Reviewer's report

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

Version: 3 Date: 10 March 2013

Reviewer: Lars Rejnmark

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Nitrates may improve bone structure and strength. Using an open-label randomized design, this investigation is a pilot (dose-finding) study aiming to identify the nitrate formulation/dose which has the greatest efficacy (as assessed by measuring bone turnover markers) and gives the least headaches. The results from the study are supposed to be used in a further randomized trial assessing whether treatment with nitrates may prevent osteoporotic fractures.

1) The introduction summarizes available studies on possible effects of nitrates on bone. Data from clinical and epidemiological studies suggest beneficial effects of treatment with nitrates on bone remodeling, bone structure and density which may result in a decreased risk of fractures. Overall, it seems that nitrates may function as a dual-acting agent causing a decreased bone resorption with a concomitant increased bone formation. This is in contrast to most other anti-osteoporotic drugs as both bone formation and resorption is decreased in response to treatment with antiresorptives whereas both are increased in response to treatment with bone formative agents. Treatment with strontium ranelate causes, however, effects on bone turnover markers similar to those reported in response to nitrates. This dual-acting effect of strontium on bone turnover markers does, however, not result in a larger reduction in risk of fractures than treatment with antiresorptives. Although no head-to-head comparisons are available, it may be that the effect on levels of bone turnover markers is more pronounced in response to treatment with nitrates than during treatment with strontium, and it seems, therefore, reasonable to study further whether such dual-acting effects in response to nitrates may be of importance.

2) 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.

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?).

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).

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.

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.

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.

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.

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

11) Figure 2. Explanations on the X-axis are missing.

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

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests