PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Does exercise impact gut microbiota composition in men receiving androgen deprivation therapy for prostate cancer? A single-blinded, two-armed, randomised controlled trial.
AUTHORS	Newton, Robert; Christophersen, Claus; Fairman, Ciaran; Hart, Nicolas; Taaffe, Dennis; Broadhurst, David; Devine, Amanda; Chee, Raphael; Tang, Colin; Spry, Nigel; Galvão, Daniel

VERSION 1 - REVIEW

REVIEWER	Katariina Pärnänen University of Helsinki, Finland
REVIEW RETURNED	27-Sep-2018

GENERAL COMMENTS	More details about what kind of qPCR analysis is done and information of what primers will be used should be provided. I would also suggest using newer methods like SWARM or DADA2 for the 16S rRNA gene analyses, since the use of 97% or 99% OTUs is becoming outdated. It should be stated that consent was obtained from the participants. For statistical analyses, I would suggest considering using R, which is more flexible than the programs suggested here for analysing microbial community data. Primer-E is not open source code, which limits the reproducibility of the results for other scientists and it is also not free to use, unlike R.

REVIEWER	Sofia Forslund MDC, Germany
REVIEW RETURNED	13-Nov-2018

GENERAL COMMENTS	This is an exciting and elegant study. You clearly contrast "as usual" with a specified intervention. This formally removes need to track compliance under intervention as well as what exercise "as usual" means, but the resulting limitations must then be clearly stated. Same as with diet tracking; you do not track that so you do not know if the intervention acts through changing eating habits etc. Need to spell this out as a limitation. You need something in the statistics setup to account for whether medication changes during the intervention. You have this data, just need to ensure it is accounted for when testing. One major interesting thing would be 1) differential efficacy in androgen blocker effect, 2) sex hormone conversion in the body,

	3) exercise intervention hormonal impacts. Neither can currently be assessed, and this is OK, but the limitation should be stated. Consider if you can doing a sex hormone panel on the samples also, in which case your scope and power can be extended into these aspects as well. If you do not, this is still valid but limitations need to be stated. Please specify further the androgen deprivation regime esp. if this is not same for all subjects.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. More details about what kind of qPCR analysis is done and information of what primers will be used should be provided.

We would like to provide this information but because the qPCR analysis will be determined based on the 16S amplicon sequence data, it's not possible to predict this. We have deliberately not provided these details, as we don't won't to be restricted to perform certain qPCR before we can make an informed decision based on the microbial community data. As a result, we have added the following text to page 10 to clarify this that reads:

"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."

2. I would also suggest using newer methods like SWARM or DADA2 for the 16S rRNA gene analyses, since the use of 97% or 99% OTUs is becoming outdated.

Since writing this manuscript, we have adopted DADA2 for our amplicon classification and the manuscript has been modified and now reads (page 10):

"Sequence read quality will be initially assessed with FastQC before demultiplexing and preprocessing by Shi7 and/or GHAPv2. Cutadapt will be used for removal of all non-biological sequences. DADA2 is then used for quality filtering, error correction, exact sequence variants (ASVs) picking. A trained naïve Bayes classifier then assign ASVs to genus/species against a curated database of microbial reference sequences such as the RDP40 or SILVA."

3. It should be stated that consent was obtained from the participants.

We have added this statement to the manuscript which reads (page 8):

"Additionally, all participants will be asked to provide informed consent prior to the start of any study activities."

4. For statistical analyses, I would suggest considering using R, which is more flexible than the programs suggested here for analysing microbial community data. Primer-E is not open source code, which limits the reproducibility of the results for other scientists and it is also not free to use, unlike R.

This is a very good point, and we have now consolidated the description of the statistical methodology and included a statement regarding the intended use of open source software, and making any bespoke code publicly available. The added statement reads (page 16):

"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."

Reviewer: 2

1. You clearly contrast "as usual" with a specified intervention. This formally removes need to track compliance under intervention as well as what exercise "as usual" means, but the resulting limitations must then be clearly stated. Same as with diet tracking; you do not track that so you do not know if the intervention acts through changing eating habits etc. Need to spell this out as a limitation.

These have been added as limitations (page 19) and reads:

“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.”

2. You need something in the statistics setup to account for whether medication changes during the intervention. You have this data, just need to ensure it is accounted for when testing.

This has been revised which now reads (page 16):

“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.”

3. One major interesting thing would be 1) differential efficacy in androgen blocker effect, 2) sex hormone conversion in the body, 3) exercise intervention hormonal impacts. Neither can currently be assessed, and this is OK, but the limitation should be stated. Consider if you can doing a sex hormone panel on the samples also, in which case your scope and power can be extended into these aspects as well. If you do not, this is still valid but limitations need to be stated.

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. This has now been stated in the limitation section of the manuscript (page 19).

4. Please specify further the androgen deprivation regime esp. if this is not same for all subjects.

This has been revised in the description of participants which now reads (page 7):

“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.”

VERSION 2 – REVIEW

REVIEWER	Katariina Pärnänen University of Helsinki Department of Microbiology Finland
REVIEW RETURNED	22-Jan-2019

GENERAL COMMENTS	The authors have addressed my concerns adequately and I recommend acceptance. I would like to note that the spelling of the Cutadapt program is wrong in the manuscript main text – "Clutadapt" is wrong and "Cutadapt" is correct.

REVIEWER	Sofia Forslund ECRC, Berlin, Germany.
REVIEW RETURNED	18-Jan-2019

GENERAL COMMENTS	Seems OK now.