**CONFIDENTIAL**

**Protocol**

**Document Type:** Clinical Study Protocol

**Trial:**  Exercise Intervention

**Sponsor:** Faculty of Medical and Health Sciences

 The University of Auckland

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 Auckland

New Zealand

**Investigators:** Dr Troy Merry

Dr Cherie Blenkiron

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**Origin Date:**  8th March 2017

# PROTOCOL SYNOPSIS

The following synopsis is provided as an overview of the study. The protocol text and appendices should be referred to for a comprehensive description.

|  |  |
| --- | --- |
| **Name of Sponsor:** Faculty of Medical and Health Sciences, The University of Auckland.   |  |
| **Title of Study:**  | The effect of high intensity exercise training on the gut microbiota in humans. |
| **Investigator(s):**  | Dr. Troy Merry, Dr. Cherie Blenkiron, Dr. Nick Gant, Julia Cree |
| **Objective:**  | To determine whether HIIT training changes the composition and diversity of gut microbiota. |
| **Methodology:** | Pre-post design |
| **Number of Subjects:**  | 40 |
| **Main Criteria for Inclusion:**  | Healthy males aged 18-50 years. Lean subjects BMI <25, overweight subjects BMI >28 |
| **Study Intervention:** | Exercise |
| **Duration of Intervention:**  | 5 weeks  |

|  |  |
| --- | --- |
| **Study Design & Visit Schedule:** | The study requires 11 visits in total (2 to the laboratory and 9 to high intensity interval training sessions)* Visit 1: screening, informed consent, food frequency questionnaire, fasted blood sample, collection of oral / buccal swab sample, aerobic fitness test to estimate maximum oxygen consumption (VO2max test) and body composition by dual energy X-ray absorptiometry (DEXA).
* Visit 2-10: High intensity exercise session (3 sessions x 3 weeks).
* Visit 11: fasted blood sample, collection of oral / buccal swab sample, VO2max test and DEXA.
* Prior to Visit 2 and 11 only: Collection of stool samples; 2 samples pre and 2 samples post exercise intervention. Participants will collect their own stool samples using the kits provided at visit 1. Stool samples will be picked up from the participants’ location within a suitable timeframe by a study investigator.
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| **Study data:** | Primary endpoint* Composition of gut microbiota as determined by analysis of stool samples

Secondary endpoints* VO2max
* Composition and diversity of oral microbiome
* Blood biomarkers of metabolism
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| **Safety:** | Adverse events will be recorded and subjects presenting with adverse events during the trial will be treated as per standard clinical practice by their local primary care, health provider or other appropriate medical facility |

# TABLE OF CONTENTS

PROTOCOL SYNOPSIS 2

TABLE OF CONTENTS 4

1 ETHICS 6

1.1 Institutional Review Board / Ethics Committee 6

1.2 Informed Consent 6

2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE 7

2.1 Principal Investigator 7

2.2 Study Initiation 7

2.3 Study Discontinuation 7

3 INTRODUCTION 8

3.1 Background 8

3.2 Study Objectives and Hypotheses 9

Objectives 9

Hypotheses 9

4 INVESTIGATOR PLAN 10

4.1 Study Design 10

4.2 Study Methods 10

4.3 Testing Procedures 11

Stool sample collections 11

Blood Collection 11

Oral / Buccal Swab Sample Collection 11

Dual Energy x-ray Absorptiometry 11

4.4 Analysis of Samples 12

4.5 Outcome Measures 12

Stool and buccal swab measures 12

Blood measures 12

Morphological Measures 12

4.6 Subject Compensation 12

5 STUDY POPULATION 13

Inclusion Criteria 13

Exclusion Criteria 13

6 STUDY PLAN 13

Sample Size and Study Centres 13

Timing of the Study 13

Summary of Study Visits 13

7 STATISTICAL METHODS 14

Endpoints 14

Primary Endpoints 14

Secondary Endpoints 14

8 ADVERSE EVENTS 15

Adverse Event Definitions 15

Serious Adverse Events 15

Reporting of Adverse Events 15

Recording of adverse events 15

12 STORAGE OF DATA 17

REFERENCES 18

# 1 ETHICS

## 1.1 Institutional Review Board / Ethics Committee

The Principal Investigator agrees to provide the Institutional Review Board/Ethics Committee (IRB/EC) with all appropriate material, including the subject information sheet and the informed consent document. The trial will not be initiated until appropriate IRB/EC approval of the protocol and the informed consent document have been obtained in writing by the Investigator and copies have been received by The University of Auckland Research Office. Appropriate reports including an annual update/report on the progress of the study by the Principal Investigator will be submitted, if required to the IRB/EC and the Research Office.

## 1.2 Informed Consent

Properly executed written informed consent, in compliance with the International Conference on Harmonization (ICH) guidelines, shall be obtained from each subject before the subject is entered into the study or before any unusual or non-routine procedure is performed that involves risk to the subject. If new information related to the study arises, subject will be asked to sign a revised informed consent document. If applicable, it will be provided in a certified translation of the local language. Signed consent forms must remain in each subject’s study records and must be available for verification by study monitors if required.

# 2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

## 2.1 Principal Investigator

**Principal-Investigator:** Dr. Troy Merry

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## 2.2 Study Initiation

Investigators must complete all regulatory documentation as required by local and national regulations. Investigators must agree to comply with the obligations detailed.

## 2.3 Study Discontinuation

A subject may withdraw consent for participation in the study at any time without prejudice. Additionally, the Investigator may withdraw a subject if, in his/her judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol. Whenever possible, the tests and evaluations listed for the End of Study visit should be carried out at the time of subject withdrawal or whenever the Investigator feels that the subject will be unable to make any further visits. A genuine effort must be made to determine the reason(s) why subjects fail to return for the necessary study visits.

# 3 INTRODUCTION

## 3.1 Background

The term gut microbiota refers to the large number of microbes which colonise the human intestine, estimated at approximately 100 trillion microorganisms, over 10 times the total number of cells in the human body ([1](#_ENREF_1)).

Whilst it is widely acknowledged that diet has an influence on gut microbiota composition, there is emerging evidence from rodent studies that exercise (specifically high intensity interval training, HIIT), may also influence bacterial diversity in the gut ([2](#_ENREF_2)). In addition, a recent study involving aerobically conditioned horses (fillies only) suggested that a bout of intense exercise may significantly alter the intestinal microflora ([3](#_ENREF_3)). This has particular importance in relation to health because gut microbiota can influence metabolism and gut bacterial diversity has been hypothesized to be a contributing factor to the development of obesity and metabolic disease. In particular, an increase in the ratio of Firmicutes:Bacteriodetes phyla was associated with obesity ([4](#_ENREF_4)), linked to the enhanced ability of the obese microbiome to extract energy from the diet ([5](#_ENREF_5)). This hypothesis has been further supported by microbiota transplant studies from obese to lean germ-free mice which promoted development of obesity in recipients ([6](#_ENREF_6)).

It is currently unclear whether exercise can influence gut microbiota composition in humans, as to date only a single cross sectional study examining the influence of exercise on gut microbiota ([7](#_ENREF_7)) has been published. This research compared the gut microbiota of elite professional rugby players (during a preseason training camp) with healthy male controls and observed that the athletes had a significantly more diverse gut microbiota than controls and lower proportions of Bacteriodetes. However, diet is a potential influence on these results, as the athletes consumed significantly more calories, protein, fat and carbohydrate than either control group. In addition, the athletes and the control group with a BMI of 25 or less has significantly higher proportions of the genus Akkermansia than the control group with a BMI of 28 or more. Lower levels of Akkermansia have previously been shown to be associated with obesity in murine models ([8](#_ENREF_8)) and also in obese and overweight pre-school children ([9](#_ENREF_9)).

High intensity exercise intervention produce physiological changes resulting in significant improvement in cardiorespiratory fitness ([10-12](#_ENREF_10)) and insulin sensitivity ([13](#_ENREF_13)) in as little as 3 sessions a week for 3 weeks. However, there is a lack of published research specifically examining the relationship between exercise and composition of gut microbiome in humans. It is possible that the modification of the composition of gut microbiota as a result of exercise may be one of the mechanisms by which exercise positively affects metabolism.

Whilst exercise and physical activity are acknowledged as a means to prevent or delay numerous chronic conditions ([14](#_ENREF_14)), however many people do not adhere to an exercise regime citing lack of time, enjoyment or motivation ([15](#_ENREF_15), [16](#_ENREF_16)). High intensity interval training (HIIT) has been demonstrated to be more potent and time efficient than exercise at a constant intensity ([16](#_ENREF_16), [17](#_ENREF_17)). Therefore, should research indicate the potential for HIIT to modify gut bacterial diversity, this may be a more effective mechanism through which to improve metabolic health than current exercise treatments.

## 3.2 Study Objectives and Hypotheses

### Objectives

The primary objective of this study is:

* To evaluate the effect of multiple sessions of high intensity exercise training on gut microbiome.

The secondary objectives of this study are:

* To determine the effect of multiple sessions of high intensity exercise training on fitness.
* To evaluate the effect of multiple sessions of high intensity exercise training on oral / buccal microbiome.
* To evaluate the effect of multiple sessions of high intensity interval training on blood biomarkers which can be used to assess metabolic health and inflammatory response.

### Hypotheses

* We hypothesise that the high intensity exercise intervention will change the composition and diversity of the gut microbiome, for example decreasing the ratio of Firmicutes:Bacteriodetes phyla.
* We hypothesise that the high intensity exercise intervention will improve fitness / exercise performance.
* We hypothesise that the high intensity exercise intervention will not significantly affect the composition and diversity of the oral / buccal microbiome.
* We hypothesise that the high intensity exercise intervention will change blood biomarkers of metabolic health.

# 4 INVESTIGATOR PLAN

This clinical study was designed by researchers at the University of Auckland (TLM, CK, NG, JMC).

## 4.1 Study Design

This study will be a pre-post intervention.

The intervention will be acute exercise and exercise training on a stationary cycle ergometer in a clinical exercise physiology laboratory.

## 4.2 Study Methods

Participants will be recruited using word of mouth, local business contacts, social media, and advertising at University of Auckland Tamaki and/or Auckland campuses. Prior to attending visit 1, participants will be given a copy of the PIS-CF and have the details of the study explained to them and will be able to ask questions. Also at this time, participants will be advised not to eat or drink anything except water after 10pm in order to be able to supply a fasted blood sample at the laboratory first thing in the morning of following day at visits 1 and 11. In addition, participants will be asked to abstain from caffeine in the 12 hr prior, and alcohol and vigorous activity in the 24h prior, to the fitness / exercise testing (visits 1 and 11).

Informed consent to participate in the trial will be obtained from the participant, and participants who meet the inclusion/exclusion criteria will be registered into the trial. The participants will be requested to visit the laboratory on 2 occasions over 5 weeks to undergo fitness, exercise testing and/or collection of samples, and attend a high intensity exercise training session on 9 occasions over a period of 3 weeks to perform exercise on a stationary bike. Each high intensity exercise session will be separated by at least 1 day and no more than 4 days.

*Visit 1:*

Participants will be interviewed to determine if they meet criteria for inclusion in the study and will be asked to provide informed consent. A fasted blood sample will be taken by a trained phlebotomist and participants will be asked to fill in a food frequency questionnaire. Participants will be asked to provide an oral swab sample. Participants will have their height and weight recorded, and undergo a cardiorespiratory fitness test (to estimate VO2max) and body composition (DEXA scan) testing. Participants will undergo the VO2max test on a cycle ergometer. Following a warm up, subjects will be asked to maintain a cycle cadence of >60 rpm during a ramp protocol until one or more of the criteria of reaching VO2max is met. This is expected to take <15 mins. Participants will be provided with a stool sample collection kits and instructed to provide at least two independent samples prior to visit 2 and visit 11 (pre and post exercise intervention). There should be a minimum of 36 hrs between collection of the 2 samples, and collection should take place at least 48 hours after the VO2max test and / or any vigorous exercise.

*Visits 2 - 10:*

Participant will be asked to perform 8-12 repeated 60s intervals of high-intensity stationary cycling (at 80-100% of VO2max output) with 75s of recovery between each repetition.

*Visit 11:*

Prior to attending visit 11, participants will be advised not to eat or drink anything except water after 10pm in order to be able to supply a fasted blood sample at the laboratory first thing in the morning of following day. A fasted blood sample will be taken by a trained phlebotomist and participants will be asked to provide an oral swab sample. Participants will have their height and weight recorded, and undergo VO2max and body composition (DEXA scan) testing.

## 4.3 Testing Procedures

### Stool sample collections

Participants will be instructed on how to collect their stool samples using the aFecal collection tube, prior to visits 2 and 11. Participants will be provided with a container with which to collect their stool without letting the sample go into the toilet. Using the spoon provided in the collection tube cap, one spoonful of feces from the sample will be placed in the collection tube, the cap tightened and the tube shaken to mix the contents thoroughly. The unused fecal material may then be flushed down the toilet and participants should wash their hands. The participants will contact the study investigator to advise that the sample is ready for pick up.

### Blood Collection

Blood samples will be collected from an antecubital vein by a trained phlebotomist using a venipuncture during visits 1 and 11. No more than 20 ml of blood will be collected per visit.

### Oral / Buccal Swab Sample Collection

An oral / buccal swab sample will be collected using the a collection tube with swab at visits 1 and 11. The swab package will be opened and swab removed, and the participant asked to open their mouth. The investigator will gently rub and rotate swab along the inside of the cheek for 5-10 seconds, ensuring that the entire swab-tip has made contact with the cheek. The swab will then be inserted into the collection tube, prior to capping and inverting the collection tube.

### Dual Energy x-ray Absorptiometry

Dual energy x-ray absorptiometry (DEXA) provides a rapid non-invasive measure of body composition based on scanning and imaging techniques. It is also an indirect method of assessment of fat free mass (FFM) and fat mass (FM). DEXA measures the density of different tissues from the different attenuation of 2 levels of x-rays and under normal hydrated conditions is reliable measure of body composition ([18](#_ENREF_18)). A rapid scan DEXA (model iDXA, GE-Lunar, Madison, WI) will be used in this study, and body composition which comprises total body fat, fat-free soft tissue and bone mineral content, will be determined from DXA whole-body scans. FFM will be calculated as the sum of fat-free soft tissue and bone mineral content. It is appropriate for use in this study since it will provide the required segmental composition data. Participants will be positioned on the DEXA table and asked to lie still during the scan. The total radiation exposure per DEXA scan is 0.03mSV which is comparable to the background radiation expose over the course of a normal day.

***Questionnaire***

The questionnaire will be administered during the screening visit to assess inclusion/exclusion criteria.

***Food Frequency Questionnaire (FFQ)***

The FFQ will be administered during the screening visit to assess habitual dietary patterns. Participants will be asked to maintain similar their current diet habits throughout the study.

## 4.4 Analysis of Samples

DNA and RNA will be extracted from buccal and fecal samples and subjected to sequencing analysis using microbe specific primer sets used to amplify regions for next generation sequencing (NGS). This analysis will NOT be able to analyse human DNA sequences/donor genetics and may be competed overseas. Blood will be analysed for hormones, cytokines, mitochondrial derived peptides, genes and metabolites associated with exercise and metabolism.

## 4.5 Outcome Measures

### Stool and buccal swab measures

Composition ( ie pH, macronutrient and micro nutrients, and energy density and metabolite levels) and Bacterial composition and activity.

### Blood measures

Blood biomarkers and metabolites associated with exercise and metabolism will be determined in blood samples such this includes peptides, hormones and substrates.

### Morphological Measures

Whole body fat and fat free mass, and bone mass will be measured with DEXA.

## 4.6 Subject Compensation

Because this study will involve 2 laboratory visits and 9 high intensity exercise sessions, it is important to compensate the subjects for the time commitments related to the study. For this reason, subjects will be given $100 worth of MTA fuel vouchers for their participation. They will also be given information about their maximal aerobic capacity, body composition, blood biomarkers, gut and oral/buccal microbiome profile as well as having 9 supervised high intensity exercise sessions. Participants will be informed that they are free to withdraw at any time however the amount of compensation they receive will be prorated based on how many of the laboratory visits they have completed.

# 5 STUDY POPULATION

Informed consent to participate in the trial will be obtained from the participant, and participants who meet the inclusion/exclusion criteria will be registered into the trial.

## Inclusion Criteria

The following inclusion criteria must be met for subjects to be eligible for study admission:

* Male
* 18-50 years
* Sedentary to moderately active (structured activity of less than 4 hours per week)
* Healthy

## Exclusion Criteria

Subjects will be excluded from the study if they meet any of the following criteria:

* Any previous or current chronic health conditions
* Diabetes
* Any previous or current gastro-intestinal or immune health conditions
* History of alcohol abuse
* Smoking
* Taking medications which may affect exercise responses
* Have taken antibiotics or non-steroidal anti-inflammatory drugs within the previous 2 months
* Any musculoskeletal injury which may affect ability to perform cycling exercise
* Have taken any probiotic supplement or supplement to improve gut health within the last 2 months

# 6 STUDY PLAN

## Sample Size and Study Centres

This will be a single centre study involving 20 subjects.

## Timing of the Study

Recruitment will commence in April 2017 and end when sufficient subjects have been recruited.

### Summary of Study Visits

Visit 1 (Screening Visit)

* Screen
* Informed consent
* Fasted blood sample
* Oral / buccal microbiome
* Height & weight
* FFQ
* body composition (DEXA)
* Maximal aerobic capacity (VO2max test)

Visits 2 - 10

* High intensity exercise session

Visit 11

* Fasted blood sample
* Oral / buccal microbiome
* body composition (DEXA)
* Maximal aerobic capacity (VO2max test)

# 7 STATISTICAL METHODS

ANOVA or non-parametric tests where appropriate and followed up with post-hoc tests will be used to determine differences in end points. A biostatistician will be consulted for analysis of microbiome data. Significance will be accepted at P<0.05.

##

## Endpoints

### Primary Endpoints

* Composition and diversity of gut microbiome

### Secondary Endpoints

* Composition and diversity of oral / buccal microbiome
* VO2max and exercise performance
* Blood biomarkers and metabolites

# 8 ADVERSE EVENTS

## Adverse Event Definitions

For this protocol, an adverse event (AE) is any untoward medical occurrence (e.g. sign, symptom, disease, syndrome, intercurrent illness, abnormal laboratory finding) that emerges or worsens relative to pre-treatment baseline, during the exercise intervention. The untoward medical occurrence may not necessarily have a causal relationship to the exercise. An AE can therefore be any unfavourable and/or unintended sign (including an abnormal laboratory result), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the exercise intervention.

## Serious Adverse Events

An adverse event occurring at any time during the study should be classified as **SERIOUS** if:

* It resulted in death (i.e., the AE caused or led to death)
* It was life-threatening (i.e., the AE placed the subject at immediate risk of death; it does not apply to an AE that hypothetically might have caused death if it were more severe)
* It required or prolonged in-participant hospitalization (i.e., the AE required at least a 24 h in-subject hospitalization or prolonged a hospitalization beyond the expected length of stay. Hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion)

## Reporting of Adverse Events

Expedited Reporting:

The Investigator will report any fatal or life-threatening adverse event to the IRB/EC within 3 working days via the Serious Adverse Event (SAE) form. A more detailed report may be required for submission to IRB/EC no later than 5 working days after the Investigator discovers the event. The Investigator will report to IRB/EC a report of any unexpected events no later than 10 working days after the Investigator first learns of the event.

## Recording of adverse events

Adverse events will be collected by observing and interviewing the participant during the study. All adverse events (serious and non-serious) will be recorded. For events that satisfy the regulatory definition of serious, serious adverse event report forms will also be completed. The description of each event will include the dates of onset and remission. Additionally, each adverse event will be assessed for severity using the protocol severity grading system (mild, moderate or severe). The SAE report will also contain information on the location, relationship to the treatment or control intervention required if any, and the outcome of the event.

Protocol Severity Grades:

The Investigator will grade all AEs according to and the following protocol severity criteria:

* **Mild - Grade 1:** event may be noticeable to subject; does not influence daily activities; usually does not require intervention
* **Moderate - Grade 2:** event may be of sufficient severity to make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed
* **Severe - Grade 3:** event may cause severe discomfort; usually interferes with daily activities; subject may not be able to continue in the study; treatment or other intervention usually needed

All participants who experience adverse events will be followed by the Investigator until there is a return to the participant’s baseline condition or until a clinically satisfactory resolution is achieved. The appropriate follow-up visits must be scheduled and the specific tests repeated or performed as necessary. Where a diagnosis is possible, it is preferable to report this diagnosis rather than a series of terms (signs/symptoms) relating to the diagnosis.

Assigning Causality:

Using the following criteria, Investigators will also assess whether there is a reasonable possibility that the intervention caused or contributed to the AE:

A. **Definitely Related:** There is a clinically plausible time sequence between the onset of the AE and study and/or exercise intervention and all other potential causes have been ruled out.

B. **Probably Related:** There is a clinically plausible time sequence between the onset of the AE and the study and/or exercise intervention; the AE is unlikely to be caused by the concurrent/underlying illness, other drugs, or procedures; and (if applicable) the AE follows a clinically consistent resolution pattern upon withdrawal of treatment or control administration.

C. **Possibly Related:** There may or may not be a clinically plausible time sequence between the onset of the AE and study and/or exercise intervention and the study and/or exercise intervention as a cause cannot be ruled out.

D. **Not related:** Another cause of the AE is most plausible; a clinically plausible temporal sequence is inconsistent with the onset of the AE and study and/or exercise intervention; and/or a causal relationship is considered biologically implausible.

# 12 STORAGE OF DATA

The Investigator will maintain the records of signed consent forms, and supporting documentation for a minimum of ten years after the study.

Data will be stored on a password-protected computer that investigators can access. Once all research data is collected names will be removed from all data sets to de-identify findings linked to minimal subject information attained from the questionnaires. Data, including research findings, will be stored electronically, and questionnaires will be stored in paper format (in a locked office at the University of Auckland). Each will be stored securely for a period of ten years.

The Investigator may withdraw from the responsibility to maintain records and transfer custody of the records to another person who will accept responsibility for them.

As part of standard research it is expected that findings will be presented at conferences and reported in peer-reviewed journal articles. All data will be presented in an anonymised fashion such that the participants would not be able to identify themselves.

# REFERENCES

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