Reviewer's report

Title: Low-dose menaquinone-4 improves gamma-carboxylation of osteocalcin in young males

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Reviewer: Katharina Nimptsch

Reviewer's report:

This is a nicely written manuscript describing the findings from a small dose-examination study, in which 15 healthy volunteers received daily Menaquinone-4 (MK-4), with weekly increasing doses. Authors observed that compared with baseline, gamma-carboxylation status of the vitamin-K-dependent bone protein osteocalcin improved at MK-4 doses of 600 µg/day or higher. It is a limitation of the study that it is no randomized placebo-controlled trial, but as I understand from the discussion, such a study design will be the next step of the research group. Authors state that 600 µg/day is a dose that can be achieved with dietary intakes, at least in Japan (with consumption of the fermented soy product natto, with is a rich vitamin K source). My main concern with the manuscript is that the conclusions drawn from the study do not always reflect the study design (i.e. no placebo-controlled trial). My specific comments are:

Major Compulsory Revisions

(1) The exact role of osteocalcin in bone health is not completely understood. This should be recognized in the introduction and/or discussion.

(2) Introduction, page 5, line 37: A larger dietary reference intake may be beneficial not only for a better carboxylation status of osteocalcin but also for other vitamin K-dependent proteins. Please refer to these briefly in the introduction.

(3) Introduction, page 5, line 44: Please state why finding the lowest dosage of supplemental MK-4 was the aim of this study. Is there a concern of toxicity?

(4) Methods, page 7, line 63: Were the blood samples fasting? If not, was fasting status accounted for?

(5) Results: Dietary vitamin K intake and serum phylloquinone as well as MK-7 decreased substantially during the study period. Do authors have an explanation for that? This should be part of the discussion.

(6) Results, page 9, lines 88-89: Please rephrase the sentence; it cannot be known whether the higher serum MK-4 is due to the supplementation with 1500 µg/day or due to the MK-4 supplementation in the preceding weeks.

(7) Dietary vitamin K intake was exceptionally low and it is unclear why dietary vitamin K intake decreased (could this be due to a decrease in the participant’s motivation to answer the questions on vitamin K-rich foods week by week?). Also, in table 2, the lower range is 0, which is practically impossible. While
weekly dietary records are a good dietary assessment instrument, the calculation of vitamin K intake only from vitamin K-rich foods has limitations. Using the dietary record data in combination with a food composition database with vitamin K values on various foods, including meat and meat products, would improve the dietary intake data. Please also give some information on the participant’s compliance in filling out the dietary records. In addition, please give more information on the face-to-face interviews on the amounts of vitamin-K rich foods participants had consumed each day (i.e. how were amounts specified; how many missing values?). Adding the questions on vitamin K-rich foods as a supplement may be helpful. Also, please specify which food composition database was used for the estimation of dietary vitamin K intake.

(8) Discussion: first sentence does not reflect observations. From the study design it cannot be known whether a one-week daily dose of 600 µg/day is effective, since it was preceded by one week of 300 µg/day.

(9) Please briefly discuss the advantages/disadvantages of using absolute ucOC or GlαOC versus UGR.

Minor Essential Revisions

(1) Methods: Please state which software was used for analysis.
(2) Discussion, page 10, lines 102-104: Please move to results section.

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