Author's response to reviews

Title: A double blind randomized placebo control crossover trial on the effect of dietary nitrate supplementation on exercise tolerance in stable moderate chronic obstructive pulmonary disease

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Version: 3 Date: 6 March 2015

Author's response to reviews: see over
We thank the reviewers for their feedback on our manuscript. The reviewers’ feedback are listed in order below and responses prepared.

Reviewer 1

- Nitrate supplementation is supposed to increase limb muscle function. However COPD patients may well be limited by their breathing so that no benefit was shown with the nitrates.
  o The literature on this is mixed – the discussion has been rewritten
- The effect size was overestimated so that the number studied was not enough to have a chance of showing any benefit
  o Other studies have shown exercise enhancement in COPD but these have had smaller sample sizes – this has been incorporated in the discussion

Reviewer 2

- Lines 84-95: I appreciate the authors’ intent of laying out and rationalizing their hypothesis. In doing so, however, their research hypothesis is lost – that is, this section would benefit from simplification. For example, “hypothesized/hypothesis” is stated 3 times – which is the actual research hypothesis (this can be lost on the reader too easily)? Please simplify this section for reader clarity without losing the important background information being presented.
  o This has been clarified
- Line 95: please clarify primary endpoint. Are you really talking about walk test distance or time to fatigue?
  o This has been clarified
- Data reporting can be enhanced with adding t-value and d.f. – though this is unlikely the convention of the journal. The study design is justified and well executed.
  o No specific guidance is currently available on the style guideline
- Lines 114-115: I’d suggest removing funding source listed in lines 114-115 and place at end of manuscript following typical convention. I see it on line 57.
  o This has been done
- Line 217: Please indicate how many patients were screened during the enrollment period and add this to the associated enrolment figure detailing how many patients were identified and reasons for not including them (declined participation etc…).
  o Corrected: “Between March 2012 and October 2013, 131 eligible participants were identified, 35 of whom agreed to participate in the study”
- I would suggest a diagram/flow chart of testing days/visits to make the methods clearer for the reader.
  o Figure 1 contains a description of testing days and visits and has been clarified
- Line 223: 4 patients were deemed “too fit”. This is not listed as exclusion criteria in the manuscript. Please rectify/better justify this. For example, at what point was it decided to exclude these patients relative to data analysis? My point being that the reader needs to be confident that there were some a priori decision making rules in place for these patients’ exclusion. Line 224: continuing with my note directly above I would suggest there is some confusion regarding the line 224 “measurement of the ESWT is limited to ...15ml/kg/min…”
  1) What is meant/the point of this statement? 2) Is not the VO2max value of 14.9 ml/kg/min
reported in Table 1 approximate to the rationale of 15 ml/kg/min being too fit? As it reads, it is not intuitive for the reader what is the thinking of the authors’ along this line of rationale.

- **Revised**
  - Line 178: please provide rationale/reference for the washout period.
    - This has been clarified (lines 193-194)
  - A majority of the results is dedicated to blood pressure and heart rate metrics (3 tables). I think better rationale about these outcomes is needed in the introduction. Currently there is minimal to none about these outcomes, though they occupy much of the readers attention in the data reporting.
    - Introduction has been altered. One table has been moved to an Additional File (online)
  - Figure 2 legend: I’d suggest reiterating the boxplot boundaries for the reader.
    - Added to text under results, lines 263-4
  - Each Figure Legend seems to have a header and then description. I’m not sure if that’s convention for the journal. I’d suggest merging these 2 descriptors to 1 succinct legend description.
    - This is convention for BMC Pulmonary Medicine (http://www.biomedcentral.com/bmcpulmmed/authors/instructions/researcharticl e, section: Figure legends)
    - Figures 2 and 3 have been merged to Figure 2
  - Figures 2 and 3: use the same axis labels between the 2 figures and arrange data in same order (Fig 2 BR then PL; Fig 3 PL then BR). Units needed on Y –axis on Fig 3.
    - Figures 2 and 3 merged into a new Figure 2
  - Lines 51-53: I’d suggest reporting a conclusion linked to the data findings, not the future directions.
    - The discussion has been rewritten to reflect this
  - “Major Addition” Needing Attention: I believe the discussion section would strongly benefit from discourse related to potential physiology that may explain the study’s negative finding. Why was there no “functional” effect of dietary nitrate on exercise performance? An elegantly crafted section along this line would benefit future research. Specifically, the introduction highlights the favorable NO effect of dietary NO3- — this suggests the authors’ expected a potential reversal in adverse exercise blood flow to be an important/primary modulating factor for exercise limitation in COPD. This notion would benefit from stronger rationale for this underlying physiological rationale for NO3- supplementation and, consequently, a measured discussion addressing the lack of exercise benefit in this light (that of exercise vascular benefit in COPD – and if the authors’ believe this was/wasn’t the case, or if this can even be supported by the data)
    - Added to discussion, but note that introduction and discussion have also been enhanced

Reviewer 3

- The research question posed by the authors was well defined, although they did not incorporate the results of at least 3 recent (smaller N) RCTs on dietary nitrate supplementation in COPD in their introduction or discussion [Kerley et al., Nitric Oxide, 2015, PMID: 25534960; Shepherd et al., Nitric Oxide, 2015, PMID: 25596150; and Berry et al., Nitric Oxide, 2014, PMID: 25445634].
These trials and their implications have been discussed in the Discussion (the reporting of these trials occurred after the completion of our study).

The methods (RCT) and sample size were appropriate and well described; however, failure to measure changes in circulating nitrate and nitrite concentrations represents an important limitation of the present study, particularly as it relates to performing 1) a priori correlative analyses between the post-dose change ESWT distance and each of plasma nitrate and nitrite levels and 2) post-hoc responder vs. non-responder analyses (e.g., were those patients whose ESWT distance improved by the MCID also those whose plasma nitrate and nitrite levels increased to the greatest extent?).

This is an important shortcoming of our study, and a section has been added to the discussion to address this.

The authors are encouraged to elaborate on the proposed mechanism(s) of action of NO in COPD in their introduction and discussion, particularly as it relates to the potential for dietary nitrate supplementation to improve exercise performance by improving the proximate source(s) of exercise intolerance in COPD, including 1) abnormal ventilatory, breathing pattern and operating lung volume responses to exercise and 2) abnormalities in peripheral locomotor muscle contractile and metabolic function.

The introductory remarks on NO/NO3 physiology have been expanded, as has the discussion.

A section has been added to the discussion.

Overall, this was a well-designed and clearly (succinctly) written paper. Unfortunately, the results do not support a role of acute dietary nitrate supplementation in the clinical management of exercise intolerance and activity-related dyspnea in patients with moderate COPD. The authors are encouraged to make a statement to this effect in the conclusions of their abstract and discussion, which (despite the negative study results) currently read as a call for future research.

The discussion has been revised to reflect this.

Abstract

Methods: include information on the dose of BR used, the duration of the intervention and the length of the washout between treatments.

§  Have altered

Results, Lines 49-50: “...11% and 6%, respectively; however, these differences did not...”

§  Have altered

Conclusions: your conclusion is too disconnected from your study results and is a bit of a tangential call for future researcher. Consider something like: “The results of this RCT do not support a role for acute dietary nitrate supplementation in the management of physical activity limitation in patients with GOLD stage II COPD.”

§  Discussion has been revised

Introduction

Line 68: “...social burden: it is estimated that 64 million people...”

§  Done

Line 75: ..., and improves submaximal exercise endurance ...

§  Done

NO levels are altered in COPD patients, see review by Malerba et al., 2014

§  The review by Malerba et al, 2014 (PMID: 24719850) is interesting: it is unclear whether NO levels per se are altered, as direct measurement of NO is difficult and FeNO levels are ‘contradictory’. It is clear that NO dynamics
are altered (by differing NO synthetase levels). And whether nitrate supplementation alters NOS dynamics is an intriguing question.

- Consider also the results of the following recent published manuscripts on dietary nitrate supplementation in COPD:
  - Kerley et al., Nitric Oxide, 2015, PMID: 25534960
  - Shepherd et al., Nitric Oxide, 2015, PMID: 25596150
  - Berry et al., Nitric Oxide, 2014, PMID: 25445634.
    - These have been incorporated in the text

- Line 87: define abbreviations (i.e., NO3-, NO2- and NO) on first use.
  - Done
- Lines 93-95: be more specific; for example, “...that 3 days of dietary nitrate supplement as beet root juice (0.6 g/day) ...”
  - Done

**Methods**

- Why did you limit your patient population to GOLD II (moderate) COPD only?
  - Added under ‘participants’
- Were any of the patients taking vasodilators?
  - No

**Results**

- From Figure 3, it appears as though 1 participant drastically decreased their walking distance (from 2000 to 500 m?), and another drastically increased their walking distance (from 700 to 2100 m?). This seems fairly extreme. Did you consider an outlier test? Did these patients exacerbate during the trial? Were HR and Borgs comparable between PL and BR in these patients? In other words, did these patients give their maximal efforts on both visits 3 and 4?
  - Excluding these participants did not change the primary endpoint of the study.
  - Medications and clinical status was recorded, these participants were not exacerbating
  - Participant 8: 2090m on placebo to 690m on BR: Borg 3 on both (no change), HR 120 on placebo and HR 110 on BR.
  - Participant 18: 840m on placebo to 2210m on BR: Borg 3 on both (no change), HR 130 on placebo, HR 115 on BR.
  - Heterogeneity seems to have been a problem in Berry et al’s study, and comment has been made on this in the discussion

- Table 1: FEV1/FVC (not FEV1/VC)
  - Rectified
- Table 1: define VO2max in list of abbreviations.
  - Rectified

**Figures**

- Figure 3 requires a Y-axis label
  - Rectified
- Figure 2 and 3 could be combined into a single figure with 2 panels since the data are complementary. In fact, Figure 3 (which shows individual subject data) should precede Figure 2 (which shows mean data).
  - Combined: now Figure 2a comprises individual subject data and Figure 2b has mean data.
• Discussion
  o Line 278-279: expand this discussion point. How are NO levels different in COPD patients relative to their healthy age, sex and physical activity matched counterparts
    § This line initially concerned measurement of NO3/NO2. The discussion has now been rewritten.
  o What’s the proposed mechanism of improved exercise tolerance following BR supplementation?
    § This is briefly covered in the new introduction
  o Line 306: The ESWT is more sensitive than the 6-minute walk test during pharmacological intervention (Pepin et al., 2010).
    § Integrated into discussion under ‘study design’ lines 314-317
  o COPD is a disease of old age. As such, consider the results of Kelly et al. (Am J Physiol Regul Integr Comp Physiol. 304: R73-83, 2013) in your discussion.
    § Discussion has been rewritten

• Study limitation: is it possible that the dose was too low? How can you explain your results relative to those recently reported by Kerley et al. 2015, where they reported significant improvements in ISWT after a single dose administration of BJ (12.9 mmol nitrate content) in COPD patients? Please also consider the recent findings by Shepherd et al. 2015 and Berry et al. 2014.
  o Discussion rewritten

• Lines 330-333: these statements are not referenced. At the very least, consider using the recent ATS statement by Maltais et al. on peripheral muscle dysfunction in COPD.
  o Removed

• Conclusion: Your “Conclusion” should be remained “Future Directions” and you should consider writing a conclusion that is commensurate with your study results (see above).
  o Conclusion rewritten