Protocol

Improving high intensity interval training response (IMPROVE HIIT) – Pilot Study

RESEARCHERS

Principal investigator: Professor Jeff Coombes
School of Human Movement and Nutrition Sciences
The University of Queensland
Email: jcoombes@uq.edu.au

Co-investigator: Camilla Williams, PhD candidate
School of Human Movement and Nutrition Sciences
The University of Queensland
Email: camilla.williams@uq.net.au

Co-investigator: Dr Luciana Torquati
School of Human Movement and Nutrition Sciences
The University of Queensland
Email: l.torquati@uq.edu.au

Co-Investigator: Dr Jonathan Little, PhD
University of British Columbia
Email: jonathan.little@ubc.ca
Phone: 1 250 8079876

Co-Investigator: Dr Nir Eynon, PhD
Victoria University
Email: nir.eynon@vu.edu.au
Phone: +61 3 9919 5615

Co-Investigator: Dr Ilaria Croci, PhD
Norwegian University of Science and Technology
Email: ilaria.croci@ntnu.no

Resources

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SUMMARY

Cardiorespiratory fitness (CRF) is one of the biggest predictors for chronic disease morbidity and mortality; however, one in five adults report little to no improvement in CRF ($\dot{V}O_{2max}$) following exercise training [1]. Variability can be attributed to a myriad of factors, such as age, sex, gender and baseline $\dot{V}O_{2max}$ [2-4]. Genetic make-up contributes to approximately 50% of $\dot{V}O_{2max}$ trainability [5]. From our systematic review, we identified nearly 100 genetic variants associated with $\dot{V}O_{2max}$ trainability [6]. Individuals can be given a gene predictor score (GPS) based on how many genetic variants they have that contribute to a high or low $\dot{V}O_{2max}$ training response; with 0 representing homozygote for an allele (genetic variant) associated with a low response to training; 1 for heterozygous and 2 homozygous for an allele (genetic variant) associated with a high response to training. A lower score can indicate a lower predicted $\dot{V}O_{2max}$ training response.

Typically, there are fewer low responders with high intensity interval training (HIIT) compared to moderate intensity continuous training (MICT), and sprint interval training (SIT) [2, 7]. Individuals with a low GPS ideally should be prescribed a HIIT intervention over other forms of training to elicit greater adaptations. Despite this, variability will still exist. Investigating other possible gene expression and enzyme/protein mediators that may positively influence results, such as the gut microbiome, may help to improve cardio-respiratory changes in 'non-responders' to high intensity exercise.

The gut has received much attention over the past few years for its increasing role in human health and disease. Recent research has found a correlation between host genetics and the gut genome [8-10]; with $\dot{V}O_{2peak}$ associated with greater gut microbial diversity [11-14]. Gut microbiome diversity is a marker of health and can be increased by the consumption of prebiotic fibre [15-18].

There has been minimal, if any, research to identify the association between the gut microbiome and its effect on $\dot{V}O_{2peak}$ trainability. More specifically, do individuals with a low or high response genetic profile for trainability have differing gut microbiota; and can this microbiome be positively influenced to elicit greater CRF changes for the host?

The overall objective of IMPROVE HIIT is to contribute to evidence-based personalised medicine. Understanding the factors that influence training variability could be used to improve individualised exercise prescription, thereby contributing to health maintenance and treatment/prevention of disease.

AIMS

Primary Aim

- To identify if altering the gut microbiome with prebiotic supplementation influences the $\dot{V}O_{2max}$ training response to HIIT

Primary Hypothesis

- Altering the gut microbiome will influence the $\dot{V}O_{2max}$ training response to HIIT

Secondary Aims

- To identify if the gut microbiome is correlated to the gene predictor score
- To detect if altering the gut microbiome improves chronic disease risk factors

Secondary Hypotheses

- Gene predictor scores associated with the HIIT-induced $\dot{V}O_{2max}$ training response will be correlated with a specific gut microbiota taxa.
- Manipulating the gut microbiome will improve chronic disease risk factors
- The gene predictor score will predict lower vs higher responders to the HIIT-induced $\dot{V}O_{2max}$ response

BACKGROUND

The worldwide prevalence of chronic diseases, such as cardiovascular disease, cancers, stroke and diabetes is rising [19]. Low cardiorespiratory fitness is strongly associated with chronic diseases and premature mortality [20-22]. To alleviate the health and economic burden associated with low cardiorespiratory fitness, health guidelines across the world recommend individuals undertake regular exercise training [19].

Improvements in cardiorespiratory fitness in response to exercise training varies greatly between individuals, with some people responding well or very well ('responders' or 'high-responders') to exercise training, whereas others only have mild increases in their cardiorespiratory fitness following similar exercise training ('low-responders') [2-4, 23, 24]. The ability to change cardiorespiratory fitness is a multifactorial trait [2, 4]. Considering cardiorespiratory fitness is one of the best integrative predictors of morbidity and mortality risk, it is important to understand how factors, such as genetics and environmental influences (e.g. training and diet), may predict the variability in response to exercise training. This knowledge could lead to targeted personalised exercise therapy to decrease the burden of chronic disease.

Predictor genes for VO_{2max} trainability

From our systematic review, 35 studies identified 97 genes that predict $\dot{V}O_{2max}$ trainability [6]. Exercise-related phenotypes are complex traits and are polygenic (i.e. influenced by many genes working together) with each genetic variant likely to be contributing a small percentage (typically less than 1%) to the overall change in $\dot{V}O_{2max}$. Approximately, 50% of the variance in $\dot{V}O_{2max}$ trainability can be attributed to genetic make-up. A gene predictor score (GPS) based on these variants can predict who may be a lower or higher responder to training. A score of '0' represents homozygote (two copies of the same allele) for the low-response variant; '1' represents heterozygous (two different alleles, with one a high response allele) and '2' represents homozygous for the high-response allele. Individuals with a low score (e.g. <9 high response alleles) may be a lower responder to training.

Possible Gene Expression Mediators

Training intervention

HIIT has a lower frequency of 'non-responders' [7]; and greater improvements in vascular function, cardiorespiratory fitness and other biomarkers compared to other forms of training [7, 25]. This training requires individuals to exercise at a high intensity (e.g. 90% of maximum heart rate) for a specific period of time (e.g. 4 minutes) with a rest/recovery period (e.g. 3 minutes) in between [26].

Exercise can alter expression of genes (messenger RNA (mRNA) and protein levels) related to cardiovascular function, such as mitochondrial function and energy use [27, 28]. Methylation (addition of methyl group to DNA regions) can increase gene expression, and affect metabolic adaptions in skeletal muscle [27]. Genes can be "bookmarked" with methyl groups to switch their expression on and off. In skeletal muscle, most genes related to metabolism are demethylated following long-term exercise training, with consequent increased expression of such genes [29]. These changes appear to be dose dependent and transient, with higher intensity exercise causing greater demethylation and gene expression compared to lower-intensity exercise [27]. This dose-response relationship might suggest that higher intensity exercise may be more effective in typical 'low-responders' to training. Working at higher thresholds might be necessary to activate certain genes and molecular pathways required to induce an exercise training response [2]. Hence, the HIIT protocol is being used in our proposed second study.

The Gut Microbiome

The microbiome is considered our second genome, and contains 150 times more genes than its host [30]. The gut microbiome is found mainly in the colon and consists predominantly of bacteria, with smaller communities of archae, eukaryotes and viruses [30, 31]. Just like the human genome, each microbiome is unique [32].

The gut is involved in many processes, such as digestion, production of essential vitamins, hormones and neurotransmitters; and gut barrier defense [30-34]. Increased gut microbiome diversity has been associated with health, and is used as a marker of health status [15-18].

Whilst the gut microbiome is shaped from birth, it can be manipulated by several factors [9, 30-33]. For example, host genetics, diet and lifestyle choices (e.g. exercise) can affect gut diversity and overall health [8, 9, 30, 33].

Host Genetics and the Gut Microbiome

Host genetics can contribute to gut communities, diet preference, inflammation and metabolism, and complex diseases such as Chrohn's Disease, type II diabetes and obesity [8, 35]. For example, the NOD2 gene is a risk locus for irritable bowel disease (IBD), with 11 IBD genetic variations associated with a reduction in the *Roseburia* genus (which helps to produce butyrate) [36]. Suggestive links have also been found between the lactase non-persistence gene (LCT) variant and *Bifidobacterium* abundance [8].

In a recent study examining over 1514 Dutch individuals, 58 SNPS at 9 loci were associated with gut bacteria ($p<5x10^{-8}$, FDR <12%). *Blautia Genu* (*Firmicutes* phylum) and *Methanobacteriaceae* members (*Euryarchaeota* phylum) had the strongest association with the host ($p<5x10^{-8}$), and are positively associated with Chrohn's disease, obesity, lipid levels and BMI [37]. Genetic variants were identified in the *APOE* gene (lipid transfer) and the *PPAR* gene (fatty acid storage and glucose metabolism) [37]; both of which are predictor genes for VO_{2max} trainability [6].

Studies have also found associations with the gut diversity and the Vitamin D receptor gene, GRIK 4 [38] and CD36 [9]. The latter two genes have been identified as possible predictor genes for VO_{2max} trainability [6].

Exercise and the gut microbiome

Endurance exercise appears to increase alpha diversity, particularly members from the *Firmicutes* phylum [13]. Mice studies have demonstrated that a more diverse gut microbiota correlates with antioxidant enzyme activities (increased catalase and GPX activity) and hydration levels, which is associated with reduced fatigue and improved performance [13]. Human studies have found athletes to have greater gut diversity compared to the general population [11]. In a study of 40 male athletes and 40 comparators, the gut microbiota was significantly more diverse in the athletes, with a greater enrichment of pathways associated with *Akkermansia* bacteria, and production of short-chain fatty acids (e.g. butyrate and propionate) ($p<0.001$) [11]. Butyrate was strongly associated with fiber intake, and propionate with protein intake [11].

In a recent human cross-sectional study [12], $\dot{V}O_{2peak}$ was related to gut microbial diversity. Fourteen phyla (and 207 genera) were found across the 39 participants (age range 18-35 years). Those with a higher $\dot{V}O_{2peak}$ had a greater number of taxa (173 vs 153 for those with a lower $\dot{V}O_{2peak}$), with 20% of the variance attributed to $\dot{V}O_{2peak}$ independent cofounders, such as diet [12]. $\dot{V}O_{2peak}$ was significantly ($p<0.05$) associated with butyrate-producing *Coprococcus, Roseburia, Adlercreutzia, Clostridiales, Lachnospiraceae* and *Erysipelotrichaceae* species[12]. Those with a higher VO_{2peak} had lower levels of lipopolysaccharide biosynthesis (which is related to inflammation), and higher levels of fatty-acid biosynthesis ($p=0.046$) [12].

Furthermore, 6 weeks of aerobic training (30-60 minutes, 3x/week) increased the fecal concentration of acetate, propionate and butyrate concentrations in 32 sedentary adults, with changes in butyrate and acetate being BMI dependent (greater changes occurred in lean participants, $p<0.05$) [39]. The butyrate regulating gene, butyral coA: Acetate CoA transferase (BCoAT), and the propionate regulating gene, methylmalonyl-CoA decarboxylase (mmdA), were positively related to the changes in short chain fatty acids following the exercise intervention ($p<0.05$); with greater concentrations associated with a higher $\dot{V}O_{2max}$, lean body mass and lower body fat at baseline (obese participants had double the concentration of BCoAT compared to lean participants) [39].

Diet (short chain fatty acids) and the gut microbiome

A high-fibre diet has shown to control appetite and weight; and reduce the risk of cardiovascular disease, colon cancer, irritable bowel disease and inflammatory bowel disease [40]. One of the gut's primary functions is fermentation of non-digestible carbohydrates (e.g. fiber) into short chain fatty acids (SCFA), including acetate, propionate, butyrate (ratio of 60:20:20 respectively); and secondary bile acids [13, 40]. The Firmicutes phylum mainly produces butyrate, whereas the Bacteroidetes phylum mainly produce acetate and propionate [40].

A review has highlighted recent animal-based research detailing the bidirectional communication that exists between the gut microbiome and host mitochondria [41]. The gut and their by-products influence energy production and redox balance directly and indirectly via transcription factors; and mitochondria can regulate functions of the gut through genetic variations [41]. For example, genetic variants in the *ND4* (A13434G) and *CYTB* (T15784C) genes (D-loop region of the host mitochondria) can affect the gut community by increasing the number of *Eubacterium* and *Rosburia* species (which are butyrate producers) [41].

SCFA can regulate the transcription factors involved in energy production and mitochondrial function [41] For example, SCFA regulate glucose and fatty acid metabolism by binding to free fatty acid receptors 2 and 3 (*FFAR2, FFAR3)* and by regulating *SIRT1* (via PGC1- α deacetylation) [41]. Butyrate can promote fatty acid oxidation and ATP production by increasing the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in brown adipose tissue and skeletal muscles [41]. Butyrate can also activate AMPK in colonocytes by stimulating the UCP2-AMPK-acetyl CoA pathway, thereby decreasing peroxisome proliferator-activated receptor gamma (*PPARG*) gene expression, and increasing the AMP:ATP ratio (which also results in increased AMPK in the liver, muscle and adipose tissues). As a result, this stimulates glucose uptake, mitochondrial FAO and oxidative phosphorylation [41]. Secondary bile acids can also control carbohydrate and lipid metabolism by regulating the *FXR* gene and G-coupled membrane protein 5 receptor (*TGR5*) gene, thereby increasing oxidative phosphorylation and fatty acid oxidation (via increasing *SIRT1* and *Fiaf,* and *PPAR-α* expression respectively). Furthermore, SCFA can regulate reactive species oxidation (ROS) and decrease TNF alpha and IL-6; both of which increase inflammation and reduce fatty-acid oxidation and glucose metabolism [41].

These factors can combine to increase performance due to greater energy availability, resistance to fatigue and better oxygen uptake [46]. Thus, can we improve the VO_{2max} trainability in low responders by targeting the gut microbiome and related genes? Whilst it seems exercise and host genetics can influence gut diversity, one of the best ways to improve gut diversity, and in particular, SCFA production, is through the ingestion of pre-and pro-biotics [15].

Pre-and-probiotics

Probiotics are 'live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host'[15]. Examples, include *Bifidobacterium infantis 35624* and *Lactobacillus rhamnosus GG* [15]. Consumption of probiotics changes the gene expression of existing gut microbiota and influences overall gut activity, as opposed to modifying the structure of the gut [15].

Prebiotics are non-digestible fibers that increase SCFA acid production; they are fermented within the intestinal microbiome, resist absorption in the upper gastrointestinal tract and stimulate the activity of beneficial bacteria (e.g. *bifidobacteria*) within the gut [15, 18, 31] [15, 18, 31, 42]. Favouring the growth of such bacteria creates a healthier gut composition that helps to prevent the growth of pathogenic bacteria that can contribute to obesity and other illnesses and diseases [42]. One of the most common types of prebiotics include fructoligosaccharides (FOS) and inulin (which include chicory).

Prebiotics are considered more stable than probiotics (e.g. unaffected by temperature and other factors) [15] and as such, is the preferred choice of supplement for this study. Inulin-type fructans (ITF), inulin and oligofructose, have been widely studied in humans due to their ability to have a bifidogenic and butyrogenic effect, and an overall positive influence on the gut microbiome composition [16, 18, 43, 44]. The combination of inulin and FOS increases the benefits than either one alone, due to its ability to stimulate Bifidobacterium genus and fecalbacterium prausnitzii (*F.prausnitzii*) numbers via cross feedings [17].

However, in human studies, there has been a mixed response to treatment prebiotics [43]. The variability in response is often dependent upon dose, host genetics, total fiber intake compared to overall kilojoule intake, chain length of the fructans, and the initial amount and type of bifidobacteria strain within the gut [16, 43, 45]. Thus, measuring SCFA in addition to microbiome diversity is warranted.

Table 1. Summary of studies using inulin type fructans

Study Design	Results	Reference
Healthy adults given either 5 or 7.5g of agave inulin/day for three weeks compared to control (no supplementation). N=29.	*Actinobacteria* and *Bifidobacterium* were significantly increased in the inulin group compared to the control group (p=0.001). Butyrate was positively associated with total fiber intake (p=0.005) and bifidobacterial abundance (p=0.08)	[46].

15 obese women received 16g of ITF per day for 3 months, compare to those (n=15) who took a maltodextrin/placebo (16g/day).	*Bifidobacterium* and *Caecalibacterium prausnitzii* were significantly increased in ITF group (p=0.01). Fecal propionate and acetate were lower in the ITF group, which correlated with a lower BMI	[47]
Jerusalem artichoke inulin or chicory inulin snack bars (7.7g/day for one week; twice daily for 2 weeks) compared to placebo (ordinary snack bars). N=15 in each group – 45 in total.	Increased prevalence of bifidobacterial and reduced Bacteroides/Prevotella in inulin group compared to a placebo ($p<0.05$). There were no changes in fecal SCFA concentrations	[48]
Critically ill patients received 7g of ITF for 7 days. n=22.	No difference in gut changes	[49].
ITF mixture compared to a placebo (6g of maltodextrin twice daily) for one week before and three weeks after radiotherapy. N=31.	Increased prevalence of *Lactobacillus* and *Bifidobacterium* species in ITF group (p=0.03).	[50].
Cross-over design with a 2-week wash out period. 4 weeks of chicory-derived inulin (12g/day), compared to placebo (12g maltodextrin/day). N=42.	*Bifidobacterium* and *Anaerostipes* abundance was increased, and *Bilophila* decreased in inulin group ($p<0.05$).	[51].

Conclusion

Our gut microbiome is predetermined by host genetics that can be manipulated through numerous external factors, such as exercise and diet. Whilst host genetics and associated gut taxa increases susceptibility to various chronic diseases and overall fitness levels, the relationship between the gut microbiome and $\dot{V}O_{2max}$ trainability is unknown.

In addition to examining the gene predictor score for $\dot{V}O_{2max}$ trainability, our study will also investigate microbiota-related genes, host genes related to gut microbiome composition, and the genes involved with the gut-microbiota cross-talk. In conclusion, we hope to collect data to help understand if manipulation of the gut microbiome can improve the training response of individuals with a low gene predictor score for $\dot{V}O_{2max}$ trainability.

RESEARCH PLAN

Improving High Intensity Interval Training Response (IMPROVE-HIIT)

Overview

Improve HIIT will examine the association between the gene predictor score for $\dot{V}O_{2max}$ trainability the gut microbiome; and to investigate whether altering the gut microbiome via diet and training influences the $\dot{V}O_2$max training response to HIIT.

Design

Improve HIIT is an 8-week randomised trial with two groups allocated to either: (1) HIIT plus placebo; or (2) HIIT plus prebiotic

Study Population

Consenting adults aged over 18 years.

Power Calculation (sample size justification)

The proposed project will be a pilot study. Considering financial resources, a sample size of 40 is possible. When using G*Power (download.cnet.com) ([52]), a sample size of 39 will achieve a medium effect size of 0.22 (using 6 predictors). This participant number is similar to a previous study examining if gut taxa is related to $\dot{V}O_{2max}$ levels [12]. A linear multiple regression model was used to include covariates that may contribute to the overall outcome (e.g. 6 predictors may include BMI, age, gender, initial $\dot{V}O_2$max, initial gut microbiome and cholesterol levels). Below are results of the power calculation:

F tests - Multiple Regression: Special (R^2 increase)

Analysis:	A priori: Compute required sample size		
Input:	Effect size f^2	=	0.22
	α err prob	=	0.05
	Power (1-β err prob)	=	0.8
	Numerator df	=	1
	Number of predictors	=	6
Output:	Noncentrality parameter λ	=	8.58
	Critical F	=	4.149097
	Denominator df	=	32
	Total sample size	=	39
	Actual power	=	0.8106

Recruitment

Participants will be recruited through Exercise Physiology Brisbane channels (where principal investigator works) channels. These channels will include current patients, Facebook, emails, face-to-face, doctor's surgeries) using referrals, email, newsletters and snowballing (see appendix for wording). Additionally, an email will be sent out to current staff and students from the University of Queensland. This process will begin once ethics has been approved and continue until 40 people have been recruited (within a 12-month time-frame).

Inclusion Criteria

- Inactive adults aged between 18 and 50 years
- Less than 60 minutes of structured exercise each week
- Signed consent form

Exclusion Criteria

- Antibiotic use 6 months prior to intervention or antibiotic use during intervention.
- Pre- or-probiotic use within four weeks of participating in study
- Pregnancy, chronic infections, auto-immune diseases and intestinal chronic conditions (e.g. IBS, Chrohn's disease, ulcerative colitis, coeliac disease).
- Existing cardiac conditions
- Recent surgery or orthopaedic conditions that prevents treadmill walking/running

- Diabetes
- Allergies to inulin (chicory root) or fructans
- Allergies to maltodextrin and other polysaccharides
- Allergies to soy, milk and egg (there maybe traces of these in maltodextrin)
- Greater than 60 minutes of exercise per week within four weeks of the study

Rationale for Criteria

Antibiotics can decrease gut microbiome diversity [35]. As such, participants on antibiotics up to 6 months prior to the study will be excluded. Moreover, pre-and probiotics may alter gut composition; participants on these supplements (up to four weeks prior) will be excluded. The age and exercise inclusion criteria have been added to create a more homogenous group. Other exclusions are included for participant safety (e.g. to avoid exacerbating existing conditions or placing the participant at a greater risk for a health complication).

Informed consent

Through the recruitment, information will include details about the study and the above-mentioned inclusion and exclusion criteria. The contact details for the Research Team will be provided.
Once participants have registered their interest and the Research Team check they meet the inclusion criteria, they will be asked to sign the informed consent form (via email or posted) and to come to the University of Queensland for the following baseline testing sessions.

Baseline testing sessions

Participants will be asked to fast prior to their first testing visit, and to bring their signed consent form.

Testing Visit 1 (60-90 minutes):

- Completion of a questionnaire assessing physical activity readiness (Australian Physical Activity Readiness Questionnaire – see appendix). Participants may be asked to for medical clearance if anything untoward is identified at this stage.
- Provision of a saliva sample (DNA sample). This will involve spitting (2ml) into a Oragene DNA collection tube (DNA Genoteck, Canada).
- Completion of a fasted blood test. A small sample of blood (two 10ml tubes, ~1 table spoon) will be collected to measure fasting glucose, triglycerides and cholesterol.
- Weight, waist and hip circumference measurements taken.
- Fat mass and muscle mass measured using a body composition x-ray. (DEXA machine).
- Provision of a Sustagen popper to participant after body composition x-ray.
- Provision of a stool collection kit and associated questionnaires. Participants will be asked to collect a stool sample the day before their second testing visit, record the date and time and immediately freeze the sample in the containers provided. Participants will be provided with detailed instructions about collection and hygiene (see appendix). Participants will be required to complete a consent from provided by the stool collection company, 'Microba'. At the time of the stool sample, participants will be required to complete a food frequency questionnaire (see appendix), and to answer questions related to their mental health (CESD) exercise habits, medical history and day of sampling (see appendix). These questionnaires are completed on-line and have been approved through Microba's ethical committee. During this time, the investigator can assist the participant to complete the necessary questionnaires. The frozen samples will be returned to the study researchers at the second testing visit. One stool sample will be sent to 'Microba' in Brisbane for analysis; and one stool sample will be stored at the University of Queensland for short-chain fatty acid analysis.

- Resting blood pressure and heart rate measured.
- Completion of a maximal exercise test ($\dot{V}O_{2max}$ test).
- Book testing visit 2.

Testing Visit 2 (30 minutes):

- Participant to provide frozen stool sample.
- Completion 24-hour diet recall (see appendix).
- Participant will be randomly allocated to one of two intervention groups.
- Supplement provided based on randomisation.
- A diary (see appendix) will be provided to monitor supplementation compliance, and to monitor exercise attendance. This diary will also include instructions on when and how to take the supplement, and to highlight that dietary habits should not be changed during the intervention period.
- Participants will be asked what days and time-frames suit best for the 6-week exercise intervention. A tentative date will be booked for their first supervised exercise session. The two-week supplementation adjustment period will begin two weeks before this date.

Randomisation

Prior to the first testing session of the first participant, sequentially numbered opaque sealed envelopes will be used to randomly allocate participants to one of two intervention groups: 1) High intensity interval training plus placebo (maltodextrin) (HIIT-M), or 2) High intensity interval training plus prebiotic powder (HIIT-P)

'HIIT-M' written on carbon paper will be inside 20 of the opaque envelopes. 'HIIT-P' written on carbon paper will be inside the remaining 20 opaque envelopes. These 40 envelopes will be shuffled several times in the presence of at least two investigators.

Participants will be given a code to deidentify them for this study. For example, participant 'a' may have the code IMPROVE HIIT_01. Once participants have been deidentified with a code, these codes will be sequentially placed on the outside of the shuffled envelopes. This procedure will be done in the presence of all investigators involved in the study.

Participants will be blinded as to which supplement they are using. Aside from the principal investigator, researchers involved in the study will be blinded as to which supplement each participant is using. Only the principal investigator will have access to the master code.

Intervention Groups

All participants will be instructed to maintain their usual diet during the intervention period. This information will be outlined in the diary they are provided during their second visit (see appendix).

1. Supplementation adjustment period

Each group will have two weeks prior to the 6-week exercise intervention to gradually increase the dose of supplementation from 2g to 12 g/day (6g/day twice daily) by the start of the 6-week intervention. This will be done to reduce unwanted side-effects in the prebiotic supplementation group, such as flatulence.

Rationale for prebiotic

The quantity of prebiotic supplement (12g/day) is based on previous research which has shown positive results ranging from 5-16g/day, over a period of 1 week up to 3 months [43]. A prebiotic fiber produced by Jackson GI Medical in America ('Prebiotin' [53]) will be used. It is currently being used in an NIH funded trial examining if Prebiotin (16g/day) assists with improving the gut microbiota and reducing inflammation and cardiovascular disease

in patients with end stage renal disease [53]. Prebiotin is currently being used in other international-based studies examining the effect of inulin on, 1) the microbiome of end stage renal disease patients treated with peritoneal dialysis, 2) inflammatory bowel disease, 3) body fat reduction in overweight and obese children, 4) cognitive impairments in schizophrenia, and 5) increasing butyrate-producing bacteria [53]. Prebiotin is made up of fructo-oligosaccharide enriched inulin (which is a combination of longer and shorter chain inulin derived from chicory root). The recommended intake is 2g/day, progressing to 12g or more per day as tolerated. This recommendation provided by Prebiotin is consistent with previous studies that have shown increased bifidogenic and potentially butyrate with a combined inulin and FOS supplementation [43, 44]. Prebiotin contains 21kJ calories per 4 grams.

Rationale for placebo
Maltodextrin has been chosen as the placebo due to its slightly sweet taste (mimicking that of the prebiotic powder). Maltodextrin has been used as a placebo in other gut-related studies [54, 55]. Maltodextrin contains 68kJ per 4g.

2. Exercise intervention and supplementation period

Following the two-week supplementation adjustment period, each group will complete a 6-week HIIT exercise intervention period in conjunction with daily supplementation of a placebo or prebiotic.

Rationale for exercise intervention

A HIIT design for 6 weeks has been chosen to maximise fitness changes within a relatively short time-frame [21], thereby maximising compliance and adherence to the study protocol.

HIIT protocol

Participants will receive 3 supervised exercise sessions each week, over a 6-week period. These sessions will involve a 10-minute warm up at 60-70% of maximal heart rate (HRmax). Modality will be on the treadmill. Proceeding this 10 minutes, participants will work at 90-95% of their HRmax for 4 minutes, followed by a 3-minute active recovery (50-70% of HRmax). This will be repeated 4 times (4x4), followed by a 5-minute cool down. Training will occur at either UQ, St. Lucia, or Exercise Physiology Brisbane, Kelvin Grove.

Intervention group 1 - HIIT plus placebo (HIIT-M).

Participants will be required to take a placebo each day for the 6-week intervention period (12g of maltodextrin (My Protein, United Kingdom). Participants will be required to complete 3 supervised sessions of HIIT per week for the 6 weeks. Participants will be asked to keep a diary.

Intervention group 2 - HIIT + Prebiotic (HIIT-P)

Participants will be required to take 12g of an oligofructose enriched inulin powder each day (Prebiotin, USA) during the 6-week exercise intervention. Participants will be required to complete 3 supervised sessions of HIIT per week for the 6 weeks. Participants will be asked to keep a diary.

3. Post intervention

At the end of the 6 weeks, participants will be asked to recomplete the three testing baseline visits (as outlined above).

A summary report of de-identified results will be available to participants in the study. Similar information will also be used within journal article submissions, abstracts, conferences and other presentation opportunities.

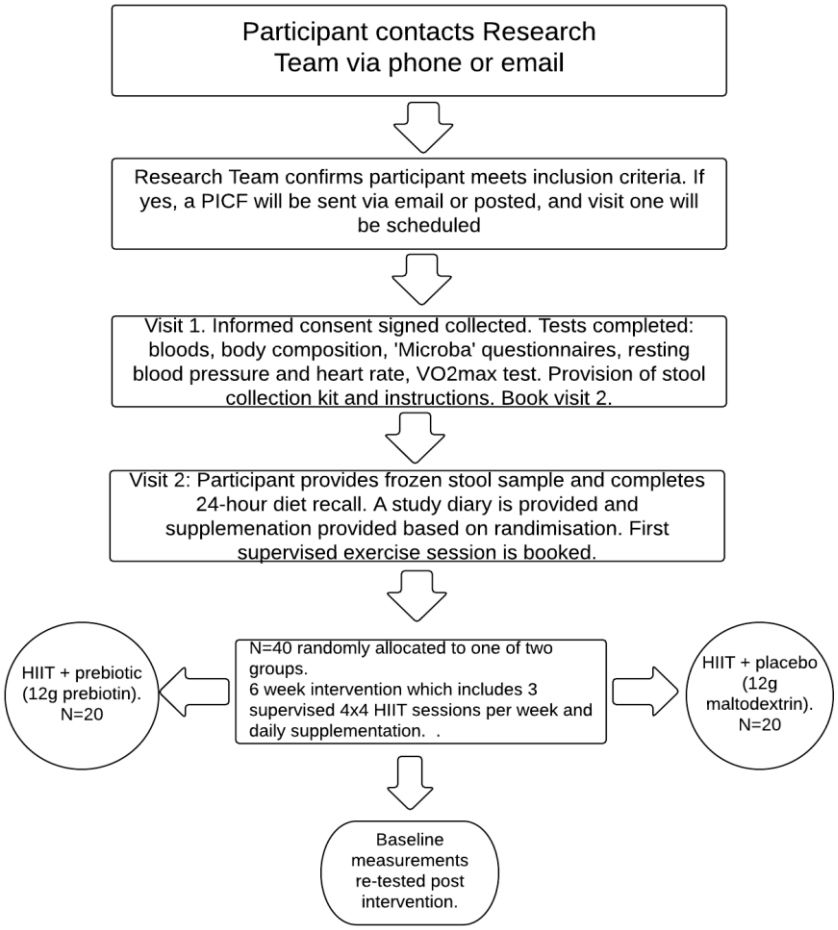

Figure 1. Recruitment and intervention process summary

Withdrawal Criteria

During initial contact (if reconsent is required), the participant will be informed of their right to withdraw at any stage of the study. If participants choose to withdraw, their DNA samples and data will be discarded/deleted.

Data Management

DNA and blood samples will be coded so only the Principal Investigator and study co-ordinators can decode them should results become available (the code will not relate to the participant in any way). If results arising from samples are published in a scientific journal, identities will not be revealed. Participants will not be referred to by name during research reports or study discussions. Once all measures have been taken, DNA, stool and blood samples will be discarded in biohazard containers.

All non-coded records will be stored in a locked filing cabinet with restricted access for a minimum of five years in a private office of Professor Jeff Coombes. Any information obtained for the purpose of this research project that can identify a participant will be treated as confidential and securely stored. All computer records will be stored on an access-restricted and file-encrypted network (UQ's Research Data Management System). Only the Principal Investigator and Co-Investigators will have access to project's documents. It will be disclosed only with permission, or as permitted by law.

Data Collection

Primary outcomes

A panel of genetic variants will be identified from a saliva sample collected at baseline via an Oragene collection kit**.** Saliva samples will be sent to the Translational Research Institute (TRI) for DNA extraction and GWAS analysis. The GWAS will target predictor genes identified from our PREDICT HIIT study.

GWAS analysis

a) DNA extraction from saliva

DNA will be extracted from the stabilised saliva samples using the QIAsymphony (Qiagen) DNA MIDI Kit (Cat.no. 937255). The yield and purity will be measured using the Trinean DropSense-96 as per the manufacturer's instructions.

b) Genotyping

100ng of DNA will be genotyped on Illumina Infinium HumanCoreExome24v1.1 arrays as per manufacturer's instructions. The arrays will be scanned on an Illumina iScan system and the raw fluorescence intensity data normalized and clustered for each sample using Illumina Genome Studio (v. 2011.1). Genotypes will be called using the standard Illumina HumanCoreExome24v1.1 cluster file.

c) GWAS

The program PLINK will used to covert the .ped and .map files into .fam, .bim and .bed files. To this data, clinical files (co-variates and phenotypes) will be added. Data is filtered via the following steps: 1) sample level (call rate, heterozygosity, relatedness, ancestry), 2) SNP level (cell rate, minor allele frequency, HWE). Two new types of data will be created: 1) principal components for capturing population-substructure, and 2) genotypes of untyped SNPs (using sanger imputation server for the imputations with HRC r1.1 as reference). Logistic regression will be performed with 'N' PCA components (N is unknown until we do the experiments). SNP coordinates are from the Genome Reference Consortium GRCh37 (hg19) build. A Manhattan plot will be created to visualise the significant genetic markers and raw density plot will be examined manually.

Change in cardiorespiratory fitness

An incremental treadmill test to exhaustion on a treadmill will be completed at baseline and 6 weeks to ascertain $\dot{V}O_{2peak}$. This test involves the participant wearing a head and mouthpiece, nose tape and nose clip. The participant walking on a treadmill at 0% gradient and 1.6km/hour (Bruce Ramp). This initial stage is used as the warm up period. During the test, speed and gradient is monitored. Heart rate and rating of perceived exhaustion is measured at the end of every minute. The speed and or gradient of the treadmill is increased every one-minute. Expired air is collected via a metabolic cart every 30 seconds during the test. Heart rate is measured via a Polar heart rate monitor and blood pressure is taken using a sphygmomanometer. The participant is encouraged to exercise until exhaustion. At exhaustion, the grade, speed and heart rate are recorded. The workload is decreased to 3km/hour with no incline to allow the participant to cool down. Following the cool down, the test is ended, and all devices are removed from the participant. $\dot{V}O_{2peak}$ is measured as the average of the two highest volumes of oxygen consumed (reported as volume per minute relative to body weight; mL/kg/min).

STAGE	TIME (min)	GRADE (%)	SPEED (km/hr)
1	1	0	1.6
2	1	5	2.1
3	1	10	2.7
4	1	10	3.4
5	1	11	3.7
6	1	12	4.0
7	1	12	4.2
8	1	13	5.0
9	1	14	5.4
10	1	14	6.1
11	1	15	6.6
12	1	16	6.7
13	1	16	7.2
14	1	17	7.7
15	1	18	8.0
16	1	18	8.5

Figure 2. VO_{2peak} test stages

Gut microbiota

Gut diversity/bioinformatics and short-chain fatty acid (SCFA) analysis will be performed on faecal samples collected at baseline and 6 weeks. Participants will be provided with a home stool collection kit with instructions. They will be asked to keep stool sample frozen until their next testing visit. After this time, the stool samples will be kept at -80 degrees Celsius until analysis. One stool sample will be kept for short chain fatty acid analysis, and two other stool samples will be sent to *Microba* for testing.

a) Short chain fatty acid analysis

Stool samples will be defrosted and weighted, with 1ml of orthophosphoric acid added to each 0.1g of sample (e.g. 0.52 g stool sample = 5.2ml of orthophosphoric acid). The sample will be vortexed at a maximum speed for 2 minutes, with 1ml added to Eppendorf tubes and centrifuged at maximum speed for 10 minutes. 250 ul of supernatant will be extracted and 250ml of ethyl acetate added. Stool sample will be refrozen at this point. Tubes will be vortexed for 1-2 minutes and centrifuged for 10 minutes at maximum speed. 150 ul supernatant will be placed into deidentified (coded) vials. Vials will be placed into a gas chromatography mass spectrometer (Thermo Fisher Scientific, USA) to examine the number of SCFA gram per gram of dry weight stool. Chromeleon Software (Thermo Fisher Scientific, USA) will be used to process the data by following the manufacturer's procedures.

b) Gut diversity/bioinformatics

The faecal samples will be sent to *'Microba'* for analysis. *Microba* will provide the bioinformatics for the gut microbiome of each participant. Human DNA reads are first removed by aligning all reads with BWA (http://bio-bwa.sourceforge.net/) to the Human Genome assembly GRCh38.p12. Species-level genome-based abundances are calculated by mapping sequencing reads using BWA to a curated private database of high-quality microbial genomes (Microba's GenomeDB), then normalising the results by sequencing depth and genome size. Relative abundances are calculated as the fraction of each genome abundance in the total of all genome abundances. Microba's GenomeDB contains genomes deposited in NCBI and additionally mined from SRA and other proprietary samples. Each genome is quality checked for high completeness and low contamination (e.g., assembly problems) using CheckM (http://ecogenomics.github.io/CheckM/). The database is de-replicated to <95% genomic nucleic acid identity (ANI), which correlates with species-level resolution. Genomes are assigned taxonomy using the new genome-based Genome Taxonomy Database (http://gtdb.ecogenomic.org/).

Secondary Outcomes

Nutrition analysis

A food frequency questionnaire (see appendix – *Microba* online questionnaire) will be collected at baseline and at 6 weeks (completed at testing visit 1). Analysis will be completed by *Microba (Australia)*. To control for diet and to assess whether there is an interaction between dietary inulin and supplement inulin, a 24-hour diet recall at baseline and post intervention (see appendix) will be collected during the second testing visit using the (The Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool developed by the National Cancer Institute (NCI)). This will be analysed using Food Works, Xyris, Australia (a nutrient analysis software).

Body composition

Body composition will be measured at baseline and 6 weeks. Measurements will include height, weight, waist and hip measurements. Circumferences will be calculated to the nearest 0.1 decimal using a measuring tape. Weight will be measure to the nearest 0.1 decimal using calibrated scales. Body fat will be measured via a Duel-energy X-ray Absorptiometry – DXA scan (Hologic QDR Series, Massachusetts, USA). The participant will be required to lay still in a supine position for approximately seven minutes.

Blood chemistry

A person trained in phlebotomy will take blood at baseline and 6 weeks. A small sample of venous blood will be collected from the superficial antecubital vein using 2 x 10ml vacutainers (red and purple vacutainers). The purple vacutainers (used for measuring whole blood and contain EDTA) will be placed on ice. The red vacutainers (contains no anticoagulant and used for serum) will remain at room temperature for 30 minutes. Before centrifuging, 3 x 300ul of whole blood will be collected for back up and placed in the freezer at -80 degrees Celsius. Remaining samples will be centrifuged at 1500G for 10 minutes at 4 degrees Celsius. Plasma and serum will be placed into 300ul aliquots and stored at -80degrees Celsius until analysis. Analysis of total cholesterol, HDL, LDL, triglycerides and glucose will be measured on an automated clinical chemistry analyser (Randox Datomer Plus) using the manufacturer's procedures.

Family and personal medical history

An adult pre-exercise screening tool will be completed prior to the intervention. *Microba* will also be measuring mental health and wellbeing using The Center for Epidemiologic Studies Depression Scale (CEDS), day of sampling history, exercise and medical history pre and post intervention.

The following table outlines the information to be collected.

Measure	Pre	Post
Cardiorespiratory Fitness		
VO_{2peak}	X	X
Resting blood pressure	X	X
Resting heart rate	X	X
Maximum heart rate	X	X
Gene Predictor Score		
Total number of variants that contribute to high or low response to training		
Diet History		
Food frequency questionnaire	X	X
24-hour diet recall	X	X
Gut Microbiota		
Diversity	X	X
Short chain fatty acid	X	X
Body Composition		
Weight	X	X

Body mass index	X	X
Waist circumference	X	X
Body fat percentage	X	X
Blood chemistry		
Total Cholesterol	X	X
HDL	X	X
LDL	X	X
Triglycerides	X	X
Glucose	X	X
DNA	X	
Family and Personal Medical History		
Family History of Cardiovascular Disease	X	
History of Illness or Injury	X	
Smoking status	X	
Medications	X	
Exercise habits	X	
CESD-R score	X	X

Statistical Analysis

Determining the association between the gene predictor score and the gut microbiome

Following on from the GWAS, a genetic predisposition score for each participant will be calculated to test for the cumulative effect of multiple alleles on $\dot{V}O_{2peak}$ training response and cardiovascular disease risk factors. Using the genetic variants identified from our PREDICT HIIT study (results yet to be published) and taking the approach used in Thomaes et al. (2011), '0' will be given for a homozygote for alleles associated with a low response to training, '1' for heterozygote for an allele associated with high response, and '2' for homozygotes of an allele associated with high response to training. The $\dot{V}O_{2max}$ scores and other covariates will be collated for each participant. Microba will provide gut bioinformatics for each participant (pre and post intervention). The butyrate production per participant will also be calculated as described above (pre and post intervention). This information (including average fibre intake per participant) will be analysed using SPSS statistical software (IBM SPSS Statistics).

Data will be tested for normality using a Shapirio-Wilk test. Where normality is not found, the Kruskal-Wallis test will be used for comparing between data sets.

To compare the mean OTUs (and other bioinformatics provided by 'Microba'), fibre intake and butyrate production for predicted low and high responders (e.g. gene predictor score), a one-way analysis of co-variance (ANCOVA) will be calculated (with age and sex as covariates).

Investigating whether altering the gut microbiome via diet and training influences the $\dot{V}O_{2max}$ training response to HIIT

The change in $\dot{V}O_{2max}$ of each participant will be calculated. Low responders will be classified by a $\dot{V}O2peak$ change of <100ml of O_2/min, and high responders by a VO2peak change of > 700ml in O_2/min (following the intervention period).

To compare the mean change in OTUs, alpha diversity, fibre intake and butyrate production to $\dot{V}O_{2max}$ training response, a one-way analysis of co-variance (ANCOVA) will be calculated (with age, sex, baseline $\dot{V}O_{2max}$ as covariates).

A multiple regression analysis will be used to evaluate the role of possible predictors for change in $\dot{V}O_{2max.}$ Predictors will be screed using a Spearman correlation matrix to find the top four significant variables to include in the multiple regression analysis (e.g. gene predictor score, sex, age, gender, BMI, fibre intake, OTUs, butyrate production, alpha diversity).

The above approach will also be used to compare changes in other biomarkers (e.g. blood tests, body fat percentage etc).

All statistical tests will be performed at a 5% significance level.

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APPENDIX

1. Adult Pre-Exercise Screening Tool

https://www.essa.org.au/wp-content/uploads/2011/09/Screen-tool-version-v1.1.pdf

2. *Microba* online questionnaires (already approved through their ethics committee)

a) Sample food frequency questionnaire

https://www.cancervic.org.au/downloads/cec/FFQs/DQES_v2_Sample-Questionnaire.pdf

b) Medical history

General		
What is your date of birth?	Month	Number
What is your date of birth?	Day	Number
What is your date of birth?	Year	Number
What is your biological sex?	choice	Male
What is your biological sex?	choice	Female
What is your biological sex?	choice	Intersex
How would you describe your ethnicity?	choice	Aboriginal
How would you describe your ethnicity?	choice	African
How would you describe your ethnicity?	choice	East Asian
How would you describe your ethnicity?	choice	South Asian
How would you describe your ethnicity?	choice	European
How would you describe your ethnicity?	choice	Mixed North American
How would you describe your ethnicity?	choice	Latino
How would you describe your ethnicity?	choice	Middle Eastern
How would you describe your ethnicity?	choice	Polynesian
How would you describe your ethnicity?	choice	Mixed
How would you describe your ethnicity?	choice	Other (please specify)
Please specify Other ethnicity		NULL
What is your height (in cm)?	number	NULL
What is your weight (in kg)?	number	NULL
Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Married
Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Living with partner
Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Widowed

Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Divorced
Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Separated
Are you now married, living with a partner in a marriage-like relationship, widowed, divorced, separated, or never married?	choice	Never married
What is the highest level of education you completed?	choice	Less than high school
What is the highest level of education you completed?	choice	High school
What is the highest level of education you completed?	choice	Professional certification
What is the highest level of education you completed?	choice	University
What is the highest level of education you completed?	choice	Masters
What is the highest level of education you completed?	choice	Doctorate
What is the highest level of education you completed?	choice	None of these
Altogether, have you smoked at least 100 or more cigarettes in your entire lifetime?	choice	Yes
Altogether, have you smoked at least 100 or more cigarettes in your entire lifetime?	choice	No
Do you now smoke cigarettes every day, some days, or not at all?	choice	Every day
Do you now smoke cigarettes every day, some days, or not at all?	choice	Some days
Do you now smoke cigarettes every day, some days, or not at all?	choice	Not at all
On average, how many cigarettes do you now smoke a day?	number	
On average, how often do you poo?	choice	More than 3 times a day
On average, how often do you poo?	choice	2 to 3 times a day
On average, how often do you poo?	choice	Once a day
On average, how often do you poo?	choice	Once every 2 days
On average, how often do you poo?	choice	Less than once every 2 days
Do you have any of the following pets?	choice	Cats
Do you have any of the following pets?	choice	Dogs
Do you have any of the following pets?	choice	Birds
Do you have any of the following pets?	choice	Rodents
Do you have any of the following pets?	choice	None of these
Have you had your appendix removed?	choice	Yes
Have you had your appendix removed?	choice	No
Have you had your appendix removed?	choice	Don't know
How would you describe your overall health?	choice	Excellent
How would you describe your overall health?	choice	Good
How would you describe your overall health?	choice	Moderate
How would you describe your overall health?	choice	Poor
How would you describe your overall health?	choice	Terrible

Cardiovascular Health

1. Including any conditions which can be controlled with medication, have you ever been told by a doctor or nurse that you have any heart or circulatory conditions? (If no, skip to question 7)	choice	Yes

1. Including any conditions which can be controlled with medication, have you ever been told by a doctor or nurse that you have any heart or circulatory conditions? (If no, skip to question 7)	choice	No
1. Including any conditions which can be controlled with medication, have you ever been told by a doctor or nurse that you have any heart or circulatory conditions? (If no, skip to question 7)	choice	Don't Know
2. What are the names of these conditions?	click-down	Rheumatic heart disease
2. What are the names of these conditions?	click-down	Heart attack
2. What are the names of these conditions?	click-down	Heart failure
2. What are the names of these conditions?	click-down	Stroke (including after effects of stroke)
2. What are the names of these conditions?	click-down	Angina
2. What are the names of these conditions?	click-down	High blood pressure/hypertension
2. What are the names of these conditions?	click-down	Low blood pressure/hypotension
2. What are the names of these conditions?	click-down	Hardening of the arteries/atherosclerosis/arteriosclerosis
2. What are the names of these conditions?	click-down	Fluid problems/fluid retention/oedema
2. What are the names of these conditions?	click-down	High cholesterol
2. What are the names of these conditions?	click-down	Rapid or irregular heartbeats/tachycardia/palpitations
2. What are the names of these conditions?	click-down	Heart murmur/heart valve disorder
2. What are the names of these conditions?	click-down	Haemorrhoids
2. What are the names of these conditions?	click-down	Varicose veins
2. What are the names of these conditions?	click-down	Other (specify)
Please specify other type of heart or circulatory conditions.		NULL
3. Including any conditions which you are controlling with medication, do you currently have any heart or circulatory conditions? (If no, skip to question 5)	choice	Yes
3. Including any conditions which you are controlling with medication, do you currently have any heart or circulatory conditions?	choice	No
3. Including any conditions which you are controlling with medication, do you currently have any heart or circulatory conditions?	choice	Don't Know
4. What are the names of these heart or circulatory conditions?	click-down	Rheumatic heart disease
4. What are the names of these heart or circulatory conditions?	click-down	Heart attack
4. What are the names of these heart or circulatory conditions?	click-down	Heart failure
4. What are the names of these heart or circulatory conditions?	click-down	Stroke (including after effects of stroke)
4. What are the names of these heart or circulatory conditions?	click-down	Angina
4. What are the names of these heart or circulatory conditions?	click-down	High blood pressure/hypertension
4. What are the names of these heart or circulatory conditions?	click-down	Low blood pressure/hypotension
4. What are the names of these heart or circulatory conditions?	click-down	Hardening of the arteries/atherosclerosis/arteriosclerosis
4. What are the names of these heart or circulatory conditions?	click-down	Fluid problems/fluid retention/oedema
4. What are the names of these heart or circulatory conditions?	click-down	High cholesterol
4. What are the names of these heart or circulatory conditions?	click-down	Rapid or irregular heartbeats/tachycardia/palpitations
4. What are the names of these heart or circulatory conditions?	click-down	Heart murmur/heart valve disorder
4. What are the names of these heart or circulatory conditions?	click-down	Haemorrhoids
4. What are the names of these heart or circulatory conditions?	click-down	Varicose veins

4. What are the names of these heart or circulatory conditions?	click-down	Other (specify)
Please specify other type of heart or circulatory conditions.		NULL
5. Again, remembering to include any conditions which can be controlled with medication, have any of these conditions lasted, or are they expected to last, for 6 months or more?	choice	Yes
5. Again, remembering to include any conditions which can be controlled with medication, have any of these conditions lasted, or are they expected to last, for 6 months or more?	choice	No
6. Which conditions are they?	click-down	Rheumatic heart disease
6. Which conditions are they?	click-down	Heart attack
6. Which conditions are they?	click-down	Heart failure
6. Which conditions are they?	click-down	Stroke (including after effects of stroke)
6. Which conditions are they?	click-down	Angina
6. Which conditions are they?	click-down	High blood pressure/hypertension
6. Which conditions are they?	click-down	Low blood pressure/hypotension
6. Which conditions are they?	click-down	Hardening of the arteries/atherosclerosis/arteriosclerosis
6. Which conditions are they?	click-down	Fluid problems/fluid retention/oedema
6. Which conditions are they?	click-down	High cholesterol
6. Which conditions are they?	click-down	Rapid or irregular heartbeats/tachycardia/palpitations
6. Which conditions are they?	click-down	Heart murmur/heart valve disorder
6. Which conditions are they?	click-down	Haemorrhoids
6. Which conditions are they?	click-down	Varicose veins
6. Which conditions are they?	click-down	Other (specify)
Please specify other type of heart or circulatory conditions.		NULL

Arthritis

7. Do you have, or have you ever had Gout?	choice	Yes
7. Do you have, or have you ever had Gout?	choice	No
8. Do you have, or have you ever had, Rheumatism?	choice	Yes
8. Do you have, or have you ever had, Rheumatism?	choice	No
9. Do you have, or have you ever had, Arthritis?	choice	Yes
9. Do you have, or have you ever had, Arthritis?	choice	No
10. Do you have, or have you ever had, Osteoarthritis?	choice	Yes
10. Do you have, or have you ever had, Osteoarthritis?	choice	No
11. Do you have, or have you ever had, Rheumatoid arthritis?	choice	Yes
11. Do you have, or have you ever had, Rheumatoid arthritis?	choice	No
12. Do you have or have you ever had any other type of arthritis?	choice	Yes
12. Do you have or have you ever had any other type of arthritis?	choice	No
Please specify other type of arthritis.		NULL
13. Do you currently have any of the following conditions?	click-down	Gout
13. Do you currently have any of the following conditions?	click-down	Rheumatism
13. Do you currently have any of the following conditions?	click-down	Arthritis
13. Do you currently have any of the following conditions?	click-down	Osteoarthritis

13. Do you currently have any of the following conditions?	click-down	Rheumatoid Arthritis
13. Do you currently have any of the following conditions?	click-down	Other (specify)
13. Do you currently have any of the following conditions?	click-down	No
Please specify other type of arthritis.		NULL

Diabetes/High sugar levels

14. Have you ever been told by a doctor or nurse that you have Diabetes? (if no, skip to question 19)	choice	Yes
14. Have you ever been told by a doctor or nurse that you have Diabetes? (if no, skip to question 19)	choice	No
15. At what age were you first told that you have Diabetes?	number	
16. What type of Diabetes were you told you have?	click-down	Type 1 (Insulin Dependent Diabetes Mellitus/Juvenile Onset Diabetes/Type A)
16. What type of Diabetes were you told you have?	click-down	Type 2 (Non-Insulin Dependent Diabetes Mellitus/Adult Onset Diabetes/Type B)
16. What type of Diabetes were you told you have?	click-down	Gestational (pregnancy)
16. What type of Diabetes were you told you have?	click-down	Diabetes insipidus
16. What type of Diabetes were you told you have?	click-down	Other (specify)
16. What type of Diabetes were you told you have?	click-down	Don't know
Please specify Other type of Diabetes		NULL
17. Do you currently have Diabetes? (If no, skip to question 19)	choice	Yes
17. Do you currently have Diabetes? (If no, skip to question 19)	choice	No
17. Do you currently have Diabetes? (If no, skip to question 19)	choice	Don't know
18. Which types do you currently have?	click-down	Type 1 (Insulin Dependent Diabetes Mellitus/Juvenile Onset Diabetes/Type A)
18. Which types do you currently have?	click-down	Type 2 (Non-Insulin Dependent Diabetes Mellitus/Adult Onset Diabetes/Type B)
18. Which types do you currently have?	click-down	Gestational (pregnancy)
18. Which types do you currently have?	click-down	Diabetes insipidus
18. Which types do you currently have?	click-down	Other (specify)
18. Which types do you currently have?	click-down	Don't know
Please specify Other type of Diabetes		NULL
19. Have you ever been told by a doctor or nurse that you have high sugar levels in your urine? (If no, skip to question 23)	choice	Yes
19. Have you ever been told by a doctor or nurse that you have high sugar levels in your urine? (If no, skip to question 23)	choice	No
20. At what age were you first told you had high sugar levels?	number	
21. Do you currently have high sugar levels?	choice	Yes
21. Do you currently have high sugar levels?	choice	No
21. Do you currently have high sugar levels?	choice	Don't know
22. Have your high sugar levels lasted, or are they expected to last, for 6 months or more?	choice	Yes
22. Have your high sugar levels lasted, or are they expected to last, for 6 months or more?	choice	No
22. Have your high sugar levels lasted, or are they expected to last, for 6 months or more?	choice	Don't know

Cancer

23. Have you ever been told by a doctor or nurse that you have any type of cancer?	choice	Yes
23. Have you ever been told by a doctor or nurse that you have any type of cancer?	choice	No
24. What type of cancer were you told you had?	click-down	Skin cancer (include melanoma, basal cell carcinoma, squamous cell carcinoma)
24. What type of cancer were you told you had?	click-down	Colon/rectum/bowel cancer (colorectal)
24. What type of cancer were you told you had?	click-down	Breast cancer
24. What type of cancer were you told you had?	click-down	Prostate cancer
24. What type of cancer were you told you had?	click-down	Lung cancer (include trachea, pleura and bronchus)
24. What type of cancer were you told you had?	click-down	Cervical cancer
24. What type of cancer were you told you had?	click-down	Cancer of other female reproductive organs (include uterus, ovary)
24. What type of cancer were you told you had?	click-down	Bladder/kidney cancer
24. What type of cancer were you told you had?	click-down	Stomach cancer
24. What type of cancer were you told you had?	click-down	Leukaemia
24. What type of cancer were you told you had?	click-down	Non-Hodgkin lymphoma
24. What type of cancer were you told you had?	click-down	Other type of lymphoma
24. What type of cancer were you told you had?	click-down	Cancer of unknown primary site
24. What type of cancer were you told you had?	click-down	Other cancer (specify)
Please specify Other type of cancer		NULL
25. Are you currently receiving treatment for your cancer?	choice	Yes
25. Are you currently receiving treatment for your cancer?	choice	No
26. Including cancer which is in remission, do you currently have cancer?	choice	Yes
26. Including cancer which is in remission, do you currently have cancer?	choice	No
27. What types of cancer do you currently have?	click-down	Skin cancer (include melanoma, basal cell carcinoma, squamous cell carcinoma)
27. What types of cancer do you currently have?	click-down	Colon/rectum/bowel cancer (colorectal)
27. What types of cancer do you currently have?	click-down	Breast cancer
27. What types of cancer do you currently have?	click-down	Prostate cancer
27. What types of cancer do you currently have?	click-down	Lung cancer (include trachea, pleura and bronchus)
27. What types of cancer do you currently have?	click-down	Cervical cancer
27. What types of cancer do you currently have?	click-down	Cancer of other female reproductive organs (include uterus, ovary)
27. What types of cancer do you currently have?	click-down	Bladder/kidney cancer
27. What types of cancer do you currently have?	click-down	Stomach cancer
27. What types of cancer do you currently have?	click-down	Leukaemia
27. What types of cancer do you currently have?	click-down	Non-Hodgkin lymphoma
27. What types of cancer do you currently have?	click-down	Other type of lymphoma
27. What types of cancer do you currently have?	click-down	Cancer of unknown primary site
27. What types of cancer do you currently have?	click-down	Other cancer (specify)
Please specify Other type of cancer		NULL

Long Term Conditions

28. Do you currently have any conditions that have lasted, or are expected to last, for 6 months or more?	choice	Yes

28. Do you currently have any conditions that have lasted, or are expected to last, for 6 months or more?	choice	No
29. Which of these conditions do you have?	click-down	Hay fever
29. Which of these conditions do you have?	click-down	Sinusitis or sinus allergy
29. Which of these conditions do you have?	click-down	Food allergy
29. Which of these conditions do you have?	click-down	Drug allergy
29. Which of these conditions do you have?	click-down	Other allergy
29. Which of these conditions do you have?	click-down	Anaemia
29. Which of these conditions do you have?	click-down	Bronchitis
29. Which of these conditions do you have?	click-down	Celiac disease
29. Which of these conditions do you have?	click-down	Chronic Fatigue Syndrome
29. Which of these conditions do you have?	click-down	Crohn's disease
29. Which of these conditions do you have?	click-down	Emphysema
29. Which of these conditions do you have?	click-down	Epilepsy
29. Which of these conditions do you have?	click-down	Fluid problems/fluid retention/oedema (exclude those due to heart or circulatory condition)
29. Which of these conditions do you have?	click-down	Guillain-Barre syndrome
29. Which of these conditions do you have?	click-down	Hernias
29. Which of these conditions do you have?	click-down	Irritable Bowel Syndrome
29. Which of these conditions do you have?	click-down	Kidney stones
29. Which of these conditions do you have?	click-down	Migraine
29. Which of these conditions do you have?	click-down	Multiple sclerosis
29. Which of these conditions do you have?	click-down	Parkinson's
29. Which of these conditions do you have?	click-down	Psoriasis
29. Which of these conditions do you have?	click-down	Stomach ulcers or other gastrointestinal ulcers
29. Which of these conditions do you have?	click-down	Thyroid trouble/goitre
29. Which of these conditions do you have?	click-down	Ulcerative colitis
29. Which of these conditions do you have?	click-down	Depression
29. Which of these conditions do you have?	click-down	Feeling depressed
29. Which of these conditions do you have?	click-down	Back – slipped disc or other disc problems
29. Which of these conditions do you have?	click-down	Back pain or back problems
29. Which of these conditions do you have?	click-down	Other (please specify)
Please specify Other type of long term condition		NULL
30. How long has the 1st condition lasted for?		Days
30. How long has the 1st condition lasted for?		Weeks
30. How long has the 1st condition lasted for?		Months
30. How long has the 1st condition lasted for?		Years
30. How long has the 1st condition lasted for?		Not applicable
31. How long has the 2nd condition lasted for?		Days
31. How long has the 2nd condition lasted for?		Weeks
31. How long has the 2nd condition lasted for?		Months
31. How long has the 2nd condition lasted for?		Years
31. How long has the 2nd condition lasted for?		Not applicable
32. How long has the 3rd condition lasted for?		Days
32. How long has the 3rd condition lasted for?		Weeks
32. How long has the 3rd condition lasted for?		Months
32. How long has the 3rd condition lasted for?		Years
32. How long has the 3rd condition lasted for?		Not applicable

33. How long has the 4th condition lasted for?		Days
33. How long has the 4th condition lasted for?		Weeks
33. How long has the 4th condition lasted for?		Months
33. How long has the 4th condition lasted for?		Years
33. How long has the 4th condition lasted for?		Not applicable
34. How long has the 5th condition lasted for?		Days
34. How long has the 5th condition lasted for?		Weeks
34. How long has the 5th condition lasted for?		Months
34. How long has the 5th condition lasted for?		Years
34. How long has the 5th condition lasted for?		Not applicable

Body Mass Index

35. Do you consider yourself to be an acceptable weight, underweight or overweight?	choice	Acceptable weight
35. Do you consider yourself to be an acceptable weight, underweight or overweight?	choice	Underweight
35. Do you consider yourself to be an acceptable weight, underweight or overweight?	choice	Overweight
35. Do you consider yourself to be an acceptable weight, underweight or overweight?	choice	Currently pregnant
36. Has your weight increased, decreased or stayed the same since this time last year?	choice	Increased
36. Has your weight increased, decreased or stayed the same since this time last year?	choice	Decreased
36. Has your weight increased, decreased or stayed the same since this time last year?	choice	Stayed about the same

Medications

Are you currently taking any medications?	
Please select all medications you are taking.	https://www.pbs.gov.au/browse/medicine-listing

c) Physical activity

International Physical Activity Questionnaire

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport. Think about all the vigorous and moderate activities that you did in the last 7 days. **Vigorous** physical activities refer to activities that take hard physical effort, make you breathe much harder than normal and make it difficult to speak if someone asks you a question (i.e.: running, fast cycling, fast swimming, heavy shovelling or digging, moving heavy loads (>20kg), etc). **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal (i.e.: jogging, dancing, gardening, brisk walking, housework, etc).

PART 1: JOB-RELATED PHYSICAL ACTIVITY

	Type
1. Do you currently have a job or do any unpaid work outside your home?	choice
1. Do you currently have a job or do any unpaid work outside your home?	choice

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time. (If 0, skip to question 4)	number

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

number

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

number

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads as part of your work? Please do not include walking. (if 0, skip to question 6) number

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

number

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

number

6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work. (if 0, skip to question 8)

number

7. How much time did you usually spend on one of those days walking as part of your work? number

7. How much time did you usually spend on one of those days walking as part of your work?

number

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you travelled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you travel in a motor vehicle like a train, bus, car, or tram? (If 0, skip to question 10)

number

9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?

number

9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?

number

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place? (if 0, skip to question 12)

number

11. How much time did you usually spend on one of those days to bicycle from place to place?

number

11. How much time did you usually spend on one of those days to bicycle from place to place?

number

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place? (if 0, skip to question 14)

number

13. How much time did you usually spend on one of those days walking from place to place? number

13. How much time did you usually spend on one of those days walking from place to place?

number

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shovelling snow, or digging in the garden or yard? (If 0, skip to question 16)

number

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

number

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

number

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard? (If 0, skip to question 18)

number

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

number

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

number

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home? (If 0, skip to question 20)

number

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

number

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

number

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time? (If 0, skip to question 22)

number

21. How much time did you usually spend on one of those days walking in your leisure time?

number

21. How much time did you usually spend on one of those days walking in your leisure time?

number

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time? (if 0, skip to question 24)

number

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?

number

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?

number

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time? (if 0, skip to question 26)

number

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

number

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

number

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?	number
26. During the last 7 days, how much time did you usually spend sitting on a weekday?	number
27. During the last 7 days, how much time did you usually spend sitting on a weekend day?	number
27. During the last 7 days, how much time did you usually spend sitting on a weekend day?	number

d) Day of sampling

1. What day/month/year did you collect your sample?

2. What time did you take your sample?

2. What time did you take your sample?

2. What time did you take your sample?

2. What time did you take your sample?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

3. According to the Bristol Stool Scale, how would you describe your most recent poo?

4. Was there blood in your stool?

4. Was there blood in your stool?

5. Have you had your colon cleansed in the last month?

5. Have you had your colon cleansed in the last month?

6. How much sleep did you get last night?

6. How much sleep did you get last night?

6. How much sleep did you get last night?

6. How much sleep did you get last night?

7. How would you describe the quality of your sleep?

7. How would you describe the quality of your sleep?

7. How would you describe the quality of your sleep?

7. How would you describe the quality of your sleep?

7. How would you describe the quality of your sleep?

8. Have you travelled overseas in the last 6 months?

8. Have you travelled overseas in the last 6 months?

8. Have you travelled overseas in the last 6 months?

8. Have you travelled overseas in the last 6 months?

9. Have you taken antibiotics/antifungals in the last 6 months?

9. Have you taken antibiotics/antifungals in the last 6 months?

a. If yes, when was the last time you took antibiotics/antifungals?

a. If yes, when was the last time you took antibiotics/antifungals?

a. If yes, when was the last time you took antibiotics/antifungals?

a. If yes, when was the last time you took antibiotics/antifungals?

a. If yes, when was the last time you took antibiotics/antifungals?

10. Are you currently taking immunosuppressants?

10. Are you currently taking immunosuppressants?

11. Are you currently taking probiotics?

11. Are you currently taking probiotics?

12. Are you currently taking hormones?

12. Are you currently taking hormones?

12. Are you currently taking hormones?

12. Are you currently taking hormones?

13. In the past week, have you taken any recreational drugs (marijuana, ecstasy, amphetamines)?

13. In the past week, have you taken any recreational drugs (marijuana, ecstasy, amphetamines)?

14. How many headaches have you had in the past week?

15. Did you have a headache the day of sampling?

15. Did you have a headache the day of sampling?

15. Did you have a headache the day of sampling?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

16. What best describes your current employment status?

17. Over the last 24 hours, did you feel nervous, anxious or on edge?

17. Over the last 24 hours, did you feel nervous, anxious or on edge?

17. Over the last 24 hours, did you feel nervous, anxious or on edge?

18. Over the last 24 hours, did you feel stressed and/or overwhelmed?

18. Over the last 24 hours, did you feel stressed and/or overwhelmed?

18. Over the last 24 hours, did you feel stressed and/or overwhelmed?

e) Mental health (CESD)

	type	display text
1. Over the last week, how often have you been bothered by any of the following problems?		
a. I was bothered by things that usually don't bother me.	choice	Rarely or none of the time (less than 1 day)
a. I was bothered by things that usually don't bother me.	choice	Some or a little of the time (1-2 days)
a. I was bothered by things that usually don't bother me.	choice	
a. I was bothered by things that usually don't bother me.	choice	Most or all of the time (5-7 days)
b. I did not feel like eating; my appetite was poor.	choice	Rarely or none of the time (less than 1 day)
b. I did not feel like eating; my appetite was poor.	choice	Some or a little of the time (1-2 days)
b. I did not feel like eating; my appetite was poor.	choice	
b. I did not feel like eating; my appetite was poor.	choice	Most or all of the time (5-7 days)
c. I felt that I could not shake off the blues even with help from my family or friends	choice	Rarely or none of the time (less than 1 day)
c. I felt that I could not shake off the blues even with help from my family or friends	choice	Some or a little of the time (1-2 days)
c. I felt that I could not shake off the blues even with help from my family or friends	choice	
c. I felt that I could not shake off the blues even with help from my family or friends	choice	Most or all of the time (5-7 days)
d. I felt that I was just as good as other people.	choice	Rarely or none of the time (less than 1 day)

d. I felt that I was just as good as other people.	choice	Some or a little of the time (1-2 days)
d. I felt that I was just as good as other people.	choice	
d. I felt that I was just as good as other people.	choice	Most or all of the time (5-7 days)
e. I had trouble keeping my mind on what I was doing	choice	Rarely or none of the time (less than 1 day)
e. I had trouble keeping my mind on what I was doing	choice	Some or a little of the time (1-2 days)
e. I had trouble keeping my mind on what I was doing	choice	
e. I had trouble keeping my mind on what I was doing	choice	Most or all of the time (5-7 days)
f. I felt depressed	choice	Rarely or none of the time (less than 1 day)
f. I felt depressed	choice	Some or a little of the time (1-2 days)
f. I felt depressed	choice	
f. I felt depressed	choice	Most or all of the time (5-7 days)
g. I felt that everything I did was an effort	choice	Rarely or none of the time (less than 1 day)
g. I felt that everything I did was an effort	choice	Some or a little of the time (1-2 days)
g. I felt that everything I did was an effort	choice	
g. I felt that everything I did was an effort	choice	Most or all of the time (5-7 days)
h. I felt hopeful about the future	choice	Rarely or none of the time (less than 1 day)
h. I felt hopeful about the future	choice	Some or a little of the time (1-2 days)
h. I felt hopeful about the future	choice	
h. I felt hopeful about the future	choice	Most or all of the time (5-7 days)
i. I thought my life had been a failure	choice	Rarely or none of the time (less than 1 day)
i. I thought my life had been a failure	choice	Some or a little of the time (1-2 days)
i. I thought my life had been a failure	choice	
i. I thought my life had been a failure	choice	Most or all of the time (5-7 days)
j. I felt fearful	choice	Rarely or none of the time (less than 1 day)
j. I felt fearful	choice	Some or a little of the time (1-2 days)
j. I felt fearful	choice	
j. I felt fearful	choice	Most or all of the time (5-7 days)
k. My sleep was restless	choice	Rarely or none of the time (less than 1 day)
k. My sleep was restless	choice	Some or a little of the time (1-2 days)
k. My sleep was restless	choice	
k. My sleep was restless	choice	Most or all of the time (5-7 days)
l. I was happy	choice	Rarely or none of the time (less than 1 day)
l. I was happy	choice	Some or a little of the time (1-2 days)
l. I was happy	choice	
l. I was happy	choice	Most or all of the time (5-7 days)
m. I talked less than usual	choice	Rarely or none of the time (less than 1 day)
m. I talked less than usual	choice	Some or a little of the time (1-2 days)
m. I talked less than usual	choice	
m. I talked less than usual	choice	Most or all of the time (5-7 days)
n. I felt lonely	choice	Rarely or none of the time (less than 1 day)
n. I felt lonely	choice	Some or a little of the time (1-2 days)
n. I felt lonely	choice	
n. I felt lonely	choice	Most or all of the time (5-7 days)
o. People were unfriendly	choice	Rarely or none of the time (less than 1 day)
o. People were unfriendly	choice	Some or a little of the time (1-2 days)
o. People were unfriendly	choice	
o. People were unfriendly	choice	Most or all of the time (5-7 days)
p. I enjoyed life	choice	Rarely or none of the time (less than 1 day)

p. I enjoyed life	choice	Some or a little of the time (1-2 days)
p. I enjoyed life	choice	
p. I enjoyed life	choice	Most or all of the time (5-7 days)
q. I had crying spells	choice	Rarely or none of the time (less than 1 day)
q. I had crying spells	choice	Some or a little of the time (1-2 days)
q. I had crying spells	choice	
q. I had crying spells	choice	Most or all of the time (5-7 days)
r. I felt sad	choice	Rarely or none of the time (less than 1 day)
r. I felt sad	choice	Some or a little of the time (1-2 days)
r. I felt sad	choice	
r. I felt sad	choice	Most or all of the time (5-7 days)
s. I felt that people dislike me	choice	Rarely or none of the time (less than 1 day)
s. I felt that people dislike me	choice	Some or a little of the time (1-2 days)
s. I felt that people dislike me	choice	
s. I felt that people dislike me	choice	Most or all of the time (5-7 days)
t. I could not get "going"	choice	Rarely or none of the time (less than 1 day)
t. I could not get "going"	choice	Some or a little of the time (1-2 days)
t. I could not get "going"	choice	
t. I could not get "going"	choice	Most or all of the time (5-7 days)

2. Over the last 2 weeks, how often have you been bothered by the following problems?

a. Feeling nervous, anxious or on edge	choice	Not at all
a. Feeling nervous, anxious or on edge	choice	Several days
a. Feeling nervous, anxious or on edge	choice	More than half the days
a. Feeling nervous, anxious or on edge	choice	Nearly every day
b. Not being able to stop or control worrying	choice	Not at all
b. Not being able to stop or control worrying	choice	Several days
b. Not being able to stop or control worrying	choice	More than half the days
b. Not being able to stop or control worrying	choice	Nearly every day
c. Worrying too much about different things	choice	Not at all
c. Worrying too much about different things	choice	Several days
c. Worrying too much about different things	choice	More than half the days
c. Worrying too much about different things	choice	Nearly every day
d. Trouble relaxing	choice	Not at all
d. Trouble relaxing	choice	Several days
d. Trouble relaxing	choice	More than half the days
d. Trouble relaxing	choice	Nearly every day
e. Being so restless that it is hard to sit still	choice	Not at all
e. Being so restless that it is hard to sit still	choice	Several days
e. Being so restless that it is hard to sit still	choice	More than half the days
e. Being so restless that it is hard to sit still	choice	Nearly every day
f. Becoming easily annoyed or irritable	choice	Not at all
f. Becoming easily annoyed or irritable	choice	Several days
f. Becoming easily annoyed or irritable	choice	More than half the days
f. Becoming easily annoyed or irritable	choice	Nearly every day
g. Feeling afraid as if something awful might happen	choice	Not at all
g. Feeling afraid as if something awful might happen	choice	Several days

g. Feeling afraid as if something awful might happen	choice	More than half the days
g. Feeling afraid as if something awful might happen	choice	Nearly every day

3. Stool collection procedures (access via http://www.microbiome-standards.org). Will be printed out for each participant.

IHMS Consortium	IHMS - QUALITY PROCEDURE SOP FOR FECAL SAMPLES FROZEN PRESERVED SELF-COLLECTION laboratory analysis handled within 24 hours to 7 days (24 hours < x ≤ 7 days)	Code : IHMS_SOP 04 V1 Version : 1 Date : 2015-04-14 Number of pages : 16 Page n° : 1	Last Contributor : Sebastian BURZ Approved by: IHMS CONSORTIUM Date : 2015-01-31

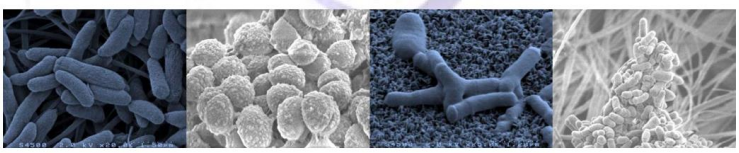
IHMS – QUALITY PROCEDURE
International Human Microbiome Standards
http://www.microbiome-standards.org

IHMS SOP 04 V1: STANDARD OPERATING PROCEDURE FOR FECAL SAMPLES

FROZEN PRESERVED SELF-COLLECTION

LABORATORY ANALYSIS HANDLED WITHIN 24 HOURS TO 7 DAYS

(24 HOURS < x ≤ 7 DAYS)



SEVENTH FRAMEWORK PROGRAMME

Authors (if you want to quote us please copy paste below): © IHMS Consortium

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4. Participant Diary

THE UNIVERSITY OF QUEENSLAND

IMPROVE HIIT STUDY - DIARY

Name:_____

Supplemenation adjustment period – 2 weeks

- ☐ Maintain your current diet – do not make any changes to what you usually eat
- ☐ 1/2 scoop = 2g; 1 scoop = 4g
- ☐ Tick in the box provided if the supplement has been taken. In the notes section, describe any side effects that you have experienced.

*AM = in the morning, PM = in the evening

Week 1

Date							
Supplement dose	☐ 2g	☐ 2g	☐ 4g	☐ 4g	☐ 4g	☐ 4g AM ☐ 2g PM	☐ 4g AM ☐ 2g PM

Notes:

Week 2

Date							
Supplement dose	☐ 4g AM ☐ 2g PM	☐ 4g AM ☐ 4g PM	☐ 4g AM ☐ 4g PM	☐ 4g AM ☐ 4g PM	☐ 6g AM ☐ 4g PM	☐ 6g AM ☐ 4g PM	☐ 6g AM ☐ 4g PM

Notes:

Exercise intervention and supplemention period – 6 weeks

Goal:

- ☐ 6g of supplement twice daily = 12g/day (1 scoop = 4g)
- ☐ Maintain your current diet – avoid making changes to what you usually eat
- ☐ 3 supervised HIIT session each week – avoid adding any extra exercise sessions

Place the date for each column. Tick in the box provided if the supplement has been taken, and if you have completed a supervised exercise session (HIIT). In the notes section, describe any side effects that you have experienced.

Week 1

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

Week 2

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

Week 3

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

Week 4

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

Week 5

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

Week 6

Date							
Supplement dose	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM	☐ 6g AM ☐ 6g PM
4x4 HIIT	☐	☐	☐	☐	☐	☐	☐

Notes:

5. Marketing Material

Email/Facebook

Would you like to know how healthy your gut is?

Would you like to know if your genetic make up indicates if you are a low or high responder to cardiorespiratory fitness training?

Would you like over $5000 worth of supervised exercise training and health checks?

Check out Camilla's new study: insert link to flyer (see next page)

Flyer wording:

THE UNIVERSITY OF QUEENSLAND

Would you like to know how healthy your gut is?

Would you like to know if your genetic make up indicates if you are a low or high responder to cardiorespiratory fitness training?

Would you like over $5000 worth of supervised exercise training and health checks?

If you are eligible, you will receive:

- ☐ 6 weeks of supervised high-intensity training sessions (University of Qld, St. Lucia)
- ☐ DNA test
- ☐ Gut micriobiome anlaysis (fecal sample anlaysis)
- ☐ Cholesterol and glucose analysis
- ☐ Body scan to measure body fat, fat mass and bone density
- ☐ 8 weeks of prebiotic supplementation (or placebo)

Who can participate?

- Inactive adults aged between 18 and 50 years
- Signed consent form

Exclusion Criteria

- Antibiotic use 6 months prior to intervention or antibiotic use during intervention.
- Pre- or-probiotic use within four weeks of participating in study
- Pregnancy, chronic infections, auto-immune diseases and intestinal chronic conditions (e.g. IBS, Chrohn's disease, ulcerative colitis, coeliac disease).
- Existing cardiac conditions
- Recent surgery
- Diabetes
- Allergies to inulin, maltodextrin, soy, milk or eggs

Interested? Contact details:

Camilla Williams
Email: camilla.williams@uq.net.au
Mobile: 07 3365 6767