabinoid concentrations and action is has been demonstrated in some studies to be associated with downregulation of the pathways that inhibit cancer cell proliferation a mechanism that may contribute to cancer progression (12). In cancer, dysregulation of the ECS including changes in endocannabinoid concentrations and action is has been demonstrated in some studies to be associated with downregulation of the pathways that inhibit cancer cell proliferation a mechanism that may contribute to cancer progression and may play a role in common cancer and cancer treatment related symptoms (12).

A recent study (14) utilising a within-subjects cross-over design and enrolling a cohort of healthy, physically-active adults (aged 18-50 years) documented eCB and affective responses to two separate exercise conditions performed on a treadmill for 50 min duration each (i.e. (i) a run at moderate intensity (70-85% of age-predicted maximal heart rate) and (ii) a walk at low intensity (50% of age-predicted maximal heart rate)). Blood was sampled immediately pre- and post each exercise condition, along with the recording of ten emotional states assessed *via* Visual Analog Scales (VAS). The study also investigated the effect of opioid (endorphin) blockade, with participants randomly assigned (1:1) to receive an opioid receptor antagonist (50 mg naltrexone administration) or placebo. Pooled analysis showed that both exercise conditions increased AEA, 2-AG, PEA, and AA, a precursor to AEA and other eCBs (all p<0.001). However, the magnitude of increase was approximately two-fold higher secondary to the running condition versus the walking condition (p<0.001). Similarly, levels of euphoria doubled in the run condition but did not change with the walking condition (p<0.001), and anxiety was significantly reduced over time in the run condition only (p=0.024). Participants were also more likely to report a ‘runner’s high’ in response to the running condition versus walking. Opioid receptor blockade did not influence the results indicating that endorphins had no significant influence on the changes in affective state. Other studies in healthy, recreationally-fit and athletic adults have noted that exercise of long duration (>40 min) and moderate intensity (70-80% max) increase one or more eCB, enhance related biochemical markers such as brain-derived neurotrophic factor (BNDF), and improves mood outcomes including tension, depression, anger and vigour (6, 15-18).

To date, the investigation of eCBs and mood responses to acute exercise in humans has been limited to young, healthy populations with no data available in any chronically diseased cohort, including patients receiving cancer treatment. Given the many psychosomatic barriers to exercise in cancer patients (4), and the fact that dysregulation of the ECS is common in chronic disease states (12) there is currently a need to better understand how exercise affects eCB and mood responses in cancer patients as compared to their healthy peers.

# **Aims/Objectives/Hypothesis**

## **Primary Objective**

To compare the effect of a single bout of exercise on eCB and affective (mood) responses in adults with cancer currently receiving chemotherapy treatment versus their healthy peers.

## **Secondary Objective**

To examine the impact of relationships between eCB and mood parameter responses to exercise in adults currently receiving cancer treatment and their healthy peers.

## **Hypothesis**

**HO 1:** There will be no significant difference in eCB responses to exercise in adults currently receiving chemotherapy treatment versus their healthy, age-matched peers.

**HO 2:** There will be no significant difference in mood responses to exercise in adults currently receiving chemotherapy treatment versus their healthy, age-matched peers

**HO 3:** There will be no significant relationship between eCB responses and mood responses to exercise in the total cohort.

## 

## **Aims**

**Aim 1:** To evaluate and compare eCB and mood responses in adults currently receiving chemotherapy treatment versus their healthy, age-matched peers.

**Aim 2:** To examine the impact of relationships between eCB and mood parameter responses to exercise in adults currently receiving chemotherapy treatment and their healthy peers

# **Participating Sites**

All research procedures will be carried out in the Supportive Care and Integrative Oncology Department (aka. the Lifehouse LivingRoom) of the Chris O’Brien Lifehouse (COBLH) located in Camperdown, NSW. Blood samples will be stored at COBLH until completion of data collection and then couriered and analysed at the National Institute of Complementary Medicine (Westmead, NSW). A total of 20 participants (10 cancer patients and 10 healthy controls) will be recruited from the COBLH.

# **Research Plan**

## **Study Design**

A controlled trial comparing the effect of a single exercise bout on eCB and mood responses in cancer patients versus age-matched, healthy peers will be implemented.

## **Sample Size**

As it is a pilot feasibility study, the decision regarding sample size is pragmatic. We aim to recruit 20 participants for this study. Where applicable, sample size estimates to inform future trials will be computed *post hoc*.

## **Study duration**

Data collection is expected to take no longer than 12 months.

Start date: March 1, 2022

End date: February 28, 2023

*How long is each participants involvement?*

* Each participant will attend a single data collection session of approximately 1.5 hours. This session will involve the completion of Informed Consent and all data collection procedures.

## 

## **Participants**

Twenty (n=20) participants, including 10 patients currently being treated for cancer with chemotherapy and 10 healthy controls, will be recruited for this study. A recruitment checklist is presented in Appendix 1a and Appendix 1b for cancer patients and healthy controls, respectively.

*Cancer patients:* Adults (>18 years) commenced chemotherapy treatment for a non-haematological cancer with a 2 or 3-weekly protocol; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, medical approval of the treating oncologist (*via* email), sufficient English to provide consent and complete study procedures. Note: See Recruitment and Screening section for detailed information about participant recruitment, Page 13.

*Healthy controls:* Adults (>18 years); gender-, age- (±5 years maximum) and BMI-matched (±3.5 kg/m2 maximum) to the recruited cancer patient; no history of systemic cancer treatment; no history of cardiac, metabolic, pulmonary, neurological and/or uncontrolled risk factors; sufficient English to provide consent and complete study procedures. Note: See Recruitment and Screening section for more detailed information about participant recruitment, Page 13.

*Exclusion criteria for all participants:*

* currently using cannabis regularly, for any reason
* any use of cannabis or cannabis products (e.g. CBD oil) within the previous 72 hours
* vigorous exercise or use of complementary therapies such as acupuncture, massage, reflexology, yoga or mindfulness training within 24 hours of the research session
* currently using anti-depressant and/or anxiolytic medication
* continuous use of steroids (i.e. outside of their standard chemotherapy protocol in cancer patients)
* presenting with any absolute or relative contraindication to clinical exercise testing, as defined by the American College of Sports Medicine (19)

## **Procedures and Outcomes**

The schedule of assessments is detailed in the Table below (See Page 12). All data will be collected in the COBLH Living Room. Baseline data collection and resting conditions will be completed in a private consultation room or quiet allocated waiting area and the exercise condition will be completed in the gym space

**Baseline data collection:**

All eligible participants will be asked to attend the Living Room at COBLH for one data collection session of approximately 1.5 hours. The session will commence by the research assistant or exercise physiologist providing a verbal description of the research to the participant, then reviewing the participant information sheet and consent form with the potential participant, and addressing any queries or concerns where possible (Note: See Recruitment and Screening section for more detailed information about participant recruitment, Page 13).After signing the informed consent declaration, the participant will select their exercise test modality (i.e. exercise treadmill or bicycle ergometer or recumbent cycle) and will be provided with an opportunity to ask any further questions about the experimental procedures.

The participant will next complete a Demographic and Medical History Form (Appendix 2) which includes the assessment of height and body mass and using standard procedures (19). Body mass index (BMI) will be computed from these outcomes. To mitigate confounding affects, the data collection session will be scheduled for a day when the participant is not receiving any prescribed cancer treatment at the COBL (i.e. non-treatment medical appointments will be excepted). Participants will also be asked to refrain from exercising at a moderate to vigorous intensity for at least 24 hours, and refrain from utilizing therapies available at the COBL such as acupuncture, massage, reflexology, yoga or mindfulness training at least 24 hours before the session. In chemotherapy patients, the data collection will be timed to be prior to the commencement of the next cycle, including prior to pre-treatment medications, e.g. steroids. Participants will be queried on their adherence to these requirements in the Demographic and Medical History Form (Appendix 2). This form will be reviewed for accuracy and completeness by the research assistant, before continuing with the data collection.

Participants will then complete the assessment of symptomology *via* the Edmonton Symptom Assessment Scale-17 (ESAS-17) (Appendix 3) and depression/anxiety *via* the Hospital Anxiety Depression Scale (HADS) (Appendix 4) (20, 21). Both scales have been validated and utilised in clinical trials involving cancer patients (20, 21).

All participants will then complete ten Visual Analog Scales (VAS); i.e. by placing a vertical slash mark on a 100mm horizontal line ranging from 0 to 100 indicating their current emotional state for: (i) pain, (ii) nausea, (iii) anger, (iv) sadness, (v) happiness, (vi) energy, (vii) fatigue, (viii) anxiety, (ix) euphoria, and (x) depression (Appendix 5). This method has been employed in a previous trial of eCB response to acute exercise in healthy adults (14), and validated and utilised in clinical trials and cancer care to identify symptoms of concern and their acute responses to treatment interventions (22)

The baseline data collection will conclude with the first blood sample being taken. All blood samples will be collected by a trained healthcare professional (HCP) into 10 mL heparinised tubes. A small cannula will be inserted in the non-dominant arm. This cannula will be accessed for repeat blood samples, and then removed at the end of the session. It will be secured safely using appropriate dressings as per standard care to ensure adequate protection from dislodging. We have previously shown that patients can exercise safely with a cannula inserted in their arm, including exercise prescribed during haemodialysis (23) and during chemotherapy infusion (24) Note: Blood sample procedures and endocannabinoids assays are further detailed below; see Page 12.

**Resting condition (30 min):** Participants will remain seated and reading a book or magazine of their choice for a 30-minute period. Mobile phones will be placed on silent and placed out of reach of the participant. Heart rate and oxygen saturation (SpO2) will be collected at the beginning of the seated rest condition and at 1 min intervals thereafter (Appendix 5). Heart rate will be measured via Polar H/9/10 heart rate monitor (Polar Electro Oy, Professorintie 5, Fi-90440 Kempele, Finland). Oxygen saturation will be recorded via iHealth pulse oximeter. Blood pressure will be collected at the beginning of the seated rest condition and at 5 min intervals thereafter using stethoscope and a manual Welsh Allyn DS66 trigger aneroid sphygmomanometer. All measurements will be performed by an exercise physiologist trained in the procedure. The VAS scales will be collected immediately after the final round of observations (i.e. heart rate, SpO2 and blood pressure) followed by the second blood sample.

**Exercise condition (30 min):** The duration of time it takes for the participant to transfer to the exercise condition will be recorded (Appendix 5). The participant will be familiarised with the equipment and then commence the exercise. Participants will perform aerobic exercise on self-selected equipment modality (Precor treadmill, Precor upright cycle or Monark RT2 recumbent cycle ergometer) and aim to attain a moderate intensity (i.e. 64-76% of age-predicted maximal heart rate (HRmax=208 – (0.7 × age)) (19) within 5 min (warm-up) and then maintain this HR intensity range (64-76% of HRmax) for 20 minutes continuously. For the final 5 min, participants will perform a cool-down with the aim of reducing heart rate to below 60% of by the end of the 30 min condition. Rating of perceived exertion (RPE), heart rate and SpO2 will be collected at 1 min intervals throughout the exercise condition. Heart rate will be measured via a Polar H9/H10 heart rate monitor (Polar Electro Oy, Professorintie 5, Fi-90440 Kempele, Finland) and Oxygen saturation will be recorded via iHealth pulse oximeter. Blood pressure will be collected every 5 min via a manual Welsh Allyn DS66 trigger aneroid sphygmomanometer using the non-cannulated arm unless contraindicated (e.g. lymphedema risk). Exercise does not stop for the collection of any data. Recommended criteria for prematurely terminating the exercise condition will be adhered to, as defined by the American College of Sports Medicine (19). At the end of the exercise bout, the third and final blood sample being collected and then the ten VAS scales will be re-assessed, followed by a question asking participants whether they had experienced a ‘Runner’s high’ (Yes / No / I don’t know), as implemented in a previous trial (14) (Appendix 5).

**Schedule of Assessments**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Baseline** | **Rest condition (30min)** | **Exercise condition (30 min)** |
| Demographic and medical information; height, weight; body mass index (BMI) | X |  |  |
| Edmonton Symptom Assessment Scale (ESAS)\* | X |  |  |
| Hamilton Anxiety Depression Scale (HADS)\* | X |  |  |
| Visual Analog Scales (VAS) | X | X | X |
| Blood Collection | X | X | X |
| Heart Rate (every minute) |  | Every 1 minute | Every 1 minute |
| Oxygen saturation (every min during rest) |  | Every 1 minute | Every 1 minute |
| Blood Pressure (every 5 min) |  | Every 5 minute | Every 5 minute |
| Experience of a ‘Runners High’ |  |  | X |
| Adverse Events | Throughout | Throughout | Throughout |

\*To be completed by the cancer patients only

**Blood Samples and Endocannabinoid Assays**

Insertion of the cannula will be performed by a trained healthcare professional, and all research-related blood samples with be collected from this cannula by this healthcare professional. Blood samples will be immediately centrifuged under refrigeration (4°C) for 10min at 2000g, and the plasma separated into aliquots before freezing at -80C. The samples will then be transferred to NICM laboratories for quantification of eCB’s by LC-MS. The LC-MS method has been previously validated for clinical trial ACTRN12617001287325. The plasma samples will be defrosted immediately prior to analysis. 500ul of plasma will be analysed with the addition of 30ul of deuterated internal standards and 300ul acetonitrile. The mixture will be vortexed and cold sonicated for 5 min. The partitioning will be facilitated by the addition of Quechers salt and centrifuged at 18000g for 5 min. The upper organic layer (150ul) will be removed to a low recovery insert and mixed with equal volume MS grade water. The eCBs will be detected using MRM monitoring and quantified in comparison to a freshly prepared standard curve. The method has been validated for the quantification of AEA, 2-AG and related biogenic lipids (AA, PEA, OEA and SEA).

**Adverse events**

Approximately 40 years of clinical trial data have shown that moderate-to-vigorous intensity aerobic exercise is safe and effective across a broad range of cancer cohorts, age ranges, and across the cancer continuum. Adverse events have been exceedingly rare and limited primarily to the usual effects of exercise including delayed onset muscle soreness and/or other musculoskeletal complaints typically resolved conservatively (2). Nevertheless, adverse events will be monitored throughout the present study, according to standard procedures. Further information on safety and adverse reporting is provided in the respective section below.

**Statistical analysis**

Statistical analyses will be carried out using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, USA). ECBs and mood will be assessed using a repeated measures ANOVA with time x group x condition as within-subject variables. All data will be included regardless of participant attrition/drop-out. Regression models will be used to evaluate relationships between eCB and mood variables of interest. Additional analyses will be determined *post hoc* by consulting a biostatistician. Statistical significance will be set at p < 0.05, and effect sizes and confidence intervals will also be reported.

## **Recruitment and Screening**

**Cancer patients:** Advertisement through banners, flyers and QR code to further study information will be developed and distributed in the outpatient areas and day therapy unit of the hospital. Medical oncologists, supportive care physician, nurses or exercise physiologist can directly refer a patient into the study. A research assistant will then review clinical records of patients, to ascertain potential eligibility and contact the patient. Eligibility will be assessed using a standardized pre-screening checklist (Appendix 1a). The research assistant will complete the recruitment checklist. The potential participant, if interested, will receive a Recruitment Advertisement (Appendix 6) and Participant Information Sheet and Consent Form (Appendix 7) to take home and review, and will be asked if they are willing to be contacted via telephone to discuss any questions and determine their interest in participating.

**Healthy controls:** Advertisement through social media, in house communications email, flyers, banner with QR code to staff and other potential control participants will be distributed throughout the hospital staff and outpatient areas. The apparently healthy controls may also be recruited by asking (verbally) the enrolled cancer patients’ if they have friends or family members that would be willing to volunteer for the study. A telephone call will be made by the research assistant to determine the healthy control’s interest in participating and their eligibility (Appendix 1b). The research assistant will complete the recruitment checklist. A Participant Information Sheet and Consent Form (Appendix 7) will be provided via email. This will include the secondary pool of healthy controls from COBLH staff; these participants will be recruited via internal advertisement (Appendix 6).

If potentially eligible participants are interested in participating, they will be scheduled to attend a data collection session and be required to provide written informed consent prior to engaging in the research procedures, as outlined previously (See *Research Plan* section)

# **Ethical Considerations**

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2007), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. To this end, no patient will be recruited to the study until all the necessary approvals have been obtained and the patient has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject.

## **Randomisation Procedure**

Not applicable.

## **Data Linkage**

Not applicable*.*

## **Confidentiality and Privacy**

A unique study number will be assigned to the patient to maintain the participant’s privacy. The trial number will only be linked to the patient's details at the institution and will not be sent offsite.  The study data will be kept in coded form and will be stored in a computerised database located at COBLH. The investigators will maintain a confidential participant identification list that allows the unambiguous identification of each participant. Consent to transfer data is sought via the Patient Information and Consent Form (Appendix 7). No identifying information will be published. It is also understood that the recipients will treat the data in accordance with all applicable privacy legislation and local policies and that recipients will not use of disclose the information outside the parameters of the agreement between them and the institution. All data (including personal data) obtained will be treated as confidential. The personal data will be stored at each study site in encrypted electronic and/or paper form and will be password protected or secured in a locked room to ensure that only authorised study staff have access. Only coded blood samples will be transferred off site for analysis at the National Institute of Complementary Medicine (Westmead, NSW).

## 

## **Data Storage and Record Retention**

Data is de-identified with each participant assigned a study code (except for personal information). Any identifying data (name, email address, phone number) is stored in a password-protected program (REDCap). Only the study investigators will have access to completed consent data and research data.

REDCap is a secure, web-based application designed exclusively to support data capture for research studies and is secured according to Chris O’Brien Lifehouse security protocols which conform to electronic data standards. REDCap provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); and 4) procedures for importing data from external sources.

All data will be collected in person, i.e. with participants attending a single 1.5hr visit at the COBLH. It is the intention of the study team to capture as much material as possible immediately into the REDCap electronics case report file (eCRF). Where it is not possible for participant-reported outcomes to be captured electronically then a paper source document will be used. If this is the case, then data from the paper CRF will be entered by investigators into the REDCap eCRF, and this will be double-checked in its entirety by an independent source. Data will be collected on the electronic REDCap system.

All REDCap eCRF entries will be considered source data if the REDCap eCRF is the site of the original recording (e.g. there is no other written or electronic record of data, or CRF). All documents will be stored safely in confidential conditions on a secure server which is routinely backed-up. On all study-specific documents, other than the signed consent forms, the participant will be referred to by their allocated study ID number and not by any participant identifiable data.

All trial documents and source documentation will be retained at COBLH for a minimum of 15 years after completion of the trial in accordance with ICH GCP Guidelines. After this time all documentation

(electronic and paper) will be destroyed

# **Tissue Sampling/Biobanking**

Blood samples will be collected by a trained clinician into 10 mL labelled (number coded) heparinised tubes. Blood samples will be processed on site at COBLH. This will the bloods being immediately centrifuged under refrigeration (4°C) for 10min at 2000g, and the plasma separated into aliquots before freezing at -80C. The samples will then be transferred to NICM (Western Sydney University) laboratories for quantification of eCB’s by LC-MS. NICM will be completing the analysis as part of a research collaboration with COBLH of which the collaboration and sharing of biological samples will be covered under the terms and agreements outlined in the Medicines Australia “Clinical Trial Research Agreement”.

The LC-MS method has been previously validated for clinical trial ACTRN12617001287325. The plasma samples will be defrosted immediately prior to analysis. 500ul of plasma will be analysed with the addition of 30ul of deuterated internal standards and 300ul acetonitrile. The mixture will be vortexed and cold sonicated for 5 min. The partitioning will be facilitated by the addition of Quechers salt and centrifuged at 18000g for 5 min. The upper organic layer (150ul) will be removed to a low recovery insert and mixed with equal volume MS grade water. The eCBs will be detected using MRM monitoring and quantified in comparison to a freshly prepared standard curve. The method has been validated for the quantification of AEA, 2-AG and related biogenic lipids (AA, PEA, OEA and SEA). The blood collection is a mandatory component of the research; however, participants have the right to withdraw from a study procedure at any time, as indicated on the PIS/Consent form.

Unused biological samples will be retained at NICM for future research whose aim will be to investigate newly discovered endocannabinoids and/or blood-based markers (inflammatory, endocrine and immune system) that have relationships with acute endocannabinoid responses.

Any research projects applying to use these samples in the future would first need approval from Ethics. All blood samples will be destroyed by NICM in an agreed process upon completion of these future projects.

# **Safety and Adverse Events**

All clinical incidents and adverse events will be reported in line with NSW Health Policy Directive (PD2017\_039) titled Safety Monitoring and Reporting for Clinical Trials Conducted in NSW Public Health Organisations (released: 27 October 2017) (24). Participants will be referred to relevant healthcare professionals if they experience any adverse events requiring further investigation or treatment.

## **Definitions**

ADVERSE EVENT (AE): is any untoward medical occurrence in a study participant at any point during assessments or interventions that does not necessarily have a causal relationship with this treatment condition.

Adverse events include the following:

* + All injuries (e.g., fall, muscle strain, fainting, etc.) related to exercise or assessments.
  + Abnormalities during physiological testing or physical examination that require clinical intervention or further investigation (beyond ordering a repeat examination).

SERIOUS ADVERSE EVENT (SAE): is any adverse event that occurs whilst the patient is performing the exercise intervention or assessment, and that:

- results in death

- is life-threatening

- requires inpatient hospitalisation

- results in persistent or significant disability or incapacity

NOTES:

* The term “life-threatening” in the definition of “serious” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
* Important medical events which may not be immediately life-threatening or result in death or hospitalization but which may jeopardize the patient or may require intervention to prevent one of the listed outcomes in the definition above should also be considered serious.

## **Eliciting Adverse Event Information**

AE and SAE will be assessed, monitored and recorded during the data collection by the research assistant. The following information will be recorded for each SAE:

• Event description

• Primary and secondary diagnoses of event (If death/hospitalisation)

• Severity / Worst Grade

• Attribution to study intervention

• Action taken

• Impact of SAE (e.g. hospitalisation details)

• Outcome of SAE including end date if recovered

## **Safety Notifications**

The Principal Investigator (JL) is responsible for reporting all SAE occurring during the study using the relevant form in *Safety Monitoring and Reporting for Clinical Trials Conducted in NSW Public Health Organisations* (released: 27 October 2017) (25)*, the Local SUSAR/USADE/URSAE Notification Form*. (25). This form will be completed by the Principal Investigator (JL) and submitted to the RGO for the site where the event occurred within 72 hours of the Principal Investigator becoming aware of the event.

## 

## **Early Termination/Withdrawal of Participants**

Participants are free to withdraw from the study at any time. All data will be included to the point of withdrawal. For each participant that withdraws with an incomplete dataset, we will aim to recruit a new participant to reach our target sample size (n=20)

# **Blinding and Unblinding**

Not applicable. Participants and investigators will not be blinded to objectives/aims of the study, nor the interventions being tested.

# **Outcomes and Future Plans**

The results of the study will be published in a peer-reviewed scientific journals and disseminated at national and international scientific conferences as well as in education workshops/in-services. *The results of the project will be presented as group data, individual data will not be available.  If individual patient data is reported or made available at the request of the HREC, medical journals or other relevant party, it will be available only as non-identifiable patient level data.*

# **Statistics**

As above. See Research Plan.

# **List of Appendices**

Appendix 1a: Recruitment Checklist (Cancer Patients)

Appendix 1b: Recruitment Checklist (Healthy Controls)

Appendix 2: Demographic and Medical History Form

Appendix 3: Edmonton Symptom Assessment Scale-17 (ESAS-17)

Appendix 4: Hospital Anxiety Depression Scale (HADS)

Appendix 5: Main Data Sheet

Appendix 6: Recruitment Flyer

Appendix 7: Participant Information Sheet and Consent Form

# **Resources**

Funding provided by Chris O’Brien Lifehouse Living Room via The SurFebruary Cancer Research Fund ($40,000.00 AUD)

# **Budget**

|  |  |  |
| --- | --- | --- |
| **Item** | **Detail** | **Cost AUD** |
| Patient associated costs and consumables | Blood collection, storage, transport, and endocannabinoid and associated biogenic lipid analysis | $27,500.00 |
| Direct salaries and wages | Research Assistant / Nurse (0.4 FTE, 16 weeks) | $12,000.00 |
| Participant reimbursement | Parking fee ($25/participant x 20) | $500.00 |
| **TOTAL** |  | $40,000.00 |

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