Reviewer’s report

Title: Effects of cow's milk beta-casein variants on symptoms of milk intolerance in Chinese adults: a multicentre, randomised controlled study

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Reviewer: Michael de Vrese

Reviewer's report:

As I wrote in my previous e-mail, the paper of Mei et al. shows, with good power/a sufficient number of participants, that cows's milk containing little of the A2-variant of the beta-casein causes less gastrointestinal complaints than conventional milk containing also the A1 variant in Chinese persons with self-declared lactose intolerance. As this result is founded by solid statistical quality, and considering that there are only few human studies addressing this topic, the paper should be published after revision.

My main criticisms include:

1. The title of the manuscript „Effects of ....... on lactase activity and symptoms of milk intolerance ......." should be reformulated more cautiously, as neither the lactase activity was measured (correctly) nor the causes of the gastrointestinal complaints have been investigated, as I will explain later. In fact, only the „Effects of .... on gastrointestinal discomfort after milk consumption ...." were shown.

2. The first publication ("Devil in the Milk") of Keith Woodford on the topic of „A1 versus A2 beta-casein (according to which BCM7 from A1-beta-casein promotes autism and possibly heart disease&diabetes in subjects with leaky gut syndrome) has led to a global, ideologically colored discussion about the harmfulness of „A1-milk". Since this discussion also always involves conclusive force, methodological weaknesses, or contradictory recommendations (e.g. WAPF AdHoc Committee2009: „Leaky gut people should avoid BCM7 until more is known"; EFSA 2009: Based on the present review of available scientific literature, a cause-effect relationship between the oral intake of BCM7 or related peptides and aetiology or course of any suggested non-communicable diseases cannot be established. Consequently, a formal EFSA risk assessment of food-derived peptides is not recommended.), the authors should include this previous and possibly also more current discussion more strongly in their introduction and / or discussion.

3. The study milk products are not sufficiently described. There is no information whether only the A1 and A2 variants were measured, or also their mutations B, C, F, G (A1) and A3, D, E (A2). In other words, it is not known, whether the milks actually contained only the A2 or A1
+ A2 variants of beta casein, whether the total A1 + B, C, F, G and A2 + A3, D, E activity was subsumed under A1 and A2, or whether part of the A1- and A2 like activities were "suppressed".

The information on the breeds of cattle from which the milk was obtained would also be helpful.

Finally, the authors should explain at some point, how they have avoided a possible BIAS based on the fact that (almost) all A1/A2 human studies have been funded by A2 milk producers.

4. Though the number of participants is indeed sufficiently large (particularly for a nutrition study), but the sample size determination should be described in more detail and with precise reasoning: please give the underlying (primary) parameter plus accompanying mean and deviation plus reference for these data. The data from the reference quoted (Jianqin et al.) refer, amongst others, to GI inflammation, which was not studied here.

5. The selection of the gastrointestinal symptoms studies by means of VAS should be explained: though all of them can occur in lactose-/milk intolerance, the underlying causes may be different ones (gas production, water inflow/osmosis, peristalsis, increased pain sensitivity, inflammation?), while in part different symptoms may derive from the same underlying causes. Therefore, the symptoms do not have the same value with respect to the characterization of the A1/A2 effects. Therefore a primary parameters should be defined and/or the symptoms should be combined, e.g. by means of multivariant methods.

The graphic presentation of one or several (combined) symptom scores or rat least a primary parameter in the RESULTS section would be better and mor informative than many (somewhat too complex and confusing) tables.

Were the participants and/or the personell in charge trained for the VAS techniques?

Were the questionnaires validated?

6. How is beta-casein A1/ BCM7 supposed to have a negative effect on gastrointestinal symptoms: reduction of lactase activity (not measured correctly, see below), prolongation of gastrointestinal transit time (not measured, see below), promotion of gastrointestinal inflammation (suitable markers were not measured, see below).

7. While the study shows a clear positive effect of A2 milk on gastrointestinal symptoms, answers to the other questions raised in title and introduction as well as further conclusions concerning the causes of acute beta-casein A1 (or rather BCM7) effects; links to lactose intolerance/ lactase activity; solving of contradictory theories or findings in the frame of the worldwide ongoing "A1/A2 milk" discussion can unfortunately not be drawn from this study, due to methodological reasons.
8. The major methodological weaknesses of this work is the inappropriate measurement of lactase activity by measuring the pp. galactose concentration in a single urine sample. The galactose concentration here depends not only on the amount of galactose absorbed (and thus the lactase activity), but also on the flow rate of galactose and the amount of excreted urine. Moreover, lactose/galactose malabsorption is defined in this paper as "an increase in the urinary galactose concentration of <0.27 mmol/L and normal galactose absorption was defined as an increase of ≥0.27 mmol/L......". This differs from the usual definition in "Western countries", so it would be desirable that the authors explain how this value was validated (by them or in the literature). This methodological weakness might explain why in the study 170 (28.3%) and 430 (71.7%) of subjects at all three sites combined were classified as lactose absorbers and lactose malabsorbers, respectively, while it is generally believed that nearly 100% of the (South) East Asian population are lactose maldigesters.

More appropriate would be the determination of the total quantity of galactose in 24(12)-h urine collections or the measurement of the course of serum galactose concentration.

9. In view of the many factors involved in lactose digestion and lactose(milk) intolerance (e.g. lactase activity, gut microbiota, formation of gas, osmotic effects, gut motility, subjective perception of pain, differences between (South) East Asian people and North / Central Europeans and descendants, etc.) and in view of the European / American readership, which is not so familiar with the Chinese situation, the study participants should have been more fully characterized before and during the experiment using (more) appropriate parameters and methods:

- **Lactase activity:** genetic and/or biochemical methods
- **Lactose(mal)digestion/-absorption:** pp serum galactose/-glucose
- **Undigested lactose+gut microbiota:** H2-breath test
- **Gut microbiota**
- **Gut motility** transit time
- **Immunity/inflammation** molecular markers; endoscopic examinations

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