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Does exercise impact gut microbiota composition of men receiving androgen deprivation therapy for prostate cancer? An exploratory study protocol.

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ABSTRACT

Introduction: A potential link exists between prostate cancer disease and treatment and increased inflammatory levels caused by dysbiosis in the gut. This study aims to examine if exercise favourably alters gut microbiota in men receiving androgen deprivation therapy (ADT) for prostate cancer. Specifically, this study will explore whether: 1) exercise improves the composition of gut microbiota and increases the abundance of bacteria associated with health-promotion; and 2) whether gut health correlates with favourable inflammatory status, bowel function, continence, and nausea among patients participating in the exercise intervention.

Methods and Analysis: A single-blinded, two-armed, randomised controlled trial will explore the influence of a 3-month exercise program (3 days per week) for men with prostate cancer receiving ADT. Sixty patients will be randomly assigned to either exercise intervention or usual care. The primary endpoint (gut health and function assessed via feacal samples) and secondary endpoints (self-reported quality of life via standardised questionnaires, blood biomarkers, body composition and physical fitness) will be measured at baseline and following the intervention. A variety of statistical methods will be used to understand the covariance between microbial diversity and metabolomics profile across time and intervention, including classical parametric and nonparametric univariate statistical methods, as well as multivariate projection models. An intention-to-treat approach will be utilised for the analyses with multiple imputations followed by a secondary sensitivity analysis to ensure data robustness using a complete cases approach.

Ethics and Dissemination: Ethics approval was obtained from the Human Research Ethics Committee of Edith Cowan University (ID: 19827 NEWTON). If exercise is proven to result in favourable changes in gut microbial diversity, composition and metabolic profile, and reduce gastrointestinal complications (inflammation, constipation, diarrhoea, and nausea) in prostate cancer patients receiving ADT, this study will form the basis of a future phase III trial.

Trial Registration: ANZCTR-12618000280202

Registration Details: 22nd February, 2018 – (prospectively registered).

Strengths and Limitations

- This study is among the first to examine the effects of an exercise medicine program on gut microbiota in men receiving androgen deprivation therapy for prostate cancer.
- Lack of follow-up past 13-weeks prohibits any assessment of sustainability of effects.
- The inclusion criteria prevents generalizability to the other types of cancer.

INTRODUCTION

Gut microbiota play a fundamental role in protection from pathogens, in the induction and function of the immune system, and in the promotion of digestion and absorption of dietary nutrients for energy production.[1-3] Indeed, perturbations of the composition and function of gut microbiota can cause gastrointestinal (GI) complications, and contribute to the pathogenesis of metabolism and disease processes such as inflammation, infection and tumours.[4-6]

Androgen deprivation therapy (ADT) is extensively used as a neoadjuvant, adjuvant and/or standalone treatment for men with prostate cancer,[7] however, survivors are burdened with persistent adverse effects of ADT including GI complications.[8-12] Despite a paucity of research into the mechanisms associated with GI diseases in prostate cancer survivors, it is hypothesised that a dysbiotic composition of the gut microbiome may mediate negative outcomes of GI complications associated with prostate cancer.[13-17] Indeed, results from murine models demonstrate that castration and antiandrogens can affect the composition of gut microbiota.[18,19] The complex, dynamic interaction between sex hormones and gut microbiome is still poorly understood.[20] Nevertheless, Markle et al (2013) demonstrated that elevated levels of testosterone in female mice results in favourable changes in microbiota, metabolomics, and inflammation, lending potential support for the role of ADT in adverse

changes to gut microbiota.[21,22] To address gut dysbiosis, especially for those with cancer, recent interventions have focused on the use of probiotics or prebiotics to alleviate symptoms[2], indicating that the manipulation of gut microbiota could be a viable therapeutic strategy for men with prostate cancer.[23,24]

Perhaps unsurprisingly, emerging evidence indicates that exercise may exert a positive effect on gut microbial composition.[2,25] In non-cancer cohorts, a link between exercise and gut microbial diversity, composition and beneficial metabolite production has been illustrated.[26-28] Of note, the ratio of Firmicutes to Bacteroidetes phyla is of particular importance, with obese individuals having a higher Firmicutes to Bacteroidetes ratio which is indicative of poorer gut health.[29,30] Importantly, it has been consistently demonstrated that men on ADT experience considerable unfavourable shifts in body composition, indicated by a significant loss of lean body mass coupled with an increase in fat mass.[31-33] Consequently, men on ADT may be at a heightened risk for this altered ratio of Firmicutes to Bacteroidetes. The shift in relative abundances of these phyla is suggested to result in an increased ability to harvest energy from food and promote low-grade inflammation.[34] Whereas, a microbiota in homeostasis and the presence of Lactobacillus and Bifidobacterium can exert anti-inflammatory effects at local and systemic levels, indicating a potential link between the gut microbiota health and inflammatory status.[35]

Metabolites are molecules that have critical roles in cellular metabolism, energy production and storage, apoptosis and signal transduction, and are affected by diet, exercise and gut microbiota amongst others. Indeed, the metabolomic profile has been suggested to be indicative of disease state in cancer and identified as a potential biomarker of the development and progression of prostate cancer.[36] Consequently, an exploration of the

relationships between changes in gut microbiota composition, metabolomic profile, inflammatory status and bowel function could have important clinical implications through enhancing positive exercise-induced effects on these outcomes through the manipulation of the composition of gut microbiota. Although the potential for exercise to modulate gut microbiota is promising, a paucity of research in the area exists, particularly in individuals at risk for gut dysbiosis. As such, no prior investigation has been conducted to determine whether gut microbiota composition is influenced by exercise in prostate cancer survivors.[37]

The aim of this exploratory study is to determine the effect of exercise medicine on gut microbiota and its metabolome in men receiving ADT for prostate cancer (PCa). Specifically, we will explore whether: 1) exercise improves composition of the gut microbiota and increases abundance of health-promoting bacterial species, thereby driving an advantageous faecal metabolomic profile, and 2) improved gut health correlates with favourable inflammatory status, bowel function, continence, nausea and appetite among patients participating in the exercise intervention. It is hypothesised that the exercise program will result in favourable changes in the composition of gut microbiota, and that these changes will be correlated with improvements in inflammatory status, bowel function and other GI issues. This study will serve to provide a better understanding of whether the gut microbiota and its metabolites can be modulated by exercise as a potential therapeutic target that can be offered for prostate cancer patients on ADT.

METHODS

Study Design

This is a single-blinded (investigators blinded to group allocation), randomised controlled trial, designed to measure the impact of a supervised exercise medicine intervention on gut

microbiota and its metabolome of prostate cancer patients receiving ADT. An 'exercise' group will receive a 12-week supervised exercise program comprising aerobic and resistance exercise undertaken three times per week. A 'usual care' group will continue to receive usual medical care during the trial. The 'usual care' group will be offered an exercise program following their control period to minimise study contamination and to reduce patient withdrawal and loss to follow-up.

Participants

Sixty men (n=60) with localised or locally advanced prostate cancer, who are currently on ADT (and expected to remain on ADT for the next three months) are eligible to enrol in this study and will be randomised to 'exercise' or 'usual care'.

Participants will be excluded if they have any visceral or bone metastases (i.e. advanced, or castrate-resistant prostate cancer); are being treated for any secondary or other cancers; previously received or currently receiving chemotherapy; currently receiving radiation therapy or any non-approved experimental therapies; and/or previously had any prior gastrointestinal surgery. Patients will also be excluded if they have taken antibiotics, prebiotics, probiotics, nutritional or ergogenic compliments or supplements within 3 months prior to study enrolment; have recently had a prostatectomy, orchiectomy, or received radiation therapy within 12 months prior to study enrolment; have any physical, mental or other contraindications to exercise; and/or cannot (or have difficulty) understanding English. Participants will be excluded if they currently engage in regular, structured physical activity (aerobic or resistance training for 2 or more times per week in the previous 3 months). All participants must receive medical clearance from their managing physician to be eligible to enroll.

Recruitment

Men with localised or locally advanced prostate cancer, who are currently receiving ADT, will be recruited by invitation of their attending specialist (i.e. urologist or radiation oncologist), who will provide clinically eligible patients with a study information sheet and referral to the study coordinator. If patients are interested in participation, and their eligibility is confirmed, they will receive an informed consent document to read and sign in the presence of a study investigator and/or clinical research coordinator prior to completing baseline measures and randomisation.

Randomisation

Prostate cancer patients will be randomly allocated in a ratio of 1:1 to two study arms: exercise or usual care, stratified by age (≤60 years, >60 years), time on ADT (≤6 months, >6 months), and prior prostatectomy or radiation therapy (Yes, No) for approximate balance between groups in order to mitigate confounding factors pertaining to variations due to ageing (i.e. differences in physical function, sarcopenia, osteopenia and osteoporosis) and to account for variations in treatment. All patients will be required to be on ADT prior to, and during the study, as per standard of care for this patient population. A research officer with no patient contact will be responsible for the randomisation of patients into groups using a computer-generated random assignment program. Study investigators and the exercise physiologists conducting testing procedures will be blinded to group allocation. Only exercise physiologists who are not part of the research team will be permitted to deliver the exercise intervention to participants in order to maintain integrity of the blinding process.

- **Insert Figure 1 here –**

Measurements

Primary and secondary endpoints will be undertaken at baseline (Week 0), and post-intervention (Week 13). All procedures and assessment tools have proven validity and reliability and are used widely throughout clinical research.

- Enter table 1 here -

Primary Endpoint

Gut Health and Dysbiosis

Gut health will be assessed through the provision of a 24-hour collection of stool sample(s), subsequently examined and analysed for microbial composition (using bacterial DNA), bacterial metabolites and for faecal calprotectin. Briefly, participants will be provided with a 24-h collection kit for collection of all bowel movements within that time. The kit includes a high performance cooler bag (Techni Ice), 2 gel ice packs (Techni Ice), collection bags, Bristol stool chart to classify stool and instructions. The cooler bag has been tested in our laboratory to maintain temperature at 4°C for 24-36 hours, and 4°C has been shown to not significantly alter faecal microbiota diversity or composition.[38] Sample storage conditions influence faecal microbiome.[38] In addition, we have also showed that there is no significant difference in faecal short chain fatty acid (SCFA) levels between cool temperature storage and immediate freezing of the stool sample at -20°C (data not shown). Samples will be returned to the laboratory within 12-24 hours following collection and stored at -80 °C in a biomedical freezer (Forma 88700 ULT; Thermo Fisher Scientific Inc., Waltham, MA, USA) until they are homogenised (combined if >1 sample over the 24h) and partitioned into aliquots for analysis.

To extract microbial DNA, samples are lysed using both mechanical and enzymatic breakdown of the bacterial cell membranes and the DNA is extracted using the QIAamp PowerFecal DNA kit (Qiagen; Hilden, Germany). Microbiome signatures are generated using the Illumina MiSeq platform using barcoded primer, V4 (515-806). Quality control samples are included in the analysis from sample collection to sequence analysis and all samples are individually screened for PCR efficiency prior to building sequence libraries. For building the sequence libraries, a PCR-free ligation protocol is deployed with sample barcodes that are never reused in our laboratory. Samples will be sequenced to a depth of minimum 30,000 reads, which is sufficient to identify microbes to a genus/species level.

qPCR will be used to verify shifts observed in the sequence data. This will provide a detailed picture of longitudinal changes in gut microbial community structure with treatment and following exercise. Sequence data/output is demultiplexed and initial sequence quality control assessments are performed with chimera's removed. The amplicon data will be analysed using a combination of bioinformatics and statistical software packages. GHAP, an in-house amplicon clustering and classification pipeline built around tools from USearch[39] and RDP[40], combined with locally-written tools for demultiplexing will be used for classification of reads into OTU's and their phylogenetic linkage. For multivariate analysis Primer7 and Permavona+ (PRIMER-E, Plymouth) will be used. Principal Coordinates Analysis (PCoA) will be deployed initially to visualise findings. Whereas, distance-based linear models (DISTLM) and distance-based redundancy analysis (dbRDA) are used for more in-depth analysis and to integrate microbiome findings with other metadata (like metabolites and diet information) that may help explain the relationship between the microbiome findings and these outcomes.

The faecal metabolome contains over 6,000 known endogenous and exogenous metabolites, comprised of many classes, each with unique chemical properties; thus no single analytical platform can be used to measure the entire metabolome in a single assay. Untargeted metabolite profiles (>2,000 metabolite features) will be measured in faecal samples using high-throughput mass-spectrometry technologies consisting of 2 complementary analytical platforms in 6 modes of operation: GC-QTOF MS (EI & CI modes) and LC-QTOF MS (Reverse Phase and HILIC Chromatography with both positive & negative ionisation modes). Resulting data will provide a comprehensive systems overview of metabolome disruption of the gut microbiota due to prostate cancer and ADT, as well as alterations resulting from the exercise intervention. All analyses will be performed at the Centre for Integrative Metabolomics & Computational Biology (CiMCB; Edith Cowan University, Perth, Australia). Additional targeted analysis methods will be used for accurate quantification of short chain fatty acids; Formate, Acetate, Propionate, Butyrate, Isobutyrate, Valerate, and Isovalerate metabolites. Faecal calprotectin will also be measured as a biomarker of inflammation to monitor gut inflammation[41].

Secondary Endpoints

Dietary Behaviour

Habitual dietary intake will be assessed using the Dietary Questionnaire for Epidemiological Studies (DQES, Version 2.0; Cancer Council of Victoria, Melbourne, Australia) which is a modified food frequency questionnaire (FFQ) relevant to cancer populations. A research assistant will supervise the completion of the DQES and use food models and charts, metric cups and spoons to increase accuracy. All participants will be instructed to maintain their habitual dietary patterns throughout the study. DQES data will be collated and batch analysed at the conclusion of the study by the Nutritional Assessment Office (Cancer Council of

Victoria, Melbourne, Australia). Dietary analysis will include food item frequency and a comprehensive nutrient intake analysis.

Biomarkers

Fasted blood samples will be collected and analysed commercially by an accredited National Association of Testing Authorities laboratory (Australian Clinical Labs, Perth, Australia) for glucose, high-sensitivity C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), cholesterol (total, high-density lipoprotein, low-density lipoprotein) and prostate specific antigen (PSA). Additional serum (SST, 8ml) and plasma (EDTA, 9ml) samples will be collected at baseline and post-intervention visits by a certified phlebotomist, and will be processed and stored in a -80°C biomedical freezer (Forma 88700 ULT; Thermo Fisher Scientific Inc., Massachusetts, USA) for subsequent metabolomics analysis at the CiMCB.

Clinical Data

Prostate cancer participant medical history, including prostate cancer diagnosis (i.e. Gleason score, stage of cancer, months since diagnosis, treatment history and time on ADT), comorbidities (i.e. hypertension, osteoarthritis, and others), concomitant medications (i.e. opioids, beta-blockers, anti-hypertensives, blood cholesterol and others), and other diagnosed chronic diseases (i.e. osteoporosis, diabetes, cardiovascular disease and others) will be sourced from patient medical records. Further, information pertaining to any related history of bowel symptoms[42], gastrointestinal symptoms[43], and other bowel-related conditions (Bristol Stool Chart)[44] will also be obtained.

Quality of life, bowel function, continence, nausea & appetite.

Cancer-specific quality of life indices will be measured using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC-QLQ-C30),[45] assessing five functional scales (physical, role, emotional, cognitive, and social functioning), three symptom scales (fatigue, pain, nausea and vomiting), a global health scale, and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhoea and financial difficulty). Prostate cancer specific quality of life indices will be measured using the EORTC-Prostate Cancer Module (EORTC QLQ-PR25),[46] assessing urinary symptoms, bowel symptoms, treatment-related symptoms and sexual functioning.

Anthropometric and Body Composition Measures

Stature will be recorded to the nearest 1 cm using a wall-mounted stadiometer (Model 222, Seca, Hamburg, DE), with body mass recorded to the nearest 0.1 kg using an electronic scale (AE Adams CPW Plus-200, Adam Equipment, Connecticut, USA). Whole body fat percentage, fat mass and lean mass will be measured using Dual-energy X-ray Absorptiometry (DXA; Horizon A, Hologic Inc., Massachusetts, USA), with whole-body and appendicular segmentations generated in accordance with Hart and colleagues.[47]

Physical Fitness

A battery of standard tests will be used to assess physical fitness, including: 1) muscle strength, using one-repetition maximum (1RM) tests for chest press, seated row and leg press exercises; 2) aerobic fitness, using the 400-metre walk test; and 3) physical function, using the timed up and go, 6-metre walk test (normal, fast and backward) and repeated chair rise.[48,49]

Exercise Program

Patients assigned to the exercise arm will attend three clinic-based supervised exercise sessions each week for 12 weeks consisting of moderate-to-high intensity resistance and aerobic exercise (interval and continuous). Each session will take approximately 60 minutes, including warm-up and cool-down, and will be supervised by accredited exercise physiologists (AEP, Exercise and Sports Science Australia). The exercise program is designed to provide optimal stimulus to the cardiorespiratory and neuromuscular systems while maximising safety, compliance and retention, thus will be tailored, progressive, periodised and autoregulated in collaboration with the patient (i.e. adjusted based on the patient's presentation at each session).

Resistance exercise will be prescribed using repetition maximums (RM), where patients will be required to perform 6-8 different resistance exercises using major muscle groups, at 6-12 RM (the maximal weight that can be lifted 6 to 12 times each set, equivalent to ~60-85% of 1RM) for 3-4 sets per exercise to achieve moderate-to-high intensity and volume. Aerobic exercise will include 20 to 30 minutes of moderate-to-vigorous intensity activity (equating to ~60-85% of estimated maximum heart rate; or a rating of perceived exertion (RPE) between 6-8 using the BORG 10 point scale[50]) involving a variety of modes including walking, jogging, cycling or rowing, using treadmills and/or stationary ergometers. Aerobic intensity will be monitored using heart rate monitors (Polar M400, H10 Sensor, Polar Electro, NSW, Australia) and an RPE chart for assessment. Patients will also be encouraged to perform additional aerobic exercise (such as brisk walking), outside of their supervised exercise clinic visits, progressing toward accruing a volume of activity consistent with national guidelines for health (i.e. 150 minutes of physical activity, or a further ~60-90 minutes self-managed). Participants will be provided log books to detail aerobic activity, which will be reviewed in the clinic each week. Flexibility exercises for all joints considered important for function and

for all muscles engaged during the session will be provided during the cool-down phase of each exercise session.

Statistical Analysis

These sample sizes (n=30 per group, totalling n=60) have been chosen to determine a moderate standardised effect (d = 0.8) for the primary and most secondary outcome measures (alpha=0.05).

A variety of statistical methods will be used to understand the covariance between microbial diversity and metabolomics profile across time and intervention, including using classical parametric and nonparametric univariate statistical methods (e.g. repeated measure ANOVA), as well as multivariate projection models (such as Principal Components Analyses, and Partial Least Squares Discriminant Analyses). The purpose of this initial stage of data analysis will be to uncover latent correlated biochemical structure in the data, mapping metabolite concentrations to metabolic pathways, and ultimately to determine candidate metabolic signatures associated with dysregulation of the gut microbiome. The resulting metabolite signatures will be integrated with quantified SCFA measurements to contextualise the results. The metabolomic and metagenomic data will be integrated using latent structure projection techniques including OnPLS48, Multiblock Component Analysis[51] and Regularized Generalized Canonical Correlation Analysis (RGCCA)[52], which extracts a minimal number of globally predictive orthogonal latent components that exhibit maximal covariance and correlation between data blocks. This will allow us to perform metabolite functional mapping, which will allow us to start unpacking the biochemical mechanism of microbiome/exercise interaction.

Statistical analyses will also include descriptive characteristics, t-tests, effect size and two-way (group x time) repeated measures ANOVA (or analysis of covariance). For categorical variables, Pearson chi-square will be used. Data will be examined using an intention-to-treat approach with multiple imputations followed by a secondary sensitivity analysis to ensure data robustness using a complete cases approach.

Patient and Public Involvement

The Exercise Medicine Research Institute (EMRI) has consumer representatives (cancer patients, survivors and their family members) who volunteer their time to provide input into research directions and study designs. Furthermore, the study clinicians (NAS, CIT, RC) consult within the major public and private hospitals in Perth, Western Australia, each with high prostate cancer caseloads, thus have used patient priorities, patient experience and patient preferences to help inform the development of the research questions and outcome measures, while aiding the study protocol to engage participants in a respectful, ethical and impactful way.

Ethics and Dissemination

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of Edith Cowan University (ID: 19827 NEWTON), with additional approval provided by the Radiation, Biosafety and Hazardous Substances Committee (RBHSC). If exercise is proven to result in favourable changes in gut microbial diversity, composition and metabolic profile, and reduce gastrointestinal complications (inflammation, constipation, diarrhoea, and nausea) in prostate cancer patients receiving ADT, this study will form the basis of a future phase III trial. These outcomes will provide further validation of exercise medicine in this population and add to efforts aimed at changing clinical practice. To reach a maximum number of

clinicians, practitioners, patients and scientists, the results of this study will be published in international, high-impact peer-reviewed clinical and academic journals. In addition, outcomes will be disseminated through national and international clinical, medical or academic conferences and oncology clinic and patient meetings. Finally, EMRI and the broader study team work closely with the Prostate Cancer Foundation of Australia (PCFA), their support groups and states offices. PCFA will assist in the translation and dissemination of the research findings to community members, and cancer support groups, while study participants will receive their individual results in addition to overall study findings.

Discussion

Prostate cancer patients are burdened with persistent adverse effects of treatment, including gastrointestinal (GI) complications.[8-12] It has been hypothesized that the hormonal consequences of ADT may have an adverse effect on the composition of gut microbiota.[22] Perturbations of the composition and function of gut microbiota can cause GI complications and contribute to the development and pathogenesis of metabolism and disease processes such as inflammation, infections and disease progression.[15] Additionally, the metabolomic profile of prostate cancer patients could be an important biomarker for disease state and progression.[36] Consequently, an examination of therapeutic strategies to induce clinically meaningful improvements in the gut microbiome, metabolomic profile and inflammatory status is warranted.

Preliminary evidence indicates that exercise, independent of diet, may result in favourable changes in gut microbial diversity and composition, which has the potential to improve overall metabolic and health profile.[53] While this research is promising, much of this area is

in its infancy, with a substantial lack of evidence in a cancer context. Given the paucity of data, coupled with the potential negative health effects of gut dysbiosis in prostate cancer, there is a clear need to directly evaluate the relationship between exercise and gut microbiota in this patient population. The protocol outlined in this paper will evaluate the effects of exercise on changes in the composition and diversity of gut microbiota and if these changes are correlated with favourable inflammatory status, bowel function, continence, nausea and appetite among patients participating in the exercise intervention.

Excess adiposity is associated with a heightened inflammatory state and gut dysbiosis.[54] Exercise has been previously demonstrated to successfully attenuate and/or reverse the accumulation of fat mass experienced by PCa patients undergoing ADT.[48,49,55] Consequently, it's biologically plausible that improvements in lean body mass and overall body composition may mediate favourable changes in the composition of gut microbiota, inflammatory status, and metabolomic profile. Moreover, gut dysbiosis has been associated with frailty in older adults.[56-58] Though a causal relationship cannot be determined, improvements in muscle strength and physical function from exercise may contribute to improvements in the composition of gut microbiota in this population. Consequently, analysis of body composition and physical fitness will provide valuable information on any factors that may mediate changes in gut microbiome from exercise.[59]

If demonstrated that this exercise intervention results in clinically meaningful improvements in gut health, the outcomes of this trial will provide innovative, new evidence that can inform the design of future phase III clinical trials to further elucidate the effects of exercise on gut health in patients with PCa. Further, any correlations between changes in the composition and

diversity of gut microbiota and inflammatory status, and gastrointestinal issues, may inform design of future research studies that further explore these relationships.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

CONTRIBUTION STATEMENT

All authors (RUN, CTC, NHH, DRT, DIB, AD, RC, CMF, CIT, NAS and DAG) contributed to the design and development of the study protocol. RUN, CTC, NHH, DRT, DIB, AD, CMF and DAG contributed to writing and editing of the manuscript. RC, CIT and NAS provided clinical input to the editing of the manuscript and will provide patient referrals to the study. All authors reviewed and approved the study protocol and have met the International Committee of Medical Journal Editors (ICMJE) recommendations.

LEGENDS OF FIGURES AND TABLES

Table 1. Schedule of assessments at baseline and post intervention

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram.png

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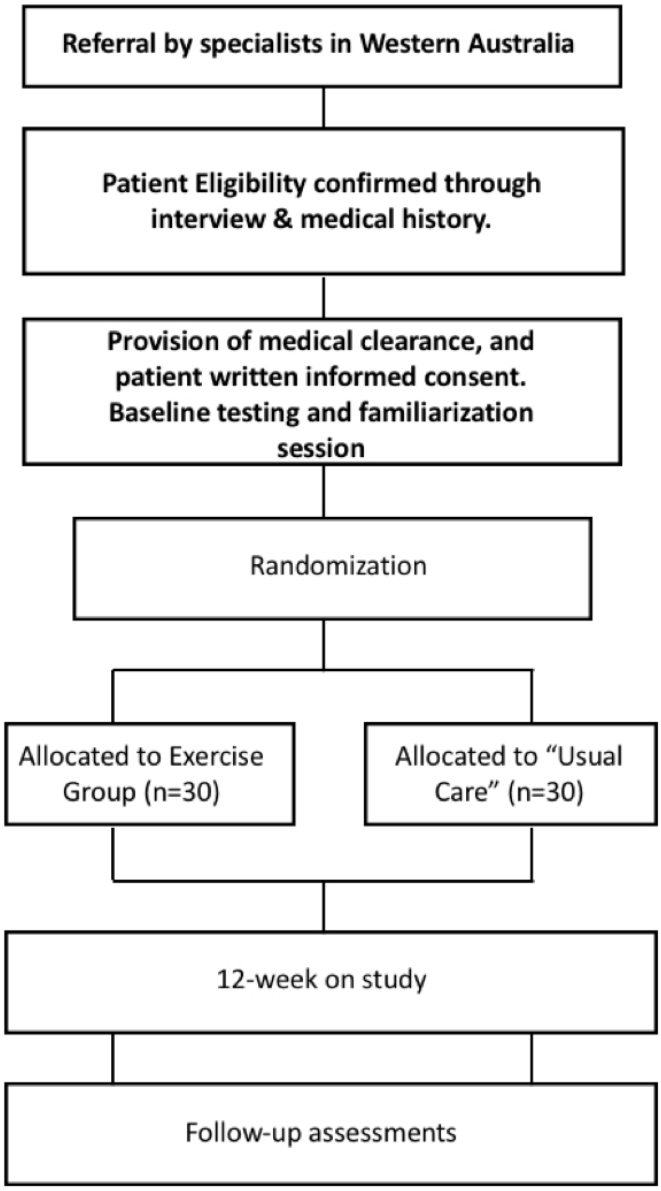

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram

Table 1. Schedule of assessments at baseline and post intervention

Measures	Baseline	Post intervention
Medical history	x	
Gut health (24-hour stool sample)	x	x
Dietary behaviour (DQES)	x	x
Blood biomarkers	x	x
Cancer specific quality of life		
EORTC-QLQ-C30	x	x
EORTC-PR-25	x	x
Body composition (DXA)	x	x
Muscle strength (1RM)	x	x
Aerobic fitness (400m)	x	x
Physical function tests	x	x

DQES: Dietary Questionnaire for Epidemiological Studies; EORTC: European Organization for Research and Treatment of Cancer.

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Does exercise impact gut microbiota composition in men receiving androgen deprivation therapy for prostate cancer? A single-blinded, two-armed, randomised controlled trial.

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ABSTRACT

Introduction: A potential link exists between prostate cancer disease and treatment and increased inflammatory levels from gut dysbiosis.' This study aims to examine if exercise favourably alters gut microbiota in men receiving androgen deprivation therapy (ADT) for prostate cancer. Specifically, this study will explore whether: 1) exercise improves the composition of gut microbiota and increases the abundance of bacteria associated with health-promotion; and 2) whether gut health correlates with favourable inflammatory status, bowel function, continence, and nausea among patients participating in the exercise intervention.

Methods and Analysis: A single-blinded, two-armed, randomised controlled trial will explore the influence of a 3-month exercise program (3 days per week) for men with high risk localised prostate cancer receiving ADT. Sixty patients will be randomly assigned to either exercise intervention or usual care. The primary endpoint (gut health and function assessed via feacal samples) and secondary endpoints (self-reported quality of life via standardised questionnaires, blood biomarkers, body composition and physical fitness) will be measured at baseline and following the intervention. A variety of statistical methods will be used to understand the covariance between microbial diversity and metabolomics profile across time and intervention. An intention-to-treat approach will be utilised for the analyses with multiple imputations followed by a secondary sensitivity analysis to ensure data robustness using a complete cases approach.

Ethics and Dissemination: Ethics approval was obtained from the Human Research Ethics Committee of Edith Cowan University (ID: 19827 NEWTON). Findings will be reported in peer-reviewed publications and scientific conferences in addition to working with national support groups to translate findings for the broader community. If exercise is shown to result in favourable changes in gut microbial diversity, composition and metabolic profile, and reduce gastrointestinal complications in prostate cancer patients receiving ADT, this study will form the basis of a future phase III trial.

Trial Registration: ANZCTR-12618000280202

Registration Details: 22nd February, 2018 – (prospectively registered).

Strengths and Limitations

- This trial comprises a comprehensive analysis of gut microbiome in individuals with prostate cancer, addressing a significant and increasingly emergent issue in this area.
- A two-armed, randomised controlled trial design to examine proof of concept and provide effect sizes to design subsequent appropriately powered trials.
- Lack of follow-up past 13 weeks prohibits any assessment of sustainability of effects.
- The inclusion criteria prevents generalizability to the other types of cancer.
- Diet and physical activity (outside of the intervention) will not be tracked and are limitations of the current design.

INTRODUCTION

Gut microbiota play a fundamental role in protection from pathogens, in the induction and function of the immune system, and in the promotion of digestion and absorption of dietary nutrients for energy production.[1 2] Indeed, perturbations of the composition and function of gut microbiota can cause gastrointestinal (GI) complications, and contribute to the pathogenesis of metabolism and disease processes such as inflammation, infection and tumours.[3-6]

Androgen deprivation therapy (ADT) is extensively used as a neoadjuvant, adjuvant and/or standalone treatment for men with prostate cancer,[6] however, survivors are burdened with persistent adverse effects of ADT including GI complications.[7-11] Despite a paucity of research into the mechanisms associated with GI diseases in prostate cancer survivors, it is hypothesised that a dysbiotic composition of the gut microbiome may mediate negative outcomes of GI complications associated with prostate cancer.[12-15] Indeed, results from murine models demonstrate that castration and antiandrogens can affect the composition of gut microbiota.[16 17] The complex, dynamic interaction between sex hormones and gut microbiome is still poorly understood.[18] Nevertheless, Markle et al.[19] demonstrated that elevated levels of testosterone in female mice results in favourable changes in microbiota, metabolomics, and inflammation, lending potential support for the role of ADT in adverse changes to gut microbiota.[19 20] To address gut dysbiosis, especially for those with cancer, recent interventions have focused on the use of probiotics or prebiotics to alleviate symptoms,[2] indicating that the manipulation of gut microbiota could be a viable therapeutic strategy for men with prostate cancer.[21 22]

Perhaps unsurprisingly, emerging evidence indicates that exercise may exert a positive effect on gut microbial composition.[2 23] In non-cancer cohorts, a link between exercise and gut microbial diversity, composition and beneficial metabolite production has been illustrated.[24 25] Of note, the ratio of Firmicutes to Bacteroidetes phyla is of particular importance, with obese individuals having a higher Firmicutes to Bacteroidetes ratio which is indicative of poorer gut health.[26 27] Importantly, it has been consistently demonstrated that men on ADT experience considerable unfavourable shifts in body composition, indicated by a significant loss of lean body mass coupled with an increase in fat mass.[28-30] Consequently, men on ADT may be at a heightened risk for this altered ratio of Firmicutes to Bacteroidetes. The shift in relative abundances of these phyla is suggested to result in an increased ability to harvest energy from food and promote low-grade inflammation.[31] Whereas, a microbiota in homeostasis and the presence of Lactobacillus and Bifidobacterium can exert anti-inflammatory effects at local and systemic levels, indicating a potential link between the gut microbiota health and inflammatory status.[32]

Metabolites are molecules that have critical roles in cellular metabolism, energy production and storage, apoptosis and signal transduction, and are affected by diet, exercise and gut microbiota amongst others. Indeed, the metabolomic profile has been suggested to be indicative of disease state in cancer and identified as a potential biomarker of the development and progression of prostate cancer.[33] Consequently, an exploration of the relationships between changes in gut microbiota composition, metabolomic profile, inflammatory status and bowel function could have important clinical implications through enhancing positive exercise-induced effects on these outcomes through the manipulation of the composition of gut microbiota. Although the potential for exercise to modulate gut microbiota is promising, a paucity of research in the area exists, particularly in individuals at risk for gut dysbiosis. As

such, no prior investigation has been conducted to determine whether gut microbiota composition is influenced by exercise in prostate cancer survivors.[34]

The aim of this exploratory study is to determine the effect of exercise medicine on gut microbiota and its metabolome in men receiving ADT for prostate cancer (PCa). Specifically, we will explore whether: 1) exercise improves composition of the gut microbiota and increases abundance of health-promoting bacterial species, thereby driving an advantageous faecal metabolomic profile, and 2) improved gut health correlates with favourable inflammatory status, bowel function, continence, nausea and appetite among patients participating in the exercise intervention. It is hypothesised that the exercise program will result in favourable changes in the composition of gut microbiota, and that these changes will be correlated with improvements in inflammatory status, bowel function and other GI issues. This study will serve to provide a better understanding of whether the gut microbiota and its metabolites can be modulated by exercise as a potential therapeutic target that can be offered for prostate cancer patients on ADT.

METHODS

Study Design

This is a single-blinded (investigators blinded to group allocation), randomised controlled trial, designed to measure the impact of a supervised exercise medicine intervention on gut microbiota and its metabolome of prostate cancer patients receiving ADT. An 'exercise' group will receive a 12-week supervised exercise program comprising aerobic and resistance exercise undertaken three times per week. A 'usual care' group will continue to receive usual medical care during the trial. The 'usual care' group will be offered an exercise program following their

control period to minimise study contamination and to reduce patient withdrawal and loss to follow-up.

Participants

Sixty men (n=60) with high risk localised prostate cancer, defined by the National Comprehensive Cancer Network as T3a disease, Gleason ≥8, or PSA ≥20,[35] who are currently on ADT (and expected to remain on ADT for the next three months) are eligible to enrol in this study and will be randomised to 'exercise' or 'usual care'. ADT can be achieved with either luteinizing hormone-releasing hormone (LHRH) agonists or LHRH antagonists, or a combination of the two. It is also acceptable for patients to receive no more than 4 weeks of anti-androgen at the initiation of their ADT to prevent flare from testosterone surge. Patients must be on continuous ADT during the study period.

Participants will be excluded if they have any nodal, visceral or bone metastases (N1 or M1 disease); are being treated for other cancers; previously received or currently receiving chemotherapy; currently receiving radiation therapy or any non-approved experimental therapies; and/or previously had any prior gastrointestinal surgery. Patients will also be excluded if they have taken antibiotics, prebiotics, probiotics, nutritional or ergogenic compliments or supplements within 3 months prior to study enrolment; have recently had a prostatectomy, orchiectomy, or received radiation therapy within 12 months prior to study enrolment; have any physical, mental or other contraindications to exercise; and/or cannot (or have difficulty) understanding English. Participants will be excluded if they currently engage in regular, structured physical activity (aerobic or resistance training for 2 or more times per week in the previous 3 months). All participants must receive medical clearance from their

managing physician to be eligible to enrol. Additionally, all participants will be asked to provide informed consent prior to the start of any study activities.

Recruitment

Men with localised or locally advanced prostate cancer, who are currently receiving ADT, will be recruited by invitation of their attending specialist (i.e. urologist or radiation oncologist), who will provide clinically eligible patients with a study information sheet and referral to the study coordinator. If patients are interested in participation, and their eligibility is confirmed, they will receive an informed consent document to read and sign in the presence of a study investigator and/or clinical research coordinator prior to completing baseline measures and randomisation. Figure 1 outlines the process of referrals, recruitment and enrolment in the trial.

- **Insert Figure 1 here –**

Randomisation

Prostate cancer patients will be randomly allocated in a ratio of 1:1 to two study arms: exercise or usual care, stratified by age (≤60 years, >60 years), time on ADT (≤6 months, >6 months), and prior prostatectomy or radiation therapy (Yes, No) for approximate balance between groups in order to mitigate confounding factors pertaining to variations due to ageing (i.e. differences in physical function, sarcopenia, osteopenia and osteoporosis) and to account for variations in treatment. All patients will be required to be on ADT prior to, and during the study, as per standard of care for this patient population. A research officer with no patient contact will be responsible for the randomisation of patients into groups using a computer-generated random assignment program. Study investigators and the exercise physiologists conducting testing procedures will be blinded to group allocation. Only exercise physiologists

who are not part of the research team will be permitted to deliver the exercise intervention to participants in order to maintain integrity of the blinding process.

Measurements

Table 1 outlines the primary and secondary endpoints that will be undertaken at baseline (Week 0), and post-intervention (Week 13). All procedures and assessment tools have proven validity and reliability and are used widely throughout clinical research.

Table 1. Schedule of assessments at baseline and post intervention

Measures	Baseline	Post intervention
Medical history	x	
Gut health (24-hour stool sample)	x	x
Dietary behaviour (DQES)	x	x
Blood biomarkers	x	x
Cancer specific quality of life		
EORTC-QLQ-C30	x	x
EORTC-PR-25	x	x
Body composition (DXA)	x	x
Muscle strength (1RM)	x	x
Aerobic fitness (400m)	x	x
Physical function tests	x	x

DQES: Dietary Questionnaire for Epidemiological Studies; EORTC: European Organization for Research and Treatment of Cancer.

Primary Endpoint

Gut Health and Dysbiosis

Gut health will be assessed through the provision of a 24-hour collection of stool sample(s), subsequently examined and analysed for microbial composition (using bacterial DNA), bacterial metabolites and for faecal calprotectin. Briefly, participants will be provided with a 24-h collection kit for collection of all bowel movements within that time. The kit includes a high-performance cooler bag (Techni Ice), 2 gel ice packs (Techni Ice), collection bags, Bristol stool chart to classify stool and instructions. The cooler bag has been tested in our laboratory to maintain temperature at 4°C for 24-36 hours, and 4°C has been shown to not significantly alter faecal microbiota diversity or composition.[36] In addition, we have also showed that there is no significant difference in faecal short chain fatty acid (SCFA) levels between cool temperature storage and immediate freezing of the stool sample at -20°C (data not shown). Samples will be returned to the laboratory within 12-24 hours following collection and stored at -80 °C in a biomedical freezer (Forma 88700 ULT; Thermo Fisher Scientific Inc., Waltham, MA, USA) until they are homogenised (combined if >1 sample over the 24h) and partitioned into aliquots for analysis.

To extract microbial DNA, samples will be lysed using both mechanical and enzymatic breakdown of the bacterial cell membranes and the DNA extracted using the QIAamp PowerFecal DNA kit (Qiagen; Hilden, Germany). Microbiome signatures will be generated using the Illumina MiSeq platform using barcoded primer, V4 (515-806). Quality control samples are included in the analysis from sample collection to sequence analysis and all samples will be individually screened for PCR efficiency prior to building sequence libraries. For building the sequence libraries, a PCR-free ligation protocol will be deployed with sample barcodes that are never reused in our laboratory. Samples will be sequenced to a depth of

minimum 30,000 reads, which is sufficient to identify microbes to a genus/species level. qPCR will be used to verify shifts observed in the sequence data and will therefore be determined post-analysis of the 16S rRNA gene data. This will provide a detailed picture of longitudinal changes in gut microbial community structure with treatment and following exercise.

Sequence read quality will be initially assessed with FASTOC before demultiplexing and preprocessing by Shi7[37] and/or GHAPv2. Clutadapt will be used for removal of all non-biological sequences[38]. DADA2 is then used for quality filtering, error correction, exact sequence variants (ASVs) picking[39]. A trained naïve Bayes classifier then assigns ASVs to genus/species against a curated database of microbial reference sequences such as the RDP[40] or SILVA.

The faecal metabolome contains over 6,000 known endogenous and exogenous metabolites, comprised of many classes, each with unique chemical properties; thus no single analytical platform can be used to measure the entire metabolome in a single assay. Untargeted metabolite profiles (>2,000 metabolite features) will be measured in faecal samples using high-throughput mass-spectrometry technologies consisting of 2 complementary analytical platforms in 6 modes of operation: GC-QTOF MS (EI & CI modes) and LC-QTOF MS (Reverse Phase and HILIC Chromatography with both positive & negative ionisation modes). Resulting data will provide a comprehensive systems overview of metabolome disruption of the gut microbiota due to prostate cancer and ADT, as well as alterations resulting from the exercise intervention. All analyses will be performed at the Centre for Integrative Metabolomics & Computational Biology (CiMCB; Edith Cowan University, Perth, Australia). Additional targeted analysis methods will be used for accurate quantification of short chain fatty acids; Formate, Acetate,

Propionate, Butyrate, Isobutyrate, Valerate, and Isovalerate metabolites. Faecal calprotectin will also be measured as a biomarker of inflammation to monitor gut inflammation[41].

Secondary Endpoints

Dietary Behaviour

Habitual dietary intake will be assessed using the Dietary Questionnaire for Epidemiological Studies (DQES, Version 2.0; Cancer Council of Victoria, Melbourne, Australia) which is a modified food frequency questionnaire (FFQ) relevant to cancer populations. A research assistant will supervise the completion of the DQES and use food models and charts, metric cups and spoons to increase accuracy. All participants will be instructed to maintain their habitual dietary patterns throughout the study. DQES data will be collated and batch analysed at the conclusion of the study by the Nutritional Assessment Office (Cancer Council of Victoria, Melbourne, Australia). Dietary analysis will include food item frequency and a comprehensive nutrient intake analysis.

Biomarkers

Fasted blood samples will be collected and analysed commercially by an accredited National Association of Testing Authorities laboratory (Australian Clinical Labs, Perth, Australia) for glucose, high-sensitivity C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), cholesterol (total, high-density lipoprotein, low-density lipoprotein), testosterone and prostate specific antigen (PSA). Additional serum (SST, 8ml) and plasma (EDTA, 9ml) samples will be collected at baseline and post-intervention visits by a certified phlebotomist, and will be processed and stored in a -80°C biomedical freezer (Forma 88700 ULT; Thermo Fisher Scientific Inc., Massachusetts, USA) for subsequent metabolomics analysis at the CiMCB. For participants that provide consent, the samples will be stored for a

duration of 5 years. We will be pursuing other research studies in advanced cancer patients, and this de-identified blood sample data may be of use to gain further insights and comparisons.For participants who do not provide consent for future storage (but do provide consent for this study), we will destroy any left-over samples following the analysis required for this study (within 6 months from time of patient enrolment).

Clinical Data

Prostate cancer participant medical history, including prostate cancer diagnosis (i.e. Gleason score, stage of cancer, months since diagnosis, treatment history and time on ADT), comorbidities (i.e. hypertension, osteoarthritis, and others), concomitant medications (i.e. opioids, beta-blockers, anti-hypertensives, blood cholesterol and others), and other diagnosed chronic diseases (i.e. osteoporosis, diabetes, cardiovascular disease and others) will be sourced from patient medical records. Further, information pertaining to any related history of bowel symptoms[42], gastrointestinal symptoms[43], and other bowel-related conditions (Bristol Stool Chart)[44] will also be obtained.

Quality of life, bowel function, continence, nausea & appetite.

Cancer-specific quality of life indices will be measured using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC-QLQ-C30),[45] assessing five functional scales (physical, role, emotional, cognitive, and social functioning), three symptom scales (fatigue, pain, nausea and vomiting), a global health scale, and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhoea and financial difficulty). Prostate cancer specific quality of life indices will be measured using the EORTC-Prostate Cancer Module (EORTC QLQ-PR25),[46] assessing urinary symptoms, bowel symptoms, treatment-related symptoms and sexual functioning.

Anthropometric and Body Composition Measures

Stature will be recorded to the nearest 1 cm using a wall-mounted stadiometer (Model 222, Seca, Hamburg, DE), with body mass recorded to the nearest 0.1 kg using an electronic scale (AE Adams CPW Plus-200, Adam Equipment, Connecticut, USA). Whole body fat percentage, fat mass and lean mass will be measured using Dual-energy X-ray Absorptiometry (DXA; Horizon A, Hologic Inc., Massachusetts, USA), with whole-body and appendicular segmentations generated in accordance with Hart and colleagues.[47]

Physical Fitness

A battery of standard tests will be used to assess physical fitness, including: 1) muscle strength, using one-repetition maximum (1RM) tests for chest press, seated row and leg press exercises; 2) aerobic fitness, using the 400-metre walk test; and 3) physical function, using the timed up and go, 6-metre walk test (normal, fast and backward) and repeated chair rise.[48 49]

Exercise Program

Patients assigned to the exercise arm will attend three clinic-based supervised exercise sessions each week for 12 weeks consisting of moderate-to-high intensity resistance and aerobic exercise (interval and continuous). Each session will take approximately 60 minutes, including warm-up and cool-down, and will be supervised by accredited exercise physiologists (AEP, Exercise and Sports Science Australia). The exercise program is designed to provide optimal stimulus to the cardiorespiratory and neuromuscular systems while maximising safety, compliance and retention, thus will be tailored, progressive, periodised and autoregulated in collaboration with the patient (i.e. adjusted based on the patient's presentation at each session).

Resistance exercise will be prescribed using repetition maximums (RM), where patients will be required to perform 6-8 different resistance exercises using major muscle groups, at 6-12 RM (the maximal weight that can be lifted 6 to 12 times each set, equivalent to ~60-85% of 1RM) for 3-4 sets per exercise to achieve moderate-to-high intensity and volume. Aerobic exercise will include 20 to 30 minutes of moderate-to-vigorous intensity activity (equating to ~60-85% of estimated maximum heart rate; or a rating of perceived exertion (RPE) between 6-8 using the BORG 10 point scale[50]) involving a variety of modes including walking, jogging, cycling or rowing, using treadmills and/or stationary ergometers. Aerobic intensity will be monitored using heart rate monitors (Polar M400, H10 Sensor, Polar Electro, NSW, Australia) and an RPE chart for assessment. Patients will also be encouraged to perform additional aerobic exercise (such as brisk walking), outside of their supervised exercise clinic visits, progressing toward accruing a volume of activity consistent with national guidelines for health (i.e. 150 minutes of physical activity, or a further ~60-90 minutes self-managed). Participants will be provided log books to detail aerobic activity, which will be reviewed in the clinic each week. Flexibility exercises for all joints considered important for function and for all muscles engaged during the session will be provided during the cool-down phase of each exercise session.

Statistical Analysis

These sample sizes (n=30 per group, totalling n=60) have been chosen to determine a moderate standardised effect (d = 0.8) for the primary and most secondary outcome measures (alpha=0.05).

A variety of statistical methods will be used to understand the covariance between microbial diversity and metabolomics profile across time and intervention, including using classical

parametric and nonparametric univariate statistical methods (e.g. repeated measure ANOVA), as well as multivariate projection models (such as Principal Components Analyses, and Partial Least Squares Discriminant Analyses). The purpose of this initial stage of data analysis will be to uncover latent correlated biochemical structure in the data, mapping metabolite concentrations to metabolic pathways, and ultimately to determine candidate metabolic signatures associated with dysregulation of the gut microbiome. The resulting metabolite signatures will be integrated with quantified SCFA measurements to contextualise the results. The metabolomic and metagenomic data will be integrated using latent structure projection techniques including OnPLS[51], Multiblock Component Analysis[52] and Regularized Generalized Canonical Correlation Analysis (RGCCA)[53], which extracts a minimal number of globally predictive orthogonal latent components that exhibit maximal covariance and correlation between data blocks. This will allow us to perform metabolite functional mapping, which will allow us to start unpacking the biochemical mechanism of microbiome/exercise interaction.

Statistical analyses will also include descriptive characteristics, t-tests, effect size and two-way (group x time) repeated measures ANOVA (or analysis of covariance, adjusted for baseline values, time on ADT and medication change). For categorical variables, Pearson chi-square will be used. Data will be examined using an intention-to-treat approach with multiple imputations followed by a secondary sensitivity analysis to ensure data robustness using a complete cases approach. Data will be completely de-identified, and subsequently coded in order to note which group / intervention a patient received; without any ability to identify the patient in any way. Any identifiable documents will be destroyed. The de-identified information will be kept electronically only, in a password locked folder, on a password locked computer, accessible only by the team investigators. All statistical techniques employed in this

study will be implemented using open source software (R or Python), which will be made publicly available at the time of dissemination.

Adverse Events

Individuals will be monitored for any adverse events during exercise testing and training and will be documented accordingly. The study team has extensive experience in delivering exercise specifically tailored to individuals with prostate cancer in a safe and efficacious manner. The risks of adverse events are low in this study and a data monitoring/safety committee has not been appointed.

Patient and Public Involvement

The Exercise Medicine Research Institute (EMRI) has consumer representatives (cancer patients, survivors and their family members) who volunteer their time to provide input into research directions and study designs. Furthermore, the study clinicians (CIT, RC) consult within the major public and private hospitals in Perth, Western Australia, each with high prostate cancer caseloads, thus have used patient priorities, patient experience and patient preferences to help inform the development of the research questions and outcome measures, while aiding the study protocol to engage participants in a respectful, ethical and impactful way.

Ethics and Dissemination

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of Edith Cowan University (ID: 19827 NEWTON), with additional approval provided by the Radiation, Biosafety and Hazardous Substances Committee (RBHSC). Any amendments to the protocol will be submitted to and reviewed the HREC and trial registration will be updated accordingly.

If exercise is proven to result in favourable changes in gut microbial diversity, composition and metabolic profile, and reduce gastrointestinal complications (inflammation, constipation, diarrhoea, and nausea) in prostate cancer patients receiving ADT, this study will form the basis of a future phase III trial. These outcomes will provide further validation of exercise medicine in this population and add to efforts aimed at changing clinical practice. To reach a maximum number of clinicians, practitioners, patients and scientists, the results of this study will be published in international, high-impact peer-reviewed clinical and academic journals. In addition, outcomes will be disseminated through national and international clinical, medical or academic conferences and oncology clinic and patient meetings. Finally, EMRI and the broader study team work closely with the Prostate Cancer Foundation of Australia (PCFA), their support groups and states offices. PCFA will assist in the translation and dissemination of the research findings to community members, and cancer support groups, while study participants will receive their individual results in addition to overall study findings.

DISCUSSION

Prostate cancer patients are burdened with persistent adverse effects of treatment, including gastrointestinal (GI) complications.[7-11] It has been hypothesized that the hormonal consequences of ADT may have an adverse effect on the composition of gut microbiota.[20] Perturbations of the composition and function of gut microbiota can cause GI complications and contribute to the development and pathogenesis of metabolism and disease processes such as inflammation, infections and disease progression.[14] Additionally, the metabolomic profile of prostate cancer patients could be an important biomarker for disease state and progression.[33] Consequently, an examination of therapeutic strategies to induce clinically meaningful

improvements in the gut microbiome, metabolomic profile and inflammatory status is warranted.

Preliminary evidence indicates that exercise, independent of diet, may result in favourable changes in gut microbial diversity and composition, which has the potential to improve overall metabolic and health profile.[54] While this research is promising, much of this area is in its infancy, with a substantial lack of evidence in a cancer context. Given the paucity of data, coupled with the potential negative health effects of gut dysbiosis in prostate cancer, there is a clear need to directly evaluate the relationship between exercise and gut microbiota in this patient population. The protocol outlined in this paper will evaluate the effects of exercise on changes in the composition and diversity of gut microbiota and if these changes are correlated with favourable inflammatory status, bowel function, continence, nausea and appetite among patients participating in the exercise intervention.

Excess adiposity is associated with a heightened inflammatory state and gut dysbiosis.[55] Exercise has been previously demonstrated to successfully attenuate and/or reverse the accumulation of fat mass experienced by PCa patients undergoing ADT.[48 49 56] Consequently, it's biologically plausible that improvements in lean body mass and overall body composition may mediate favourable changes in the composition of gut microbiota, inflammatory status, and metabolomic profile. Moreover, gut dysbiosis has been associated with frailty in older adults.[57-60] Though a causal relationship cannot be determined, improvements in muscle strength and physical function from exercise may contribute to improvements in the composition of gut microbiota in this population. Consequently, analysis of body composition and physical fitness will provide valuable information on any factors that may mediate changes in gut microbiome from exercise.[60]

LIMITATIONS

Despite the novel study design, several limitations should be acknowledged. Though a dietary intervention and/or more rigorous tracking throughout the study would be interesting, limitations in funding, resources and feasibility make it difficult to do this in the current study. An interesting aspect would be to examine the differential efficacy in androgen blockade, sex hormone conversion in the patient and subsequent exercise intervention effects. However, this is not possible in the current study and thus a limitation of the investigation is the ability to link androgen kinetics directly to the primary outcomes. Additionally, although this study will be one of the first to examine changes in gut microbiota with exercise in men with ADT, the lack of follow-up precludes an analysis of the sustainability of outcomes.

CONCLUSIONS

If demonstrated that this exercise intervention results in clinically meaningful improvements in gut health, the outcomes of this trial will provide innovative, new evidence that can inform the design of future phase III clinical trials to further elucidate the effects of exercise on gut health in patients with PCa. Further, any correlations between changes in the composition and diversity of gut microbiota and inflammatory status, and gastrointestinal issues, may inform design of future research studies that further explore these relationships.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

CONTRIBUTION STATEMENT

All authors (RUN, CTC, NHH, DRT, DIB, AD, RC, CMF, CIT, NAS and DAG) contributed to the design and development of the study protocol. RUN, CTC, NHH, DRT, DIB, AD, CMF and DAG contributed to writing and editing of the manuscript. RC, CIT and NAS provided clinical input to the editing of the manuscript and will provide patient referrals to the study. All authors reviewed and approved the study protocol and have met the International Committee of Medical Journal Editors (ICMJE) recommendations.

LEGENDS OF FIGURES AND TABLES

Table 1. Schedule of assessments at baseline and post intervention

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram

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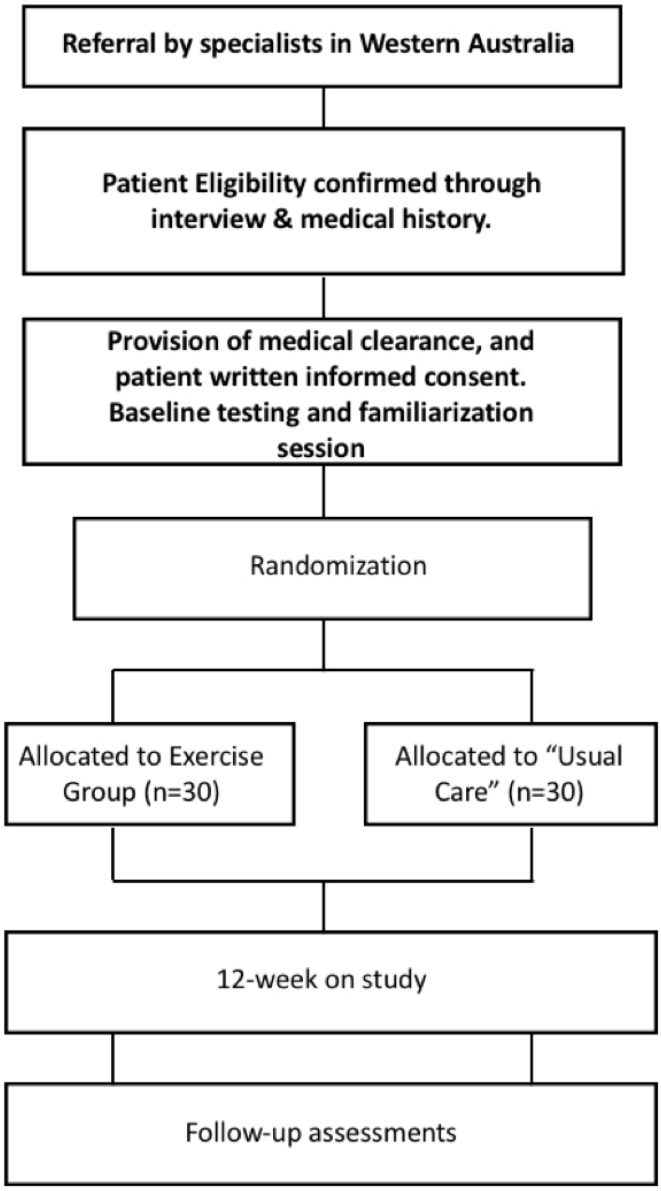

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, www.anzctr.org.au
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	19
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 & 19
	5b	Name and contact information for the trial sponsor	19
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18 & 19

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	18 & 19
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13 & 14
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 & 7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13 & 14
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13 & 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	20
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7 & 8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14 & 15
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14 &15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14 & 15
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15 & 16
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15 & 16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2 & 16
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19 & 20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15 & 16
	31b	Authorship eligibility guidelines and any intended use of professional writers	15 & 16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.