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ADAPTATION TO LACTOSE INTOLERANCE MAY NOT BE ACHIEVED BY LONG TERM INGESTION OF A MULTI- SPECIES CONTAINING PROBIOTIC: AN EXTENDED PRELIMINARY STUDY

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ABSTRACT: ***Continued consumption of lactose in intolerant people can lead to symptomatic adaptation, putatively through colonic bacterial changes. Whether adaptation can be achieved in vivo by expanding lactic acid producing bacterial populations without metabolic adaptation is still unclear. We used the multispecies high concentration probiotic VSL3 in 43 participants to attempt to clarify this issue. Four groups of lactase non-persistent subjects underwent a single blinded 42 day trial with 50g lactose challenges at the beginning and end of intervention. The groups were as follows: Control (N=12), Consumption of 60ml milk BID (N=12), VSL3 900x10^9, BID (N=9) and VSL3+ 60ml milk BID (N=10). Breath hydrogen and symptoms were recorded for 4.5Hrs with each test. A 3-day recall diet survey targeting lactose was used to control for spontaneous alterations in lactose intake prior to each test and showed no substantial changes. Analysis by ANOVA of changes in hydrogen or symptoms showed no significant effect in any of the groups. In conclusion symptomatic adaptation to lactose in lactase non persistent subjects is not corrected by VSL3 and may require additional metabolic priming to bacterial population expansion. Further evaluation of the mechanism of colonic adaptation is warranted.***

KEY WORDS: Adaptation, Lactose Intolerance, and Probiotics

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INTRODUCTION

The genetic polymorphism of intestinal lactase persistence (LP) and non-persistence (LNP) divides the world's adult population respectively into those who can and those who cannot digest the milk sugar, disaccharide, lactose. While the ability to split lactose into glucose and galactose is a dominant trait, (Scrimshaw and Murray, 1988; Kruse et al., 1988) the majority of the world's populations lose 90% of the intestinal enzyme lactase by childhood (Scrimshaw and Murray, 1988; Wang et al., 1998). As a result, consumption of dairy foods can lead to intolerance, manifested by bloating, gas production, cramps and in the more severe cases, diarrhea. Continued intake, however improves tolerance (colonic adaptation). This is clinically defined by reduced measured breath hydrogen, improved symptoms of intolerance and increased measured fecal ß- galactosidase compared with the unadapted state (Hertzler and Savaiano, 1996). It is hypothesized to be caused by an expansion of several species of lower intestinal bacteria as well as an enhanced capacity to metabolize lactose (i.e. metabolic adaptation). Since there is less measured hydrogen, the putative primary species responsible are lactic acid producing bacteria (LABs) (Hertzler et al., 1997). Many species of LABs are thought to exert health benefits to the host (Montrose and Floch, 2005; Heyman and Menard, 2002).

Probiotics (exogenously consumed live bacteria which provide health benefits to the host) and prebiotics (undigested foodstuffs, usually carbohydrates which upon reaching the colon promote the growth and / or metabolism of specific bacteria and provide health benefits to the host via these two effects) have gained wide scientific acceptance for being therapeutically or prophylactically relevant in a variety of gastrointestinal (Marteau et al., 2001; Fedorak and Madsen, 2004; Myllyluoma et al., 2005; Loguercio et al., 2005) and non gastrointestinal (Hoesl and Altwein, 2005; Weston et al., 2005; Meurman, 2005; Rautava et al, 2005) diseases. In this wider context, improvement in lactose intolerance is deemed to be a beneficial effect of probiotics (Montrose and Floch, 2005; Heyman and Menard, 2002; Guarner et al., 2005). The concept, however, mainly applies to fermented dairy products such as yoghurt (Adolfsson et al., 2004) and kefir (Hertzler and Clancy, 2003) or perhaps the addition of LABs to milk (Levri et al., 2005). These products typically cause fewer symptoms because they contain less lactose and exert other intestinal effects (Adolfsson et al., 2004; Shermak et al., 1995).

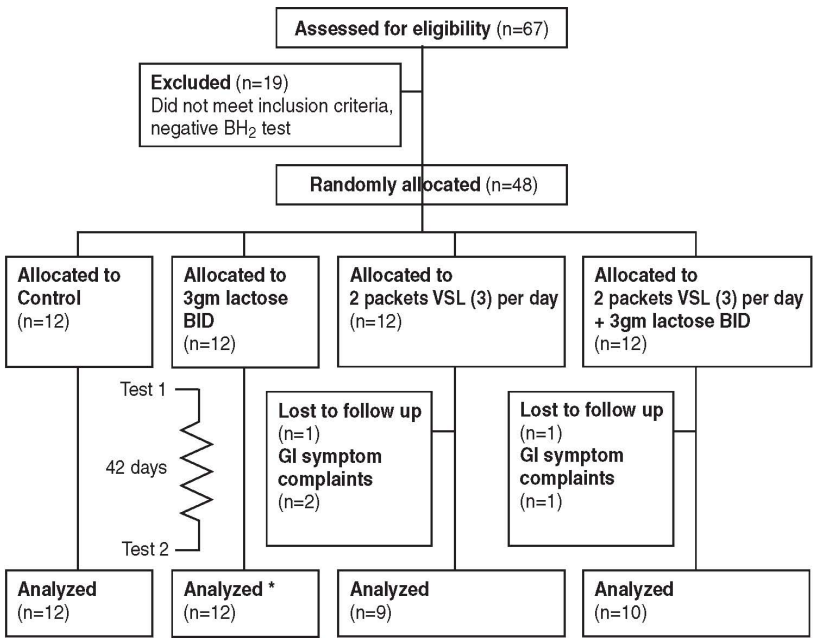
To date, consumption of probiotics without pre-stimulation by substrate (lactose), has not demonstrated improved lactose

intolerance. There have been however, only a few attempts (Hove et al., 1994; Saltzman et al., 1999; Yesovitch et al., 2004) and these could be criticized for being of short duration, which may not have provided for adequate establishment of bacteria in the lower intestine. Improved features of tolerance would shed light on a basic question whether expanded populations of LABs alone (without continuous metabolic stimulation) could account for diminished hydrogen production on lactose rechallenges. We therefore sought to determine if ingestion of a high dose multi species containing probiotic VSL (3) over a longer time period could achieve this goal.

METHODS

This study was approved by our institutional Research and Ethics Committee and all participants gave signed informed consent. They were recruited based on suspicion or knowledge of lactose intolerance and were paid a small compensation fee. Some had been previously tested, others belonged to ethnic groups having a high probability of lactose maldigestion or had a strong perceived history of intolerance. Only healthy subjects were recruited. A history of antibiotic use one month prior to the study, use of narcotics or motility altering agents and pregnancy were exclusion criteria. Each participant underwent two sets of lactose challenge tests (see below), separated by 42 days. Four groups were established and the study data was analyzed blindly. The four groups were as follows: Group one was a control (no intervention), Group 2 was asked to add 60ml of milk twice a day (3 gm lactose BID) for the 42 days, group three received probiotic VSL (3) 2 packages/day and group four received the same dose of VSL (3) and was asked to also consume an additional 60ml of milk twice a day between tests. The study protocol is outlined in figure 1.

Figure 1 The outline of the protocol and interventions by groups is displayed.



The multi-probiotic VSL (3) (Sigma-Tau Pharmaceuticals, Gaitherburg, Maryland,) contains 4 species of lactobacilli (*L. casei, L. plantarum, L acidophilus* and *L. bulgaricus*), 3 species of Bifidobacteria (*B. longum, B. breve and B infantis*) and one strain of Streptococcus salivarius subspecies thermophilus. Each packet as described by the manufacturer contained 900 x 10^9 freeze dried bacteria in the form of a white powder. A random check of viability of packets was carried out and confirmed qualitative live species of LABs as reported in our initial study (Yesovitch et al., 2004). Packets were stored at 4C until used by participants and were instructed to do the same at home. They consumed the contents of each packet in water or juice beginning with 1 packet/day for 3-4 days and increasing the dose to BID by the end of the first week. Participants were asked to return empty packets at the end of 42 days, and were monitored with periodic phone calls to ensure they were compliant with each of the interventions.

All participants completed a 3 day lactose dietary intake (27 items) survey prior to each test that was based on a previously validated questionnaire (Cooper et al., 1995). No attempt was made to alter their usual intake of lactose and in fact the purpose was to evaluate whether any changes were made prior to retesting. In the two groups in which 3g lactose BID was added to the general diet, we asked participants not to include it in calculations with their regular consumption of lactose. The questionnaire results from each test were compared.

After an overnight fast and a low carbohydrate supper, participants presented themselves to our breath hydrogen laboratory. Participants were prohibited from smoking and eating or drinking throughout the extent of breath hydrogen measurements. Quiet sitting, reading or light walking was permitted, as was the consumption of water ad libitum. Participants filled out a structured demographic questionnaire in addition to that which targeted lactose intake (mentioned above). Breath hydrogen and symptoms were recorded at 30 minute intervals (see below).

Breath hydrogen was measured with a validated hand-held H_2 electrochemical sensor (EC60 vitalograph hydrogen breath monitor, Bedfont Scientific Ltd, United Kingdom) (Metz et al., 1976; van der Klei-van Moorsel et al, 1984). The model uses a sealed electrochemical sensor which can detect $H_{2\,in}$ parts per million (ppm) v/v in a range of 0 ppm to 2000 ppm. The average of 3 breaths at each 30 minute interval was measured for a total of 4.5 hours. After an initial baseline measurement (not exceeding 20 ppm), readings were taken and corrected by subtracting the baseline value from that measured at each interval. 50 gm lactose mixed in 200-250 ml of water was used as the challenge agent. The ½ hour results of hydrogen for 4.5hrs were summed (Σ 4.5 hr BH) and used in calculations. All subjects met the criteria of positivity of H_2 >20 ppm above baseline.

Subjects recorded symptoms at each 30 minute interval for the total of 4.5 hours. The three cardinal

symptoms of bloat, gas and cramps were graded on a scale with a range of 0 to 3. In this scale, 0 represented none, 1 represented a mild presence of the particular symptom, 2 represented definite discomfort and 3 represented a severe form of the symptom. Diarrhea, considered to be the most severe expression of sugar intolerance, was rated 0 or 1. The sum of scores at each 30 minute interval for the total of 4.5 hrs (Σ 4.5 hr total symptom score TSS) was used in calculation.

Statistical analysis

Descriptive analyses are reported as means +/- SD for normally distributed continuous variables and medians and ranges (IQR) for skewed distributions. Categorical variables are reported as percentages. We compared change scores for lactose intake, H2, TSS in the four groups using ANOVA, and explored differences between groups using the post-hoc Scheffe test.

RESULTS

Demographic features of all 43 enrolled participants are shown in Table 1. The two groups receiving VSL (3) or VSL (3) and milk were somewhat older and included more women. Lactose intake did vary somewhat between pre test lactose intakes among the four groups. In fact the VSL (3) group took in the most. However, repeat evaluation before the 2nd set of tests did not show appreciable differences between test 1 and test 2 for any of the groups [Pre test 2 median (IQR) lactose intake: Milk; 3(2.2), None; 2.9(6.9), VSL (3); 9.3(16.8), VSL (3)+milk; 3.3(2.0)]. These calculations in the 2 milk intervention groups excluded the additional BID intake of 30ml of milk (3g lactose BID) before the second test.

Individual symptoms of bloating, cramps, gas and diarrhea before and after interventions are displayed in figure 2. The results are combined from each group since overall effects were not significantly different among the groups and we did not specifically evaluate individual symptoms pre and post intervention.

In table 2 we show the numerical differences between each Test 1 and Test 2 with respect to Ó 4.5 hr BH_2; Ó 4.5 hr TSS. While numerical differences were observed, the variation among individuals was large. No statistically significant change in either breath hydrogen or symptom score was observed. In an exploratory analysis comparing the different groups against each other using the Scheffe test, post- hoc test there was no trends towards significant differences between any two subgroups despite the large differences(p ranges 0.27-0.99). While most subjects in the VSL (3) groups complained of nausea in the first week, 3 subjects (2 in the VSL (3) only group and 1 in the VSL (3) + milk group) withdrew from the study due to perceived intolerance of the product. However after approximately 1 week, all other participants adjusted their intake of VSL (3) (by changing the time of intake or the medium in which they consumed the probiotics) or became used to it such that symptoms were minimal. No other subject withdrew later from these 2 groups.

Figure 2. Individual symptom scores of 4 combined groups are shown with median, 10-25% ranges below and 75-90% ranges above. The angled portions of the wasp waist graph represent 95% confidence intervals about the median. The Ï% above and below 10 and 90% limits represent outliers. Individual symptoms before and after interventions are defined as B-bloating, C-cramps, G-gas and D-diarrhea. Overall comparisons did not reveal any significant changes in symptoms between groups. This is because we tested change in symptoms between groups, not pre-post symptoms in a particular group.

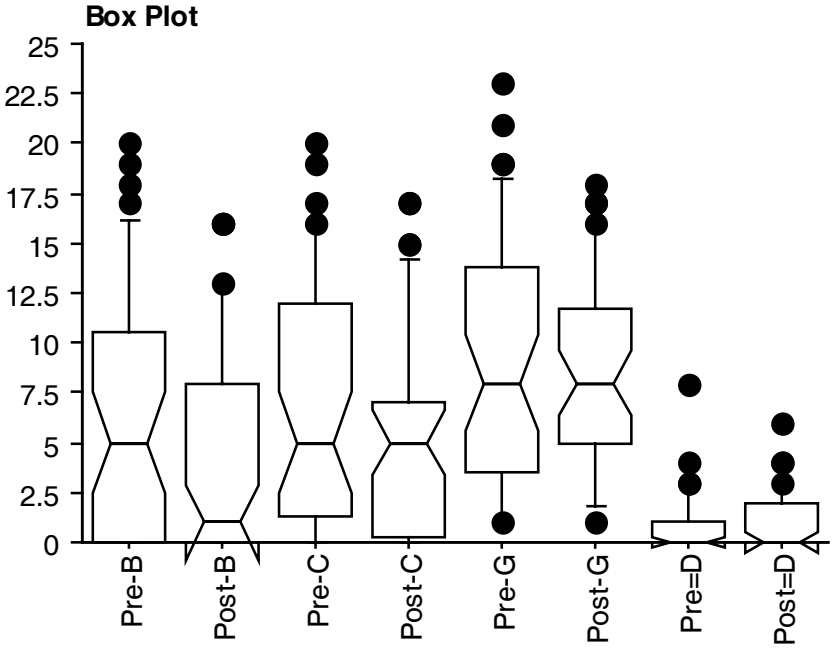


Table 1 Demographic features of 43 participants included in final analysis and divided by intervention. Results for age given as mean ± SD. Results for daily lactose intake are given as median and inter quartile range(IQR).

Group (N)	Age yrs	M : F	Pre-Test 1 lactose intake g/d
Milk (12)	27.8 ± 9.8	7 : 5	2.5(5.3)
None (12)	27.8 ± 12.16	6 : 6	5.0(10.3)
VSL(3)(9)	34.4 ± 11.8	3 : 6	7.4(13.2)
VSL(3)+milk (10)	35.6 ± 14.7	3:7	4.1(5.8)

Table 2 Differences in Σ breath hydrogen (4.5 Hr) and Total symptom scores (4.5 H4) for each of the interventional groups. All results are expressed as means ± SEM. None of the observed changes was statistically significant.

Group (N)	Change in Σ 4.5 Hr BH_2 (ppm)	Change in Σ 4.5 Hr TSS (ppm)
Milk (12)	23.7 ± 40.5	0.6 ± 3.9
None (12)	-19.6 ± 48.4	-6.8 ± 4.5
VSL(3) (9)	46.1 ± 109.0	0.67 ± 1.9
VSL(3) + milk (10)	-69.7 ± 96.2	-10.5 ± 3.96

DISCUSSION

The main finding of this study is that metabolically unstimulated exogenously provided multi-species containing probiotic VSL3 does not improve clinical features of lactose intolerance. A second finding is that a daily lactose intake of approximately 10-13 g does not lead to clinical colonic adaptation to lactose intolerance.

Continued lactose consumption in LNP populations improves features of lactose intolerance(Habte et al., 1973; Sadre and Karbasi, 1979; Villar et al., 1988) and is associated with increased fecal β-galactosidase (Hertzler and Savaiano, 1996). This process could be due to either emerging prominence of lactobacilli and bifidobacteria in fecal flora (i.e. prebiotic effect) or metabolic adaptation or both. All 8 species of probiotics in VSL3 possess β-galactosidase(Anaerobe Laboratory Manual 3rd ed, 1975). At least 3 of these species have been used in vitro experimentally and demonstrate good activity for lactose metabolism (Jiang and Savaiano,1997a, Jiang and Savaiano, 1997b,Welman and Maddox,2003).The species of Streptococcus is used for the making of yogurt (Martini et al,1991).

As far as we are aware, this is the fourth study to attempt to reproduce improvement in lactose intolerance by separately providing a probiotic and a lactose challenge. Hove et al., 1994, showed that simultaneous ingestion of *L acidophilus* and *B. bifidum* together with a 50 g lactose challenge did not result in improvement of breath H_2 despite recovery of bacteria in the stool. Saltzman et al., 1999, using the probiotic L. acidophilus B92FO4 (with high β-galactosidase content) between lactose challenges, also showed there was no significant effect despite recovering bacteria in stool. Our group published a small pilot study using 2 doses of VSL(3) over a 17 day period however it may still not have been a long enough time interval to detect reduction of hydrogen and symptoms(Yesovitch et al., 2004) . In the case of VSL3 an adequate time for embedding in the lower intestine was found to be greater than 20 days(Venturi et al., 1999).Therefore 42 days employed in the current study should have delivered adequate numbers of bacteria. Contrary then to our expectations, we did not detect any improvement in lactose intolerance and reduced hydrogen.

While there are still relatively few trials to base generalizations, taken together the studies using a variety of common probiotic species suggest that clinical colonic adaptation may not be reproduced solely by supplying exogenously provided LABs. As such, there may be a dissociation between lactose responsive fecal microflora and the changes of symptoms and breath hydrogen. Further evidence is reported in a few studies. Fructooligosaccharides increase proliferation of bacteria, but there is no reduction in symptoms or breath hydrogen(Stone-Dorshow and Levitt, 1987). Alternatively, Tannock et al 2004 reported enhanced oligosaccharide metabolism and increased fecal β-galactosidase without altered microbial populations in volunteers. Breath hydrogen and symptoms were not reported however. Therefore the clinical features of adaptation (as reported by Herzler and Savaiano) are not interdependent for general prebiotic effect. Further trials with high β-galactosidase containing probiotics could confirm or repudiate these notions.

Lactose seems to represent one of the few prebiotics, which is able to self-adapt. To date, adaptation to another prebiotic has been observed only with lactulose (Flurie et al.,1993, Szilagyi et al., 2001), which is derived from lactose(Montgomery and Hudson, 1930). Symptomatic improvement however has been also attributed, at least in part to placebo effects of repeated lactose challenge (Briet et al, 1997). That only a part can be attributed to a learning process is suggested by the failure to show uniform

symptomatic improvement in all 4 groups in the current study. Furthermore, the inverse relationship of symptoms during lactose challenge to pretest dietary lactose intake, analyzed in a blinded fashion also suggests that symptomatic improvement is more than a placebo effect(Szilagyi et al, 2005).

Adaptation did not occur however in our subjects with daily intakes of 10-13g lactose (based on the questionnaire and the addition of extra milk). This suggests that lactose must be consumed in higher doses to reach the lower intestine and induce enhanced metabolism even in the presence of an expanded population of LABs. These results are consistent with previous studies. In Japanese women a single ingestion of 10g lactose did not induce diarrhea(Oku et al, 2005). There was no clinical adaptation to lactose ingestion at 10 g/day but did occur at 20g or more/day(Szilagyi et al., 2005). A single dose of 15g/day of lactose increased fecal populations of lactobacilli and bifidobacteria in Japanese volunteers(Ito and Kimura, 1993).

There are some limitations to our study. First, we have no direct evidence of lactase function in the multispecies of probiotics given. We do have indirect evidence that all included species should possess β-galactosidase and several have been used in other in-vitro experiments successfully. Second, we did not have stool microbiological data to support the claim that subjects consumed their assigned intervention. However, all participants returned the correct number of empty packets of probiotics and many complained of some symptoms in the VSL3 consuming groups only suggesting that participants were compliant. Third, our sample size was small. Sample size calculations were based on a previous study that showed a beneficial effect of lactulose on lactose maldigestion between test 1 and test 2(n=9 per group)(Szilagyi et al 2001). While we lost several participants for a variety of reasons, no statistically significant trends regarding reduced hydrogen (the main putative marker of bacterial metabolism) were noted. As a result we do not think that more subjects would have altered outcome.

Assuming these limitations have been overcome, it is still not apparent why increasing populations of LABs (consumate lactose metabolizers) does not lead to improved metabolism of lactose and decreased breath hydrogen and symptoms. Perhaps the naturally occurring increase in LABs occurs at the expense of other lactose-consuming bacteria, and the reduction of breath hydrogen and symptoms only occurs if these other species are reduced in number. For example, Clostridia, bacteroides and E. coli all consume lactose, but unlike LABs, they produce large amounts of hydrogen (Ushijima et al., 1983; Ushijima and Seta, 1991; Chinda et al., 2004). By simply increasing LABs without providing lactose, these other bacterial counts remain high. Therefore, although the lactose osmotic load within the colon may be reduced, the hydrogen production remains high and metabolic adaptation would not be observed. Since measured hydrogen production correlates with symptoms((Szilagyi et al., 2001; Szilagyi et al., 2005; Ladas et al., 1982) it is possible that hydrogen per se contributes to some of the symptoms of intolerance.

In conclusion, we note that expanding populations of specific LABs by exogenous supply of probiotics does not reproduce other attributes of colonic adaptation.

It is possible that other species of high β-galactosidase containing probiotics in future studies may achieve better results. However, concomitant metabolic adaptation may be required as well to achieve this goal. A dose of 10 -13g of lactose a day may not achieve a threshold for colonic spillage and allow such metabolic changes to take place. Further studies of colonic adaptation may shed light on the biological significance of this clinical observation.

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