

The effect of oral supplementation with *Lactobacillus reuteri* or tilactase in lactose intolerant patients: randomized trial

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Abstract. – **Background:** Lactase enzyme supplements and probiotics with high β -galactosidase activity may be valid treatment options for the lactose intolerance. Aim of this study was to assess whether supplementation with tilactase or *Lactobacillus reuteri* when compared to placebo affects hydrogen breath excretion and gastrointestinal symptoms in lactose intolerant patients during lactose breath test (H₂-LBT).

Methods: Sixty lactose intolerant patients participated in the study and were randomized to three 20 patients-treatment groups: tilactase group (tilactase 15 minutes before control H₂-LBT); placebo group (placebo 15 minutes before control H₂-LBT); *Lactobacillus reuteri* group (LR) (LR b.i.d. during 10 days before control H₂-LBT). The outcomes were LBT normalization rate, and influences of treatments on both mean maximum hydrogen concentration and clinical score.

Results: LBT normalization rate was significantly higher in tilactase and LR groups with respect to placebo. Tilactase was significantly more effective than LR in achieving LBT normalization ($p < 0.01$). Both significant reduction of mean peak H₂ excretion and improvement of the mean clinical score were observed in tilactase and LR groups after treatment with respect to placebo ($p < 0.0001$). Tilactase was significantly more effective than LR in reducing both mean peak hydrogen excretion and mean clinical score.

Conclusions: In lactose intolerants, tilactase strongly improves both LBT results and gastrointestinal symptoms after lactose ingestion with respect to placebo. *Lactobacillus reuteri* also is effective but lesser than tilactase. This probiotic may represent an interesting treatment option for lactose intolerance since its use is simple and its effect may last in the time after stopping administration.

Key Words:

Lactose intolerance, Lactose breath test, Tilactase, *Lactobacillus reuteri*.

Introduction

The lactose intolerance is a common condition affecting a large proportion of the world population¹; its prevalence varies among different countries². In Europe, the prevalence increases towards South and East, reaching 70% in southern Italy³.

Lactose intolerance is characterized by the absence⁴ or drastically reduced levels of the intestinal lactase⁵. The lactase enzyme is a β -galactosidase that is present on the brush border of the enterocyte, responsible for the hydrolysis of lactose to the monosaccharides, glucose and galactose^{6,7}.

Sixty percent of patients are asymptomatic and are defined as “lactose malabsorbers”⁸; when after ingestion of lactose containing foods the typical symptoms such as abdominal pain, bloating, flatulence, diarrhoea occur they are considered “lactose intolerant”⁹.

The diagnosis of hypolactasia is currently based on the hydrogen¹⁰ lactose breath test (H₂-LBT)¹¹, a non invasive and simple test. Nowadays, the usual behaviour for this condition consists of the avoidance of milk and dairy products from the diet. However, this restriction leads to a reduction of intake of substances such as calcium, phosphorus and vitamins, and may be associated with decreased bone mineral density¹².

Several therapeutic approaches have been proposed in the last years¹³⁻¹⁴ the most promising being the supplementation by exogen lactase¹⁵ and more recently the use of probiotics with a β -galactosidase activity¹⁶⁻¹⁷. Exogenous lactase are obtained from *Aspergillus oryzae*¹⁸ (Lactdigest®, Italchimici, Pomezia, Rome, Italy) or from *Kluyveromyces lactis* (Silact®, Sofar, Trezzano, Milan, Italy) and are able to break down lactose

into glucose and galactose to allow a better absorption¹⁹. The use of exogenous β -galactosidase in lactose malabsorbers, also when added at mealtime, is efficacious and free of side effects²⁰.

More recently the administration of probiotics endowed with a β -galactosidase activity was useful to treat patients with lactose intolerance²¹.

Probiotics are defined as live micro-organisms that when given in adequate quantities will have a health benefit on the host²². Many bacterial families with over 500 species house our gastrointestinal tract²³ with the highest concentration in the colon²⁴. It has been demonstrated that malabsorbed lactose is salvaged by the distal ileal and colonic lactic acid bacteria. Lactic acid bacteria belong to the family of *Lactobacillus*, *Bifidobacterium*, *Staphylococcus*, *Enterococcus*, *Streptococcus*, and ferment lactose to produce lactate, hydrogen, methane, and short chain fatty acids²⁵.

In the process of fermentation, microbial lactase, present in lactic acid bacteria, initially breaks down unabsorbed lactose by hydrolysis to its monosaccharides, glucose and galactose, that may be absorbed. However, there is a huge variability in the amount of lactase activity in different probiotics²⁶. *Lactobacillus reuteri* is an interesting lactic acid bacteria able to stimulate the immune response in gastrointestinal tract²⁷, to normalize the intestinal permeability in atopic dermatitis²⁸, to improve acute diarrhoea²⁹, to reduce the incidence of antibiotic associated side-effects. In addition, it has been demonstrated that it is able to survive after the contact with gastric acid and bile salt³⁰.

Aim of the present study was to assess the effect of supplementation with tilactase when compared to placebo or *Lactobacillus reuteri* on hydrogen breath excretion and gastrointestinal symptoms in lactose intolerant patients during a standard H₂-LBT.

Materials and Methods

This prospective trial was performed at the Gastroenterology and Internal Medicine Out-patient Unit of Gemelli Hospital, the teaching hospital of the Catholic University of the Sacred heart of Rome, Italy. The report of this trial follows the recommendations of the Consort Statement for the quality of reports of parallel group, randomized trial³¹.

Patients: Eligibility Criteria

Consecutive patients referring to our Unit from September to December 2007 for the presence of gastrointestinal symptoms after lactose ingestion were evaluated. Patients positive to H₂-LBT were considered eligible for the study.

All the patients agreeing to participate signed an informed consent form before admission, after a full explanation by the investigators.

Exclusion criteria were: age < 18 or > 65 years; diagnosis of small intestinal bacterial overgrowth (as assessed by abnormal glucose breath test); history of allergy to milk proteins.

The study was performed in accordance with the Declaration of Helsinki, and approved by the local Ethics Committee.

Lactose Breath Testing

To minimise the basal hydrogen excretion, patients were asked to have a carbohydrate-restricted dinner on the day before the test and to be fasting for at least 12 h on the testing day. Before starting the test patients did a mouth wash with 20 ml of chlorhexidine 0.05%. Smoking and physical exercise were not allowed for 30 min before and during the test. End-alveolar breath samples were collected immediately before lactose ingestion. Then a dose of 25 g of lactose was administered and breath samples were taken every 30 min for 4 h using a two-pack system. Samples were analyzed immediately for H₂ using a model Quintron Gas Chromatograph (Quintron Instrument Company, Milwaukee, WI, USA). Results were expressed as parts per million (p.p.m.).

H₂-LBT was considered positive for lactose malabsorption when an increase in H₂ value more than 20 parts per million (p.p.m.) over the baseline value was registered³².

The mean time interval between the baseline testing and the testing after intervention was 42 days (ranging 30-60 days).

Randomization

The enrolled patients were randomly assigned to one of the following three treatment groups, using a computer-generated number sequence, generated by a statistician:

1. Tilactase group: tilactase (*Lacdigest*[®], Italchimici, Pomezia, Italy), 4 pills (9.000 U) 15 minutes before the control LBT;
2. Placebo group: placebo, 4 pills 15 minutes before the control LBT.

- Lactobacillus reuteri* group (LR): *Lactobacillus reuteri* (Reuterin[®], Nóos, Rome, Italy), 2 pills (4×10^8 CFU) b.i.d. during the 10 days preceding the control LBT.

Symptoms Assessment and Study Outcomes

For a total of 8 h (during the H₂-LBT and during the following 4 h), all patients were invited to fulfil a diary where recording the eventual occurrence of intolerance symptoms (bloating, abdominal pain, flatulence and diarrhoea) whose severity was assessed by a visual analogical scale (the score ranging from 0, absent to 10, severe). For each patient, a mean clinical score was calculated by the mean the partial score of each symptom. The same procedure was performed for the initial (baseline) H₂-LBT and during the testing after intervention.

The primary outcomes were the H₂-LBT normalization rate, and the effects of the treatments on both mean maximum H₂ concentration and gastrointestinal symptoms.

In the patients assigned to the LR treatment group, the compliance to the treatment was evaluated by an interview performed after the end of the therapy (at the time of the control H₂-LBT), and by a pill count of the drugs boxes returned at the same interview. Low compliance was defined as more than 20% of pills returned.

Sample Size

The sample size was calculated to warrant a significance level of 5% and a power of 80%, in

order to detect significant differences in the incidence of normalization between Placebo, Tilactase and LR groups. Assuming that the increased normalization in the Tilactase group is of 90% respect to placebo, we hypothesized to detect a reduction of 45% of normalization in the LR respect Tilactase group. This determines a sample size of 20 patients for each group, using EpiInfo3.3 statistical software for Windows.

Statistical Analysis

The one-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used to assess differences among the three randomisation groups at baseline and after treatment. χ^2 or Fisher's exact tests were used to detect differences in LBT normalization rate and in incidence of side effects. *P* values <0.05 were considered to be significant.

Data concerning H₂ excretion and clinical score were expressed as means \pm SD.

All scatter plots were carried out using Statistical Package for Social Sciences (SPSS12.0) for Windows.

Results

Figure 1 summarizes the study flow chart. A total of 60 patients (6 males, 54 females, mean age 32 ± 5 years) participated in the present study. All patients completed the study.

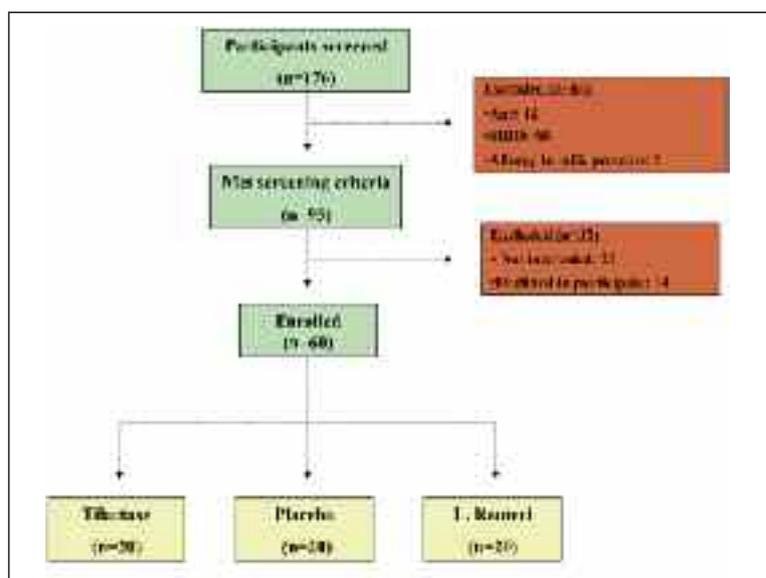


Figure 1. Study flow.

In the LR treatment group, the compliance was excellent: more than 95% of them took all the prescribed number of pills for the 10-days treatment. In particular, in the LR group no relevant side effects were reported (2 patients mild diarrhoea, 1 patient mild constipation).

Demographics and baseline characteristics (baseline mean maximum H₂ concentration and mean clinical score) of the studied population are reported in Table I.

Eighty (80%, 16/20) of the Tilactase group patients, 35% (7/20) of the LR group and none (0/20) of placebo group were negative to the control LBT respectively. Both tilactase and LR groups showed a significantly higher LBT normalization rate with respect to placebo group ($p<0.001$ and $p<0.01$ respectively). In addition, tilactase treatment was significantly more effective than *Lactobacillus. reuteri* in achieving LBT normalization ($p<0.01$).

No differences were found between the baseline mean maximum H₂ concentration before the treatment in the Tilactase group (31.8 ± 8.3 p.p.m.), in the LR group (32.7 ± 9.0 p.p.m.) and in the placebo group (30.7 ± 8.7 p.p.m.) ($p=0.76$; Table I). After treatment, a significant reduction of mean peak H₂ excretion was observed in the tilactase group (14.7 ± 8 p.p.m.) and in the LR group (23.1 ± 7.85 p.p.m.) with respect to baseline values; no modification was found in the placebo group (31.7 ± 8.3 p.p.m.) ($p<0.0001$; Figure 2). In addition, tilactase treatment was significantly more effective than LR in reducing both mean peak hydrogen excretion ($p<0.01$). Figure 3 shows longitudinal data about H₂ values observed at the control LBT, from time 0 (baseline) to time 9 (4 h after lactose ingestion) in each patient of the three treatment group. Figure 4 shows longitudinal data about H₂ values observed at the control LBT, from time 0 (baseline) to time 9 (4 h after lactose ingestion) in tilactase group.

The three treatment groups were similar about the mean intensity of all gastrointestinal symp-

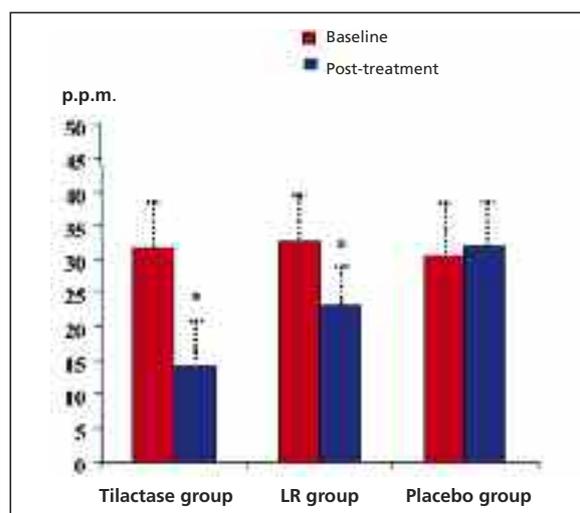


Figure 2. Mean maximum H₂ concentration during LBT at baseline and after treatment. Tilactase Group (tilactase); LR group (*L. reuteri*); Placebo group (placebo).

toms and for the mean clinical score at enrolment (mean clinical score: $p=0.98$; Table I). A significant improvement was observed after treatment with respect to the baseline values for all gastrointestinal symptoms evaluated (Table II) and for the mean clinical score in the tilactase group and in the LR group, whereas no significant modification was found in the placebo group (mean clinical score: $p<0.0001$; Figure 5). In addition, tilactase treatment was significantly more effective than LR in reducing all gastrointestinal symptoms and the mean clinical score (mean clinical score, the tilactase group versus the LR group: $p<0.0001$).

Discussion

The lactose intolerance³³, caused by reduced lactase activity in the intestine, is a common gastrointestinal disorder³⁴. Its symptoms induce

Table I. Demographics and baseline characteristics (mean maximum H₂ concentration and clinical score at enrolment) of the studied population. Data are means \pm SD.

	Tilactase group (n=20)	LR group (n=20)	Placebo group (n=20)
Age	32 \pm 9	33 \pm 11	32 \pm 12
Females	18	16	17
Mean maximum H ₂ Concentration (p.p.m)	31.8 \pm 8.3	32.7 \pm 9.0	30.7 \pm 8.7
Mean clinical score	7.25 \pm 1.35	7.29 \pm 1.23	7.25 \pm 0.97

Table II. Gastrointestinal symptoms at baseline and after treatment. Tilactase group (tilactase); LR group (*L. reuteri*); Placebo group (placebo). *Statistically significant. Data are means \pm SD.

	Bloating	Abdominal pain	Flatulence	Diarrhoea
Tilactase group baseline	8.95 \pm 0.75	8.05 \pm 1.05	6.05 \pm 2.01	5.95 \pm 1.60
Tilactase group after treatment	4.00 \pm 1.97*	2.00 \pm 1.38*	0.25 \pm 0.44*	0.20 \pm 0.41*
LR group baseline	9.10 \pm 0.85	7.95 \pm 0.82	6.00 \pm 1.68	6.10 \pm 1.58
LR group after treatment	6.95 \pm 0.88*	6.90 \pm 1.07*	3.95 \pm 1.35*	2.95 \pm 2.07*
Placebo group baseline	9.05 \pm 0.68	7.90 \pm 1.16	5.95 \pm 1.27	6.10 \pm 0.79
Placebo group after treatment	8.95 \pm 0.60	7.10 \pm 0.72	5.15 \pm 0.93	5.90 \pm 0.85

many people to avoid the consumption of milk³⁵, that is a major calcium source in the diet of western countries³⁶.

In this preliminary study we tested whether the probiotic *Lactobacillus reuteri* was effective for the treatment of lactose intolerance, thus comparing it with the supplementation by exogenous enzyme, that is a validated treatment option. The principal limitation of the present study is that the probiotic arm was not controlled. The principal reason of this was that the two treatment groups varied a lot in terms of posologic scheme. To achieve effective results using a probiotic, it has to be administered for more days to be sure to colonize the gut.

Both treatment schemes (tilactase or *Lactobacillus reuteri*) were found to be useful for the treatment of lactose intolerance, since they significantly improved either H₂-LBT results or associated symptoms with respect to placebo, the

effect being stronger with tilactase than with *Lactobacillus reuteri* treatment.

The improvement in the breath test results that we observed after treatment strongly correlated with the decrease of the clinical score, thus underlying the fact that clinical symptoms are strictly related to the presence of the condition of lactose malabsorption. However, in patients with lactose intolerance, the after treatment clinical improvement is to be considered more relevant than the normalization of breath test or the decrease of H₂ peak after lactose ingestion. It is clear that, since there are not known adverse effects of lactose maldigestion other than acute gastrointestinal symptoms, the major goal of the treatment is to improve the clinical picture characterizing the lactose intolerance. For this reason, nowadays intolerant and not malabsorber patients without symptoms should be treated³⁷.

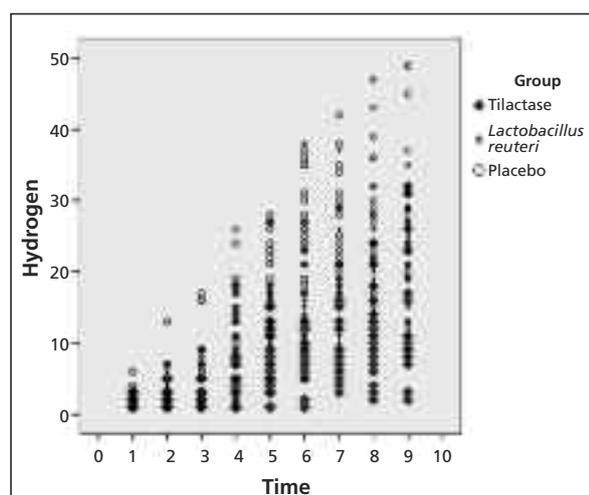


Figure 3. Longitudinal data about H₂ breath test values from time 0 (baseline) to time 9 (4 h after lactose ingestion) for each patient of Tilactase group (A), LR group (B), and placebo group (C) observed at the control LBT.

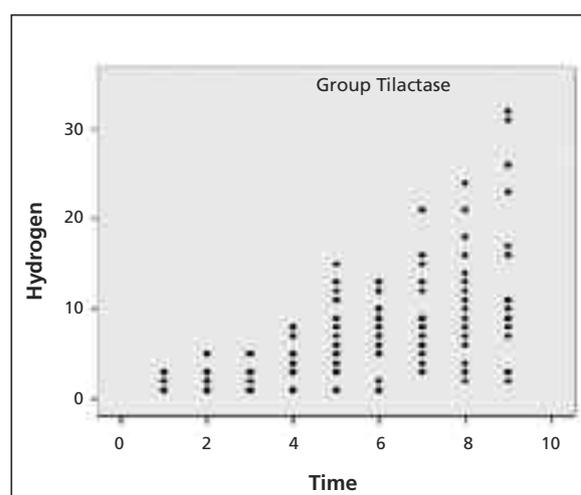


Figure 4. Longitudinal data about H₂ breath test values from time 0 (baseline) to time 9 (4 h after lactose ingestion) for each patient of Tilactase group observed at the control LBT.

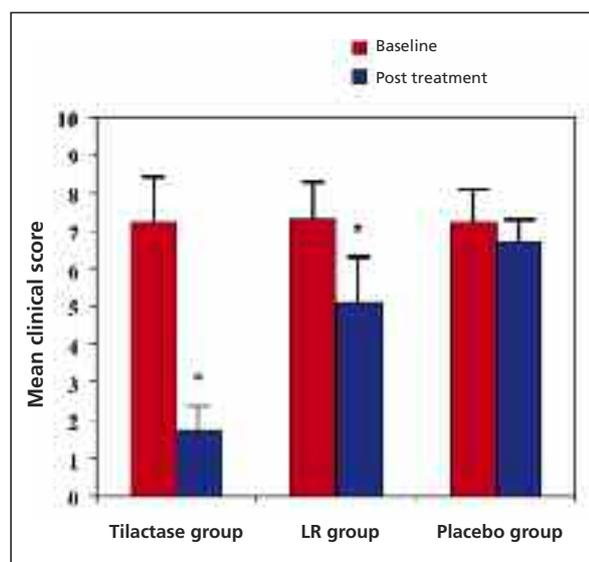


Figure 5. Mean clinical score at baseline and after treatment. Tilactase Group (tilactase); LR group (*L. reuteri*); Placebo group (placebo).

Our data showed that the supplementation by exogenous β -galactosidase at mealtime is an effective treatment for LI³⁸.

Another possible approach to improve lactose malabsorption relies on the consumption of viable lactic acid bacteria³⁹. In fact, recent reports showed that some probiotics are able to reduce bloating in patients with lactose intolerance, possibly via the presence of microbial lactase activity within lactic acid bacteria⁴⁰.

At the best of our knowledge, this is the first study assessing the effect of the probiotic *Lactobacillus reuteri* in the treatment of lactose intolerance. We found that the administration of *Lactobacillus reuteri* at standard dosage in the 10 days preceding the LBT affects lactose digestion, since it improves either LBT results or mean clinical score with respect to the baseline and to placebo group. We may hypothesize that *Lactobacillus reuteri* adheres to the biofilm in the proximal small intestine and here exhibits its β -galactosidase effect.

In our study tilactase treatment resulted significantly more effective than *Lactobacillus reuteri*. It appears hard to explain the reasons of the smaller response observed after treatment by *Lactobacillus reuteri* with respect to that observed in the group treated by tilactase. The great variability in the level of lactase enzyme deficiency in lactose intolerant patients could be one of the possible explanations.

It is well known that hypolactasia, or lactase deficiency, exists in three distinct forms: congenital, primary and secondary⁴¹. Congenital lactase deficiency is associated with the least lactase activity. Lactase nonpersistence, instead, occurs in the majority of humans and is characterized by the partial and reversible loss of lactase activity⁴²⁻⁴³. Thus, we can hypothesize that patients with a higher reduction of the enzyme may benefit only by the supplementation of the exogenous enzyme at meal time, while patients with a mild deficiency could benefit by probiotics also.

The use of *Lactobacillus reuteri* for the treatment of lactose intolerance is endowed with some potential advantages with respect to tilactase supplementation. In the case of tilactase treatment, the patient should always calculate the amount of the exogenous enzyme to assume on the basis of the amount of lactose present in the food they want to eat. In addition, tilactase effect is strictly confined to the time of its ingestion, so the drug should be taken every time the patients is going to eat lactose containing products. On the other hand, the probiotic is administered at a standard dosage, regardless of the dosage of lactose the patients are going to ingest; in addition, its effect may persist after stopping the drug assumption, since the β -galactosidase activity exerted by the probiotic should persist for all the duration of the gut colonization. This time probably vary patient by patient, and it is unknown at present.

Further studies are needed to assess the role of *Lactobacillus reuteri*, in particular concerning both the best dosage and duration of this treatment associated to a better digestion of lactose. In addition, it could be very interesting to investigate other possible effects of probiotics such as the immunomodulation on colonic microbiota or their influence on visceral sensitivity, since both may affect symptoms in patients with lactose malabsorption.

A greater understanding of the complex topic concerning lactase deficiency, lactose intolerance and symptom generation would help clinicians to treat patients more effectively.

Study Highlights

What is Current Knowledge

Avoiding milk and dietary food for lactose intolerance may lead to osteoporosis.

The supplementation by exogen lactase is useful to treat lactose intolerance in absence of the risk of osteoporosis.

What is New Here

Lactobacillus reuteri was used for the first time as a treatment of lactose intolerance.

Lactobacillus reuteri treatment significantly improved lactose digestion with respect to placebo; however its effects were lower than those observed with tilactase supplementation.

The potential advantage of *Lactobacillus reuteri* compared to Tilactase treatment is that the probiotic is administered at a standard dosage and that its effect may persist after stopping the drug assumption.

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Acknowledgements

This work has been supported by an unrestricted grant provided by Fondazione Ricerca in Medicina, Italy.