Author's response to reviews

Title: Nitrates and Bone Turnover [NABT]: trial to select the best nitrate preparation.

Authors:

  Roxana C Bucur (roxana.bucur@wchospital.ca)  
  Lauren S Reid (lauren.reid@wchospital.ca)  
  Celeste J Hamilton (celeste.hamilton@wchospital.ca)  
  Steven R Cummings (scummings@sfcc-cpmc.net)  
  Sophie A Jamal (sophie.jamal@utoronto.ca)

Version: 4 Date: 15 April 2013

Author's response to reviews: see over
MS: 3996909718079803
Nitrates and Bone Turnover [NABT]: trial to select the best nitrates preparation
Roxana C Bucur, Lauren Reid, Steven R Cummings and Sophie A Jamal

Please find below the response to reviewers comments

Scientific Editor's Comments:

1. "The authors are to be commended for the use of headings as a framework around which to describe details of the study. In some places, however, the third level headings (those posed as questions) detract somewhat from the readability of the paper in that the text is very broken up. The authors may wish to consider rephrasing the questions and turning them into the first sentences of paragraphs where appropriate to improve flow whilst maintaining the ordered structure.

We have substantially reorganized and reduced the number of subheadings throughout the manuscript

2. Please add the study setting to the abstract".

The study setting was added to the abstract (page 1).

3. Editorial requests:
   1) Please ensure the title conforms to journal style for study protocol articles. The title should follow the format __________: study protocol for a randomized controlled trial.?

Editorial requests:1) Please ensure the title conforms to journal style for study protocol articles. The title should follow the format __________: study protocol for a randomized controlled trial.?

We have changed the title as requested (page 3).

Reviewer #1: Lars Rejnmark

1) Section 1.6. “Risk to the safety of the participants involved in the trial”. This section reports that “The only risk in this trial is headaches”. According to the summary of product characteristics (SPC) of investigated nitrates, several other side effects are common during treatment with nitrates. This should be acknowledged in this section. As nitrates may cause postural hypotension, it may be of importance to systematically collect data on episodes of falls, as this may compromise potential beneficial effects of nitrates on bone.
We have added a section (page 12, paragraph 1) noting the SPC. We will be asking about these and other adverse events during follow up telephone calls. We have stated this explicitly in our protocol (page 12, paragraph 3).

3) Section “2.1 What is the proposed trial design?” The study is designed as an open-label randomized trial comparing 5 formulations of nitrates for their effects on bone turnover markers and headache. However, it is stated that a placebo group (using placebo ointment) also is included. As the study is open-labeled, those in the placebo group are presumably aware of the fact that they are being treated with placebo. Compliance to a known ineffective treatment may be worrying. Recruitment of participants to the placebo group is not stated (randomly selected from all eligible women?).

We have clarified this point; the women randomized to ointment preparation are blinded to whether they received the active NTG or placebo. They will be unblinded, if wish, upon study completion. The information was added in the manuscript.(page 11, paragraph 4)

4) Section 2.5 “Planned inclusion/exclusion criteria” Subjects with a history of angina or cardiovascular disease are excluded. It should be specifically stated whether women with mild hypertension are excluded. This is of importance as they may be treated with beta-blocking agents or ACE-inhibitors which may interact with nitrates (potentiate hypotensive effects).

Subjects diagnosed with mild hypertension are not excluded from the trial. At the first visit, all prescription medications will be noted and if needed we will perform additional analyses to investigate potential drug interactions.

5) Section 2.7 “What is the proposed frequency and duration of follow up? – treatment phase”. It is stated that daily intake of calcium and vitamin D will be assessed and supplementary calcium and vitamin D (CaD) will be provided to women with a daily intake below recommendations. This may turn out to bias study results. Presumably, bone turnover in those with a low daily intake of CaD is higher than in women with an adequate intake. Accordingly, providing supplement with CaD to those with a low habitual intake may itself lower levels of bone turnover markers. The investigators should consider one of two solutions: A) during randomization, women who are in need of extra CaD should be equally allocated to the different study groups. B) Women with a low daily CaD intake should receive supplements with CaD for at least 3 months before entering the study.

We recognize that this as a limitation of the study and may bias our findings against the effects of the nitrates. However our findings will be more generalizeable (we will be able to estimate the effect of nitrates together with calcium and vitamin D). Also, we are randomly assigning treatments and do not expect systematic bias based on calcium and vitamin D intake. Finally, we
will perform a sensitivity analysis at the end of the study to account for baseline differences in calcium and vitamin D intake.

Minors:

6) Abstract: The duration of the treatment period for the 210 women included in the treatment phase of the trial should be specifically stated.

The duration of the treatment was stated in the abstract.(page 2)

7) The section “1.4 Relevant Prior Studies. a. In vitro studies” – this section mainly reports sources of NO, but not the results from published in vitro studies on bone – only a reference is stated. Either the heading of the sub-section should be changed or results from cell studies should be reported.

N/A as we have modified our subheadings.

8) The section “b. In vivo animal studies” – The first part of this section actually reports results form in vitro studies – should be moved to the section on in vitro studies.

See above.

9) Section 1.3.e.iv: the heading of this section is “Headaches” – the last part of the section reports, however, result on BMD from a RCT – this should be moved to the section on effects of nitrates on BMD. It is stated that there has been a “personal communications from members of the Data Safety Monitoring Board” –in order to improve transparency, name of those providing this communication, as well as date of communication should be stated. This is in accordance with acknowledging the names of the statisticians helping with sample size calculation(later in the MS).

We have moved this section (page 9, paragraph 4) Unfortunately we do no have the names and dates for the DSMB we would be happy to delete or modify this section based on editorial preference

10) The section on trial management may not be of that much interest to the general reader. Should be shortened substantially.

Section 4 (previous section 3) Trial Management was shortened.

11) Figure 2. Explanations on the X-axis are missing.
Please find enclosed Figure 2 revised with the explanations on x and y axes

12) Tables included in the supplementary file: references are missing (It should be stated which papers the tables have been published in)

Please find enclosed revised Trial appendices with the references inserted for the tables.

Reviewer #2: Morten Karsdal

1) The experimental trials look very promising. I look forward to see that. Please remember that it is absolutely pivotal that participants provide morning fasting blood samples, as bone resorption markers are affected by approximately 50% by food intake.

We are aware of the effect of fasting on bone markers. The participants will be asked before the first and last visit (when blood is drawn) about fasting. If the fasting requirements are not met, the blood will be drawn the next day.

Sincerely,

Sophie A Jamal, MD, PhD, FRCPC
Associate Professor of Medicine, University of Toronto
Research Director, Multidisciplinary Osteoporosis Program, Women's College Hospital
Scientist, Women's College Research Institute