Author’s response to reviews

Title: Hypocaloric diet supplemented with probiotic cheese improves body mass index and blood pressure indices of obese hypertensive patients - a randomized double-blind placebocontrolled pilot study

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Author’s response to reviews: see over
COVER LETTER and Answers to referees comments

Thank you very much for the thorough work with our manuscript and constructive criticism. We try to provide you with the necessary answers.

Q1 The composition of the product and the placebo concerning the probiotic strain but also the other strains (i.e. “cheese” or “starter” strains) should be provided (to properly interpret results and ascribe them to the probiotic itself, the reader should know that the composition of the probiotic and control products differed only for the probiotic strains. One may imagine that adding a strain may interfere with the others).

Answer:
This paragraph Page 6 describing the probiotic and control cheese has been rewritten.

p.6 in “Cheese preparation” please change the text omitting In brief, ..... for Harmony® [22]. Two similar cheeses were prepared on the basis of regular Edam-type cheese with a starter C92 (CSK Food Enrichment, Netherlands). To produce the probiotic cheese the *L. plantarum* TENSIA was added to the cheese milk in amounts of 1.5·10^{11} CFU/g before renneting. Both cheeses (probiotic and control) were similarly ripened for 4 weeks at 10-12°C and 80-85% relative air humidity. The microbial composition of test and control cheeses did not differ in the counts and prevalence of non-starter microbiota. In probiotic cheese the viability of *L. plantarum* TENSIA was assessed before the human trial. The energy provided by consumption of 50 g of each cheese was 175 kcal. The composition and energy value (1512 kcal) of a standard hypocaloric diet is depicted in Table 1.

Q2 What was the statistical hypothesis of this trial? How was the number of subjects calculated when designing the trial? What was the primary endpoint for this calculation? What was the mode of randomization? Was the disequilibrium in number of probiotic and control subjects planned?

Answer:
In our pilot study the hypothesis was that probiotic cheese comprising *L. plantarum* TENSIA under the hypocaloric diet reduces significantly some markers of metabolic disease. The changes in the manuscript have been performed.

p.6 in “Study population” the change has been made (yellow highlight) The number of expected hospitalized patients (approximately 40 patients for 3 months) relevant to inclusion criteria was postulated.

p. 7 in “Study population” the change has been made (yellow highlight) The primary outcomes were defined as a significant (p<0.05) decrease of arterial blood pressure and a significant (p<0.05) decrease of body mass index (BMI).

p. 7 in “Study population” the change has been made (yellow highlight) Study participants were randomly allocated according to SPSS 17 for Windows into two groups. The 40 patients were randomly divided into treatment and control groups according to the mode: 2 patients of treatment group vs. 1 patient for control group (Fig.1).
Please add a sentence in “Study protocol”

p.8  We have added to p.8 a description of the method for body composition

The body composition, e.g. fat and muscle mass and total body water content were estimated by bioelectrical impedance analysis (InBody 720, Korea).

Q4 Excluded subjects should be presented in the result section and not in the statistical method.
Answer:
We have made the change from p.12 “Statistical evaluation” to p.13 for “Results” section

p.13
Spearman’s partial correlation analyses for both control and TENSIA groups combined was applied to 36 individuals because the urine samples were missing in 4 controls

Q3 What were the questions and hypothesis behind studying body water content and markers of protein catabolism?

Answer:
It was essentially expected that the low calorie diet should intervene into all three main branches of human metabolism and the body composition: carbohydrates, lipids, proteins and water content to lower the BMI and help in treatment of metabolic syndrome. The effect in carbohydrate metabolism was shown by 18% reduction of blood glucose similarly in test and control groups; the beneficial impact on lipid metabolism was shown by reduction of cholesterol and its components, still; in protein metabolism the shift in increased urea content of blood was seen only in test group, still without statistical relevance compared to control values. Besides, the range of increased content of urea was in normal boarders. That there were no profound changes in protein metabolism shows the stability of uric acid level.

In „Results“ section we rephrased the results of body composition depicted in Table 4.

Concerning the body composition, a decrease of the total water content (p=0.001) was found only in the probiotic group, however, the data of water, fat and muscle mass and waist-to-hip ratio did not differ between probiotic and control groups.

Q5Q6Q8 Water loss explains the difference between the probiotic and control. What was the % of subjects receiving diuretics in both groups?
What is the relevance for obese subjects to lose water at three weeks of consumption?

The authors interpret this as “quite promising” and this should be discussed more critically (for example, can one predict if the effect might persist on a longer term basis than 3 weeks). The health consequences of the excess of markers for protein catabolism with this food product should be discussed (potentially deleterious?).

Answer:
The moderate water loss has been considered an important effect in treatment of metabolic syndrome as much as the treatment of high blood pressure (one component of metabolic syndrome) has been always performed in 3 main directions: ACE inhibitors, CA channel blockers and diuretics In Table 2 it has been shown that in probiotic group the diuretics were administered to 2 patients (8%) and in control group to 1 (7%, the percentages not shown ). Thus the DIURETIC treatment was similar (Table 2).
We have added in “Discussion” section of manuscript a paragraph: … with some starvation.

It is possible that higher counts of lactobacilli in patients consuming cheese with *L. plantarum* Tensia caused the decrease of the pH of gut. This could be accompanied with some increase of protein putrefaction and blood urea content, previously described in experimental animals and metabolic surgery [54-56]. However, in our patients the increased urea values were still kept in normal ranges and also no shifts in uric acid were detected relevant for excess of protein catabolism towards health impairment. Moreover, the functionality of kidneys and liver was not altered by the 3 weeks treatment with hypocaloric diet and probiotic cheese. The possibility to prevent water retention and hypertension with the hypocaloric diet supplemented with probiotic cheese seems worth larger clinical and translational studies.

**Q** Does the strain has diuretic effect? or what could be the mechanism for losing water content, and what could be done to progress in this knowledge (main result of the present trial) in future studies?

**Answer:**
The diuretic effect of the strain has not been studied yet. In future we plan to perform a well-designed clinical study with a large list of biomarkers.