Author's response to reviews

Title: Postprandial effects of calcium phosphate supplementation on plasma concentration-double-blind, placebo-controlled cross-over human study

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Author's response to reviews: see over
Revision Letter

Dear Editorial Board,

Thank you for reviewing the article “Postprandial effects of calcium phosphate supplementation on plasma concentration-double-blind, placebo-controlled cross-over human study” (MS: 2044406486865323). Listed below are the point-by-point replies to the reviewer’s comments accompanied by explanations of the changes in the manuscript.

Yours sincerely,
Dr. Gerhard Jahreis

Reviewer 1

There are, however, several issues that need to be dealt with, but which should present no difficulties for the authors.

• First, although the authors provide information with respect to the total daily load of calcium phosphate, and the Tables give the load size for the oral tests, both the text and the Abstract are silent on the size of the calcium or phosphorus loads used for the single test dose for which they were able to assess the time course following ingestion. This is a vital piece of information and is essential for interpreting the results as well as for comparing with the reports of other studies, as in the first paragraph of the Discussion section.
We thank reviewer 1 for pointing out this important aspect. In the abstract and in the material and methods section (line: 23, 73) the amount of additional calcium is given as approximately 1g/d. In Table 2, we have added an additional row for the real intake of calcium and phosphorus from the CaP supplement after analysis.

• The calculation of the AUC for both calcium and phosphorus is incorrect, inasmuch as the AUC should be based on the increment/decrement above/below baseline, not on the total concentration of calcium or phosphorus. The authors can see this very clearly when they contrast the fact that there was a significant elevation in serum calcium and a significant decrease in serum phosphorus; yet if they compare the AUC values between placebo and calcium phosphate, I suspect they would find no significant difference, i.e., using AUC only they would have had to report no effect for either serum calcium or serum phosphorus. On the other hand, I predict that the incremental AUCs will differ significantly, and therefore, will contain important information about the response. The current AUC illustrations should be revised.

We calculated the AUC from 0 to 240 min for the increment in plasma calcium and phosphate and revised the illustrations in Figure 3.

Minor Comments

• Phosphorus is misspelled throughout the manuscript.

The misspelled words have been revised (line: 204, 206, 370)

• The authors omit an important, if not actually demonstrated, mechanism for the hypophosphatemia following ingestion. That is the secretion of calcitonin which follows from release of gastrointestinal hormones upon ingestion of a meal. This would have produced an immediate cessation of osteoclast work, and hence a fall in release of phosphorus into the blood from bone. The calcitonin effect is likely the cause for the phosphorus decrement in the placebo group. That, and the immediate increase in renal phosphorus clearance that accompanies a fall in PTH, would be predicted to lower serum phosphorus concentration appreciably. This strikes the reviewer as substantially more plausible than hydration, which, had it been the cause
of the drop in serum phosphorus, should have produced a corresponding drop in red cell count.

_We are grateful for this comment. We have now included this possible mechanism in the discussion section (line: 214-222)._

• The paper would be enhanced if the authors would calculate and present the Ca × P ion product at all time points around the test meal.

_This is a good point. We calculated the calcium-phosphate product and it is presented in the results section (line: 167-179)._ 

• Although the authors quote a study by Heaney with respect to phosphorus absorption, they might also wish to look at the papers by Rafferty et al. (J Food Sci 72:152-158, 2007) or Heaney et al. (Calcif Tissue Int 46:300-304, 1990). Both contain additional information about measured phosphate absorption.

_Thank you for suggesting these two publications. We incorporated them in the introduction section (line: 62-65)._ 

Reviewer 2:

Major Compulsory Revisions
1. By the participants screening, 30 people were excluded. The authors should specify the study criteria in "Methods".

_We now specify the study criteria in lines 82/83. For the information of reviewer 2, it was necessary that the participants stay at the blood withdrawal center for at least 5 hours. The 30 excluded interested persons could not manage this study criterion._ 

2. For the purpose of preventing osteoporosis, calcium is often taken by aged people, especially by aged women. The subjects of the present study are, however, young men. Can the present method be a preventive measure against osteoporosis
for older (female) people? The authors should add some descriptions about this matter by citing literatures, or their original speculations.

This is a valid point. However, the aim of the present study was to evaluate the short term effects of a single and repeated administration of calcium phosphate in general. Therefore, we used participants in good health and this is more likely the case with young participants. Because of the elaborateness of the study, we could only manage 10 participants. So a mixed study population was not preferable. We chose men since they do not undergo a menstrual cycle.

To obtain information regarding the effect of calcium phosphate on the metabolism of postmenopausal women, it is necessary to create and undertake a new study.

Discretionary Revisions
1. If possible, the amounts of milk intake during the washout period should be added, since milk may affect the absorption of calcium.

   Thank you for this point. In the wash-out period, the participants could eat whatever they wanted, despite dietary supplements. Hence, we have no information about milk or milk product intake during this period. In the defined diet, we provided one milk product for the participants, but no milk.

2. In the present study, calcium was incorporated in bread. Comparison with precedent studies employing other forms of calcium (e.g., tablet, granule) concerning bioavailability should be added to "Discussion", if possible.

   In the first part of the discussion we compared our results with other studies. To facilitate a comparison, we added the calcium source used in these studies (line: 189, 192).