Randomized control trials

A combination of acid lactase from *Aspergillus oryzae* and yogurt bacteria improves lactose digestion in lactose maldigesters synergistically: A randomized, controlled, double-blind cross-over trial

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**Article info**

Background & aims: Lactose digestion can be improved in subjects with impaired or completely absent intestinal lactase activity by administration of lactase preparations and particularly of acid lactase, which is active in the stomach, or by yogurt containing live lactic acid bacteria. It is the question, if lactose digestion can be further enhanced by combining these two approaches.

Methods: We investigated in a randomised, placebo-controlled, double-blind, 5-arm crossover study on 24 lactose malabsorbers with variable degrees of lactase deficiency if different lactase preparations and freeze-dried yogurt culture affect gastrointestinal lactose digestion after consuming moderate amounts of lactose (12.5 g) by assessing hydrogen exhalation over 6 h. Furthermore, symptoms of lactose intolerance (excess gas production, abdominal pain, diarrhoea or nausea) were assessed using validated questionnaires.

Results: All preparations increased lactose digestion and reduced peak hydrogen exhalation by /C0 27% (yogurt), /C0 29% (3300 FCC units lactase), /C0 33% (9000 FCC units lactase), /C0 46% (3300 FCC units lactase plus yogurt culture combined), as compared with placebo ( /C0 p < 0.001, Friedman test). The combination preparation had not only the strongest effect, but also showed the lowest variance in H2-exhalation values (less malabsorbers with no reduction of H2-exhalation) Apart from this, both the higher dose lactase and the combination preparation significantly reduced the symptoms most closely associated with H2-exhalation, namely flatulence and abdominal pain, respectively.

Conclusions: The combined administration of freeze-dried yogurt cultures and acid lactase increases lactose digestion more than either freeze-dried yogurt cultures or acid lactase alone, and more lactose malabsorbers benefited from this effect.

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**Abbreviations:** FCC, food chemical codex; cfu, colony forming units.

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1. **Introduction**

Primary lactose malabsorption, which is accompanied by a continuous drop in lactase activity during childhood and adolescence and hypolactasia in adulthood, is the normal physiological process in humans. If gas production due to microbial fermentation of undigested lactose and/or increased water retention in the intestine through osmotically active substances (lactose, short-chain fatty acids) lead to intestinal complaints, such as flatulence, "wind", pain, osmotic diarrhea and other unspecified symptoms, one uses the term (primary) lactose intolerance. [1–3]

In the case of lactose intolerance the common therapeutic approach tends to exclude milk products from the diet [4,5], despite the fact that this may have nutritional disadvantages [6] and although many lactose-intolerant subjects tolerate small amounts (~200 mL) of milk [7,8].

Moreover, some dairy products like cheese contain little or no lactose, whereas yoghurt is often tolerated better than milk. This is on the one hand due to the fact that lactose has in part already been hydrolysed during fermentation and on the other hand due to the microbial lactase of the starter cultures which after ingestion can support intestinal lactose digestion. Conventional yoghurt shows the best tolerability in comparison with other fermented dairy products [9–11]. Other therapeutic strategies include exogenous lactase or the use of lactose pre-hydrolysed milk [11], prolongation of gastrointestinal transit time, [8,12,13] or attempts to modify intestinal microbiota and enhance colonic adaptation by chronic lactose ingestion [14–17].

The use of enzyme preparations allows milk consumption without adverse effects. If a so-called acid lactase (from *Aspergillus oryzae*) is used, which is active in the acidic pH range (maximum activity at pH 2.5–5.5) and thus highly effective in the stomach while retaining approximately 20% of its activity in the small intestine, the enzyme can be consumed immediately with a lactose-containing meal [18–20].

The degree of lactose hydrolysis depends on the amount of lactase administered, the residual lactase activity which still exists in the intestine of the subjects, on the accessibility of the lactose in the chyme, and on the exposure time of lactose to lactase. The microbial lactase of the starter cultures -- on the other hand -- is most effective when after ingestion of a fermented milk product the starter bacteria survive passage through the stomach intact but are then destroyed in the small intestine by digestive enzymes and bile acids, so that the active enzyme is released there [5,21,22].

To answer the question whether the concomitant consumption of acid and yogurt lactase improves lactose digestion more than eating the two enzymes alone and does this reduce lactose intolerance symptomacy, a randomised, double-blind, controlled clinical cross-over study on adults with more or less impaired lactase activity has been performed.

2. **Materials and methods**

2.1. **Subjects**

Of 40 originally invited thirty subjects, 19 men (mean age 27.8 ± 6.2 years) and 11 women (31.5 ± 10.5 years), mostly belonging to ethnic groups with prevailing lactose malabsorption (south-east Asia, Africa), who considered themselves to be lactose malabsorbers, were enrolled in the study after checking inclusion and non-inclusion criteria.

Inclusion criteria were: self-assessed lactose malabsorption, >18 years of age, willingness to participate in all four test days and informed written consent.

Non-inclusion criteria were: participation in a clinical trial with a drug or medical device within the last 30 days, a surgery affecting the current health state within the last 3 months, known metabolic or gastrointestinal diseases (except lactose malabsorption) and/or medication, affecting gastrointestinal motility and/or absorption and metabolism of dietary ingredients, particularly lactose, psychiatric diseases, epilepsy, risk of suicide, eating disorders (e.g. anorexia, bulimia), alcohol or drug abuse and other exclusion criteria at the discretion of the investigator.

The study was approved by the local Ethics Advisory Committee at the Medical Association Schleswig–Holstein, Bad Segeberg, Germany, and carried out according to the Helsinki declaration. Participants gave written informed consent prior to the study.

2.2. **Study design**

To investigate the effects of various lactase preparations, lyophilized lactic acid bacteria and a combination of lactase and lactic acid bacteria, on (a) lactose digestion in lactose malabsorbers (primary target parameter) and (b) on the accompanying intestinal complaints (secondary target parameters), a placebo-controlled, randomized, double-blind cross-over study with five arms was performed.

The study comprised an initial screening visit and five experimental days, separated by 4 wash-out periods of two weeks each. During the study, participants were asked not to change their dietary habits and to abstain from taking vitamins, minerals and other supplements. At the beginning of each experimental day, after an overnight fast, a first breath sample was taken from the subjects. Thereafter, they ingested one of the five test preparations in randomised order. The lactose dose given (12.5 g) was less than for a classic lactose malabsorption test (50 g or 25 g, respectively). Such a lower dose, which is closer to the amount of lactose usually consumed with foodstuffs in daily routine, causes — if at all — far less complaints in lactose malabsorbers and is — according to the author’s experience — completely sufficient for the purpose of this study. The purpose here was to examine if there was improved lactose digestion and thus an attenuated hydrogen exhalation following consumption of the lactase-containing test preparations, while the classic lactose malabsorption test examines with <5% error the probability that there is lactose malabsorption.

2.3. **Experimental diets**

The test preparations were provided in capsules of identical appearance. The study participants were given one capsule per experimental meal together with 150 mL milk to which 5 g of lactose was added. This lactose-fortified milk contained thus approximately 12.5 g of lactose. The test preparations contained either:

- a) 1 × 10⁹ cfu *Streptococcus thermophilus* plus 1 × 10⁹ cfu *Lactobacillus delbrückii* ssp. *bulgaricus* in one capsule,
- b) 3300 FCC/capsule acid lactase from *A. oryzae*
- c) 9000 FCC/capsule acid lactase from *A. oryzae*
- d) a combination of test preparations (a) and (b) in one capsule
- e) the filling material (dicalcium phosphate) of the lactase capsules (placebo).

A conventional yoghurt culture (*S. thermophilus* and *L. delbrückii* ssp. *bulgaricus*) was used as a source of neutral microbial lactase (groups a and d), because both strains show a particular high lactase activity.

Test preparation b) and c) are commercially available dietary supplements (Lactrase 3300 and 9000, Vitacare GmbH & Co. KG,
Bad Vilbel, Germany) for people with lactose intolerance. Their β-galactosidase activity (3300 or 9000 FCC/capsule, respectively) was measured according to the FCC-III method. They have been sold in German pharmacies for many years, therefore, their safety for clinical applications is regarded as guaranteed.

Randomization of study participants as well as packaging and coding of the test preparations were performed by subjects otherwise not involved in the study.

For randomization purpose 30 unsorted sets of four unique numbers in the range from 1 to 4 (according to the 4 experimental diets) have been generated online [Research Randomizer (Version 4.0); http://www.randomizer.org/form.htm] and assigned to the study participants in order of the patient numbers.

2.4. Breath hydrogen test

The measurement of lactose digestion and the proof of lactose malabsorption were conducted with the aid of a hydrogen breath test (Microlyzer model 12, QuinTron Instruments, Milwaukee, USA). Since microbial carbohydrate fermentation in the large intestine leads among other things to the release of hydrogen (H₂) in ~90% of the general population, the increase in hydrogen concentration in the breath is a measure for the amount of undigested lactose in the large intestine and hence is also a measure of lactase deficiency in the small intestine. An increase in the peak hydrogen concentration in the breath of ≥20 ppm above the basal value after consumption of 50 or 25 g lactose is regarded as the criterion for lactose malabsorption. The period (≥2 h) between lactose consumption and the increase in H₂ concentration in the breath is equivalent to the gastric and small-intestinal transit time.

The advantage of the hydrogen breath test compared with the glucose or galactose tolerance test, in which the postprandial increases in glucose or galactose are measured after consumption of 50 or 25 g lactose, lies in the fact that the hydrogen measured here, released during microbial action in the large intestine, is at the same time a major cause of the common lactose intolerance symptoms, namely flatulence and/or abdominal pain. In other words, if hydrogen production by the intestinal flora is too low the degree of lactose malabsorption might be underestimated, but at the same time the persons affected have also less and/or weaker lactose intolerance symptoms or even none at all.

Another advantage of the hydrogen breath test, in contrast to the measurement of lactase activity in intestinal biopsies, is that it is non-invasive. Furthermore, while a genetic test only measures the genetic predisposition for primary lactose malabsorption and thus does not provide any indication of whether primary lactose malabsorption does already exist [23], the hydrogen breath test determines primary lactose malabsorption and any potential secondary lactose malabsorption (e.g. in the case of Crohn's disease, ulcerative colitis or coeliac disease).

2.5. Measurement of lactose digestion

In order to avoid too much burden on the test subjects, postprandial breath hydrogen measurement was limited to 6 h. At this time point microbial lactose fermentation and H₂ production in the large intestine of many subjects was still not complete, and consequently the total amount of exhaled hydrogen could not be determined. Therefore, the peak hydrogen concentration (which records 95% of the effect measured by the area under the breath hydrogen curve) was used as the parameter for the investigation of lactose digestion.

Furthermore, since the test subjects received markedly less lactose than in the classical malabsorption test, and since it was not a question of demonstrating lactose malabsorption in this study, also persons who showed less than 20 ppm, but 13 and more ppm H₂ in their expiratory breath in the placebo phase were included in the study.

2.6. Measurement of lactose intolerance

The participants were asked to fill out questionnaires about their gastrointestinal symptoms during the 5 experimental days. The questionnaire used was based on a score [24,25], which was used and evaluated in several of our studies [26]. It contained questions about the frequency, intensity and duration of flatulence, the frequency, intensity and duration of abdominal pain, stool frequency and consistency, and the frequency, intensity and duration of nausea. Each item was quantified on a scale of 0 (no symptoms) to 6 (unbearable symptoms). In the case of stool frequency and stool consistency, where deviations on both sides of the "normal situation" could be expected, “0” means “once a day, well-shaped stools”, whereas “6” indicates maximal deviation from normality either in the direction of constipation or diarrhoea.

However, these parameters are of varying predictive value for characterizing lactose intolerance. Thus, in the case of less pronounced lactose intolerance and/or a lower lactose test dose in comparison with the classical lactose malabsorption test, the parameters of flatulence ("wind") and abdominal pain correlate particularly well with the extent of lactose malabsorption, whereas diarrhoea tends to be induced by higher lactose amounts, and the cause of nausea and its predictive value as a lactose intolerance symptom is not well evaluated [27,28]. For this reason, all participating lactose malabsorbers were classified as intolerant if their pain score was greater than zero based on the questionnaire in the placebo phase.

2.7. Statistics

Due to the skewed distribution of the H₂ exhalation data and symptom score values, non-normal distribution was assumed. For this reason, and because the number of test participants was too small to apply the central limit theorem, nonparametric statistical test procedures were chosen and the values were presented as medians and 25th/75th percentiles. Because the medians of the intestinal complaints score values arrived often at “0”, due to the large number of symptom-free lactose malabsorbers, the score means plus standard deviation were given additionally.

Breath hydrogen and symptom score values were statistically assessed by Friedman tests followed by multiple post hoc Wilcoxon comparisons without Bonferroni corrections. This approach is justified, since only few comparisons were performed, which had been defined before the start of the study, and beyond that each comparison was related to its own question.

2.8. Sample size

In earlier investigations of the author [7,27], peak breath hydrogen concentrations of about 30 ± 11 ppm (mean ± standard deviation) had been observed after consumption of 12–15 g lactose. The drop in peak breath hydrogen concentration required to fall below the threshold value of 20 ppm associated with lactose malabsorption must therefore be > 10 ppm. Based on a relevant difference of the mean of 10.2 ppm, a standard deviation of 11 ppm, \( \alpha = 0.05 \) and \( \beta = 0.1 \), a sample size of 24 persons is required.

3. Results

Out of the 30 subjects originally enrolled in the study and randomized, one man dropped out because of “loss to follow up” and
five subjects proved to be true lactose absorbers (according to the criteria of the breath hydrogen test) during the course of the study and were therefore excluded from statistical analysis (twenty-five of thirty is equal to the approximately 83% prevalence of lactose malabsorption in these ethnic groups). Twenty-four subjects were analysed per protocol (Fig. 1).

### 3.1. Breath hydrogen measurements

The results of the breath hydrogen measurements demonstrated a significant improvement in lactose digestion, i.e. a significant reduction in peak level ($p < 0.009$) of the hydrogen exhalation curve, through administration of exogenic lactase compared to placebo (Table 1).

All lactase-containing preparations reduced the hydrogen exhalation by approx. 25–50% in the order: yogurt culture < 3300 FCC lactase < 9000 FCC lactase < combination preparation. However, only the differences between placebo on the one hand and the 3300 and 9000 FCC lactase preparation or the combination preparation on the other hand, but not the lyophilized yogurt culture, were “significant” according to multiple Wilcoxon comparisons.

Moreover, the overall range of the hydrogen values, i.e. the standard deviation (data not shown) as well as the 25th/75th percentiles, were roughly 50% less after consumption of the combination preparation than after the other lactase preparations. This was mainly because more subjects benefited from the mixed lactase product than from one of the other lactase mono-preparations.

### 3.2. Symptoms

Of the symptoms reported in the questionnaires, only the “intensity of flatulences, intensity of abdominal pain, stool consistency and intensity of nausea” could be used for statistical evaluation, because the study period was too short to record completely the frequency and duration of symptoms (Tables 2 and 3). Of these symptoms, only abdominal pain ($p = 0.033$; Friedman test) and flatulences ($p = 0.076$; Friedman test) were reduced to a relevant degree by 30–60% or 20–40%, respectively (relative to the respective means) through ingestion of the lactase-containing preparations. Multiple post hoc comparisons using Wilcoxon tests showed that the severity of abdominal pain was only significantly reduced by the combination preparation, whereas the beneficial effects on wind were only significant after administration of the 9000 FCC lactase-containing preparation (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Breath-H₂ (peak maximum)</th>
<th>Breath-H₂ (median (25/75th percentiles))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>24</td>
<td>240.0 (165.3/300.0)*</td>
<td>2.0 (0.0/2.0)*</td>
</tr>
<tr>
<td>Yoghurt culture</td>
<td>24</td>
<td>17.5 (11.5/33.0)*</td>
<td>2.0 (0.0/2.0)*</td>
</tr>
<tr>
<td>3300 FCC acid lactase</td>
<td>24</td>
<td>17.0 (8.0/25.8)*</td>
<td>1.0 (0.0/2.0)*</td>
</tr>
<tr>
<td>9000 FCC acid lactase</td>
<td>24</td>
<td>16.0 (8.8/24.8)*</td>
<td>1.0 (0.0/2.0)*</td>
</tr>
<tr>
<td>Combination preparation</td>
<td>24</td>
<td>14.0 (9.0/18.0)</td>
<td>1.0 (0.0/1.0)</td>
</tr>
</tbody>
</table>

* Statistical analysis on the basis of the area-under-the-curve (AUC) showed a $p < 0.023$ (data not shown).

### Table 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Abdominal pain (strength)</th>
<th>Flatulences (strength)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>median (25/75th percentile)</td>
<td>mean ± SD median (25/75th percentile)</td>
</tr>
<tr>
<td>Placebo</td>
<td>24</td>
<td>0.0 (0.0/1.3)*</td>
<td>0.7 ± 0.9</td>
</tr>
<tr>
<td>Yoghurt culture</td>
<td>24</td>
<td>0.0 (0.0/0.0)*</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td>3300 FCC acid lactase</td>
<td>24</td>
<td>0.0 (0.0/1.0)*</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td>9000 FCC acid lactase</td>
<td>24</td>
<td>0.0 (0.0/1.0)*</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>Combination preparation</td>
<td>24</td>
<td>0.0 (0.0/0.0)</td>
<td>0.3 ± 0.5</td>
</tr>
</tbody>
</table>

* Values not sharing superscripts (*,*) within columns are significantly different from each other (Friedman-test followed by multiple Wilcoxon comparisons).

**Fig. 1.** Participant flow.
from the concurrent administration of both enzymes, because an effective lactase hydrolysis in the stomach by the acid lactase may compensate for a weak release of microbial lactase in the small intestine and vice versa.

This might be the reason for the significantly lower mean hydrogen exhalation in study participants receiving the combination preparation as well as the lower number of subjects which did not or only slightly benefit from lactase administration. The advantage of the combination preparation was thus not only its greater contribution to lactase digestion, but also that it was effective in a larger number of the lactase malabsorbers.

Overall the study complied with the setting of the ethics committee: neither the medians or means, respectively, of the individual symptom scores nor – with few exceptions – the individual score values exceeded a value of 1–2 (on a scale of 0–6) and no diarrhoea was observed. But despite these restrictions, the present study shows that the lactase preparations used decreased not only H2 exhalation but reduced also symptoms of lactose intolerance, namely exactly those, which are most closely associated with intestinal gas production, i.e. flatulence and abdominal pain [16,17,28].

The reduction in abdominal pain by –45% (9000 FCC lactase) to –70% (combination preparation) compared with placebo can certainly be considered as relevant, although only the reduction by the combination preparation was significant. Flatulence (wind) was only significantly reduced by 9000 FCC lactase (~37% compared with placebo).

The statistical reliability of these results is somewhat impaired by the fact that even in the placebo group only weak symptoms and in many malabsorbers no symptoms at all were observed and only a small part of the symptom scale was “utilised”. The use of the Friedman test appears to be compelling for formal statistical reasons. From a physiological point of view, one may doubt the repeated measurement character of the symptom score values recorded at the various study days in each and the same person, since the composition of the intestinal microbiota and above all the concentrations of the hydrogen-producing bacteria may vary considerably from study day to study day. This depends less on the individual person than on individual meals and other factors that were not controlled for here.

On the other hand, the results of the study are consistent with what one would expect from physiological considerations. The finding that only flatulence and the severity of abdominal pain were significantly reduced, but not the other symptoms (stool consistency and nausea) which are more closely associated with intestinal concentration of undigested lactose and its osmotically active or toxic fermentation products [16,17,28,29], is most convincingly explained with the low dose of lactase administered.

In addressing the primary question of the study, it can be concluded that the combined administration of yogurt bacteria and acid lactase not only increases lactase digestion and reduces gastrointestinal complaints in lactose malabsorbers more strongly than it was to be expected from the simple addition of the individual lactase activities, but also that this enables more lactose malabsorbers to profit from this effect. Thus, the clinical relevance of the study lies in the fact that the results allow nutritionists, medical doctors and clinics to derive effective dietary recommendations for lactose-intolerant patients which do not want to refrain from the consumption of conventional milk and dairy products, but in whom the usual alternatives, e.g. consuming fermented dairy products or commercially available lactase preparations, are not or only moderately successful [30].

Thus, all taken together, administration of acid lactase and particularly in combination with yogurt bacteria represents a valid

### Table 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Stool consistency</th>
<th>Nausea (strength)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>median (25/75th percentile)</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>Placebo</td>
<td>24</td>
<td>0.0 (0.0/1.0)*</td>
<td>0.6 ± 1.1</td>
</tr>
<tr>
<td>Yogurt</td>
<td>24</td>
<td>0.0 (0.0/0.3)*</td>
<td>0.5 ± 1.1</td>
</tr>
<tr>
<td>3300 FCC acid lactase</td>
<td>24</td>
<td>0.0 (0.0/0.3)*</td>
<td>0.4 ± 0.9</td>
</tr>
<tr>
<td>9000 FCC acid lactase</td>
<td>24</td>
<td>0.0 (0.0/0.3)*</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>Combination preparation</td>
<td>24</td>
<td>0.0 (0.0/1.0)*</td>
<td>0.4 ± 0.9</td>
</tr>
</tbody>
</table>

* p = 0.993

** Quantified on a scale of 0 (no symptoms) to 6 (unbearable symptoms). In the case of stool consistency “0” means “once a day, well-shaped stools”, whereas “6” indicates severe diarrhoea.

** Values not sharing superscripts (*, †) within columns are significantly different from each other (Friedman-test followed by multiple Wilcoxon comparisons).

### 4. Discussion

In order to fulfill the requirements of the ethics committee, namely to avoid strong symptoms of lactose intolerance and above all diarrhoea in the study participants, only 12.5 g lactose per test was administered to the subjects [29]. Moreover, only subjects with an increased risk of lactose malabsorption (or intolerance) at the moment of inclusion in the study.

This and the outcome of the study overall permit the conclusion that a relevant part of the study participants had lost their intestinal lactase activity not yet completely. This conclusion is based on the fact, that in most subjects, hydrogen exhalation never exceeded 30 ppm, whereas in earlier studies of the author [7] in subjects with lactase activity not yet completely. This conclusion is confirmed by the fact, that in most subjects, hydrogen exhalation never exceeded 30 ppm, whereas in earlier studies of the author [7] in subjects with moderate successful [30].

Due to the different sites of main action of microbial and acid lactase, lactose maldigesters may benefit in a synergistic manner the small intestine.

The statistical reliability of these results is somewhat impaired by the fact that even in the placebo group only weak symptoms and in many malabsorbers no symptoms at all were observed and only a small part of the symptom scale was “utilised”. The use of the Friedman test appears to be compelling for formal statistical reasons. From a physiological point of view, one may doubt the repeated measurement character of the symptom score values recorded at the various study days in each and the same person, since the composition of the intestinal microbiota and above all the concentrations of the hydrogen-producing bacteria may vary considerably from study day to study day. This depends less on the individual person than on individual meals and other factors that were not controlled for here.

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Thus, all taken together, administration of acid lactase and particularly in combination with yogurt bacteria represents a valid
therapeutic strategy with objective and subjective efficacy and without side effects.

Sources of funding

Sponsor of the study was the Vitacare GmbH & Co. KG, Bad Vilbel, Germany who also supplied the lactase enzyme and yogurt bacteria used in the study.

Statement of authorship

CL was Principal Investigator; MV, CL and JS designed research; CL, BO, ES, FR, and AT conducted research; MV analysed data; MV wrote the paper; MV had primary responsibility for final content. All authors read and approved the final manuscript.

Conflicts of interest

MV carried out breath hydrogen measurements for the Vitacare GmbH & Co. KG, Bad Vilbel, Germany and also gave statistical advice for this company. None of the other authors had any personal or financial conflicts of interest.

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None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2014.06.012.

References
