ABSTRACT: Consumption of milk containing Lactobacillus rhamnosus GG (LGG) has been shown to reduce caries risk in children. We examined whether long-term consumption of juice containing LGG and calcium lactate gluconate (CaLG) affects the caries risk in a double-blind, placebo-controlled setting. The study comprised 530 healthy children, aged 3-6 years. They consumed 5 days a week for 7 months either carrot-pineapple juice containing LGG (Valio Ltd, Finland; 5x10⁶ cfu/ml) and CaLG or control juice. The children’s oral health status was assessed at the baseline and in the end of the study, and salivary Streptococcus mutans counts four times during the study. The caries risk and the need for dental care increased, and high S. mutans counts decreased in both study groups. At the end of the study, the caries risk was not lower (baseline-adjusted OR=0.71, 95% CI 0.38-1.32, p=0.281), but the need of dental care was smaller (baseline-adjusted OR=0.58; 95% CI 0.34-1.00; p=0.050), and there were more children who had all the six sextants healthy (baseline-adjusted OR=1.69, 95% CI 0.99-2.89; p=0.056) in the LGG-CaLG juice group compared to the control juice group. To conclude, supplementation of juice with LGG and CaLG might be beneficial for dental health in children.

KEY WORDS: Calcium, Caries risk, Children, Lactobacillus GG

INTRODUCTION

Dental caries is a chronic infectious disease and common in childhood. Although dental health of children in Finland and other Western countries has improved markedly during the past decades (Rytömaa et al., 1980; Seppä et al., 2002), the children with caries are more severely affected than before (Vehkalahti et al., 1997; Mattila et al., 2000). Use of probiotic bacteria to reduce caries has been under increased interest in recent years. Lactobacillus rhamnosus GG (LGG) is one of the most studied probiotic bacteria and shown to have an inhibitory activity against bacterial species including Streptococcus spp. (Silva et al., 1987; Meurman et al., 1995). In laboratory studies LGG has been shown to reduce or not to ferment sucrose and lactose (Saxelin, 1997; Haukioja et al., 2008). In addition, LGG reduces the adhesion of Streptococcus mutans to saliva-coated hydroxyapatite beads (Wei et al., 1992; 2002), possibly by its coaggregation in the dental biofilm (T wetman et al., 2009), which confirms the anticariogenic effect of LGG.

We have previously shown that milk containing LGG reduces caries risk in children (Näse et al., 2001). Furthermore, we found that a short-term consumption of cheese containing LGG and Lactobacillus rhamnosus LC 705 showed a trend towards reduction of the risk of the highest level of S. mutans bacteria (Ahola et al., 2002). Also other probiotic bacteria have shown similar positive effects on dental caries (Caglar et al., 2005; 2006; 2008). However, cheese and milk themselves are known to have positive effects on dental caries (Harper et al., 1986; Krobicka et al., 1987; Vacca-Smith and Bowen, 2000; Drummond et al., 2002). In the present study we assessed whether the anticariogenic effects of probiotics could even be seen when using sugar-containing juice, which itself has cariogenic potential, as the vehicle of probiotic administration.

Excessive consumption of acid-containing drinks such as soft drinks and juices is associated with an increased risk of dental erosion (Jensdottir et al., 2004; Moazzez et al., 2000). Although water and milk are considered better for dental health, some children occasionally prefer juices. Therefore, formulating sugar-containing acidic drinks with reduced cariogenic and...
erosive potential is a likely approach to support dental health. Enrichment of acidic drinks with minerals such as calcium and phosphorus to reduce their erosive potential has been under increased interest in recent years (Artin et al., 2005; Jensdottir et al., 2005; West et al., 2004). To our knowledge, formulation of an acidic drink with both probiotic bacteria and minerals has not been studied earlier. Therefore, the aim of this double-blind, placebo-controlled intervention study was to examine whether long-term consumption of a juice enriched with LGG and calcium lactate gluconate (CaLG) affects the development of caries and caries risk in day care children.

MATERIALS AND METHODS

Subjects
Thirty day care centres, in similar socio-economic areas in the cities of Espoo and Vantaa, Finland, participated in this randomized, double-blind, placebo-controlled multi-centre study. Altogether 530 healthy children aged 3-6 years volunteered to take part. The parents gave their written informed consent prior the study on behalf of their children. The study was ethically conducted in accordance with the Declaration of Helsinki and the Ethics Committee of the Helsinki City Health Department approved the study protocol.

Intervention
The intervention lasted seven months from September 2002 to May 2003. The children were randomised to the LGG-CaLG juice group or to the control juice group according to a computer generated randomly permuted block-method. In randomisation the block size of four was used, and it was stratified according to age (3-4 years or 5-6 years) and the randomisation the block size of four was used, and it was computer generated randomly permuted block-method. In randomisation the block size of four was used, and it was stratified according to age (3-4 years or 5-6 years) and the S. mutans count (0-1 or 2-3, where 0: no growth, 1: =10^3 CFU/ml, 2: 10^4 CFU/ml, 3: =10^5 CFU/ml).

During the intervention the children drank either carrot-pineapple juice (Gefilus®, Valio Ltd, Helsinki, Finland) enriched with LGG (5 x 10^6 CFU/ml of strain LGG, ATCC 53103) and calcium (CaLG 120 mg/100g), or control carrot-pineapple juice without LGG and calcium. The juices were delivered to the day care centres in cartons labelled as “M” and “R” juices. The study personnel (unless T. P.), the day care personnel, the assistants took samples from the tongue surface by using the Dentocult SM Strip mutans® slides (Orion Diagnostica, Espoo, Finland). The S. mutans counts were measured using the following categories: 0: no growth, 1: =10^3 CFU/ml, 2: 10^4 CFU/ml, 3: =10^5 CFU/ml.

Statistical methods
Calculation of the sample size was based on the assumption that the use of LGG-CaLG juice results in 44% reduction in dental caries risk. As a reference data we used our previous study which showed an odds ratio of 0.56 for LGG in preventing caries (Näse et al., 2001). We assumed that after the intervention period the proportion of moderate or high caries risk would be 27% in the LGG-CaLG juice group vs. 40% in the control juice group. It was further estimated that with a two group continuity corrected, Chi-square test with a 0.050 two-sided significance level would show a 80% power in the detection of the difference between the LGG-CaLG juice group and control juice group (OR=0.56) when the sample size in each is 228. To take the possible dropouts into consideration, 265 subjects per group were randomized.

The primary outcome measure was the caries risk measured in the primary (dmft) and permanent (DMFT) teeth were analysed. The caries risk was considered “low” if no caries was detected and the S. mutans count was <10^5 CFU/ml; “moderate” if the child had either a dmft/DMFT or initial caries score >0 or the S. mutans count ≥10^5 CFU/ml; and “high” if the child had a dmft/DMFT or initial caries score >0 and S. mutans count ≥10^5 CFU/ml.

Saliva samples were cultured on agar and LGG was identified by colony morphology, lactose fermentation test and characteristic cell shapes (Yli-Knuutila et al., 2006).

Lactobacillus rhamnosus GG measurements
Salivary LGG was analyzed at baseline and in the end of the study by using both clinical and microbiological data (S. mutans counts). The sums of decayed, missing and filled teeth in the primary (dmft) and permanent (DMFT) teeth were analysed. The caries risk was considered “low” if no caries was detected and the S. mutans count was <10^5 CFU/ml; “moderate” if the child had either a dmft/DMFT or initial caries score >0 or the S. mutans count ≥10^5 CFU/ml; and “high” if the child had a dmft/DMFT or initial caries score >0 and S. mutans count ≥10^5 CFU/ml.

Examination of oral health
Information about the children’s dental health and dietary habits were collected using a structured questionnaire filled in by the parents. The study coordinator checked the completed questionnaires, and supplied missing data with help of the parents. During the study, the parents recorded daily in a symptom diary any illnesses, dental appointments, and medications of the children.

The children’s oral health was clinically examined at baseline and in the end of the study by an experienced dentist (H. Y.-K.) according to the World Health Organisation criteria from 1987. The use of fluoride varnish was forbidden during the intervention, but all necessary dental treatment and fluoride toothpaste were allowed.

The S. mutans counts were evaluated four times during the study: at inclusion, at baseline (0 months), in the middle (3.5 months), and in the end of the study (7 months). The samples were taken in the day care centres from all the children and always at the same time, i.e. one hour after breakfast or lunch. The research assistants took samples from the tongue surface by using the Dentocult SM Strip mutans® slides (Orion Diagnostica, Espoo, Finland). The S. mutans counts were measured using the following categories: 0: no growth, 1: =10^3 CFU/ml, 2: 10^4 CFU/ml, 3: =10^5 CFU/ml.

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The primary outcome measure was the caries risk measured in the
end of the study. In the final analyses the classes “moderate” and “high” were combined. Logistic regression analyses were performed to compare the LGG-CaLG juice group to the control juice group with respect to moderate/high caries risk or other binary response variables measured in the end of the study. The corresponding baseline status was included as a categorical covariate. As the baseline-adjusted analysis requires complete data, the main analyses include the subjects who participated in all study phases (baseline, middle and end). The results are given as odds ratios (OR) with 95% confidence intervals (CI).

The baseline characteristics were analyzed using t-test for independent samples or Chi-square test, when appropriate. The analyses were performed using SPSS (Version 17.0, the SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics and compliance

Of the 530 study children, 265 were allocated to the LGG-CaLG juice group and 265 children to the control juice group (Figure 1). Thirty-seven children (14%) in the LGG-CaLG juice group and 43 children (16%) in the control juice group dropped out.

The study groups were comparable with respect to the children’s oral health habits and dietary habits (Table 1). However, children in the control juice group used slightly more xylitol products (98% in the control juice group vs. 95% in the LGG-CaLG juice group, p=0.061).

The average daily consumption of juice in the LGG-CaLG juice group was 92 (SD 0.33) ml and in the control juice group 95 (SD 0.31) ml with no difference in the compliance (46% and 47%).

Salivary LGG was analyzed twice in a subgroup of study children (total n=362, LGG-CaLG juice group n=179, control juice group n=183). At baseline, 9.5% of subjects in the LGG-CaLG juice group had detectable amounts of LGG in saliva. At the end of the study the figures were 42.5% versus 17.5% (baseline-adjusted OR=4.09; 95% CI 2.44 to 6.86; p<0.001).

Streptococcus mutans counts

Only few children showed S. mutans infection (Table 2). During

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TABLE 1. Characteristics of the study children, randomized to consume either juice enriched with Lactobacillus GG and calcium lactate gluconate (LGG-CaLG juice group, n=235) or control juice (control juice group, n=238). Figures are percentages or means (range).

<table>
<thead>
<tr>
<th></th>
<th>LGG-CaLG JUICE GROUP</th>
<th>CONTROL JUICE GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls, %</td>
<td>51/49</td>
<td>53/47</td>
</tr>
<tr>
<td>Age, years</td>
<td>5.5 (3.4-6.9)</td>
<td>5.6 (3.3-6.9)</td>
</tr>
<tr>
<td>Oral health habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brushing the teeth, %</td>
<td>&lt;1/day: 3</td>
<td>&lt;1/day: 4</td>
</tr>
<tr>
<td></td>
<td>1/day: 63</td>
<td>1/day: 64</td>
</tr>
<tr>
<td></td>
<td>2/day: 33</td>
<td>2/day: 32</td>
</tr>
<tr>
<td>Parental help brushing, %</td>
<td>83</td>
<td>80</td>
</tr>
<tr>
<td>Age when tooth brushing started, months</td>
<td>11 (0-48)</td>
<td>11 (0-48)</td>
</tr>
<tr>
<td>Use of fluoride toothpaste, %</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>Use of other fluoride products, %</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Use of xylitol-products, %</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>Using dummy, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Age when dummy eating stopped, months</td>
<td>17 (0-48)</td>
<td>17 (0-48)</td>
</tr>
<tr>
<td>Using baby bottle, %</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Age when baby bottle use stopped, months</td>
<td>18 (0-48)</td>
<td>18 (0-72)</td>
</tr>
</tbody>
</table>

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2. The number of children with different Streptococcus mutans scores at the baseline, in the middle and in the end of the study in groups consuming either juice enriched with Lactobacillus GG and calcium lactate gluconate (LGG-CaLG juice group, n=211) or control juice (control juice group, n=220). 1S. mutans scores 10^-10 CFU/ml; 2S. mutans scores 10^-10 - 10^0 CFU/ml; 3S. mutans scores >10^0 CFU/ml.

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<table>
<thead>
<tr>
<th></th>
<th>LGG-CaLG JUICE GROUP</th>
<th>CONTROL JUICE GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0-1</td>
<td>Baseline 190 (90%)</td>
<td>199 (91%)</td>
</tr>
<tr>
<td>Class 2</td>
<td>10 (4.7%)</td>
<td>14 (6.4%)</td>
</tr>
<tr>
<td>Class 3</td>
<td>11 (5.2%)</td>
<td>7 (3.2%)</td>
</tr>
<tr>
<td>Class 0-1</td>
<td>Baseline 196 (93%)</td>
<td>199 (92%)</td>
</tr>
<tr>
<td>Class 2</td>
<td>10 (4.7%)</td>
<td>13 (5.9%)</td>
</tr>
<tr>
<td>Class 3</td>
<td>5 (2.4%)</td>
<td>5 (2.3%)</td>
</tr>
<tr>
<td>Class 0-1</td>
<td>Baseline 199 (94%)</td>
<td>207 (94%)</td>
</tr>
<tr>
<td>Class 2</td>
<td>8 (3.8%)</td>
<td>10 (4.5%)</td>
</tr>
<tr>
<td>Class 3</td>
<td>4 (1.9%)</td>
<td>3 (1.4%)</td>
</tr>
</tbody>
</table>

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CaLG juice and control juice groups were 92 (SD 0.33) and 95 (SD 0.31) ml with no difference in the compliance (46% and 47%).

Salivary LGG was analyzed twice in a subgroup of study children (total n=362, LGG-CaLG juice group n=179, control juice group n=183). At baseline, 9.5% of subjects in the LGG-CaLG juice group and 12.0% in the control juice group had detectable amounts of LGG in saliva. At the end of the study the figures were 42.5% versus 17.5% (baseline-adjusted OR=4.09; 95% CI 2.44 to 6.86; p<0.001).

Streptococcus mutans counts

Only few children showed S. mutans infection (Table 2). During
the intervention, the number of children with high S. mutans counts (score 3) decreased from 5.2% to 1.9% in the LGG-CaLG juice group and from 3.2% to 1.4% in the control juice group. We combined moderate and high S. mutans count groups (scores 2 to 3) for further analyses. In the middle of the study, the number of children in moderate and high count groups had decreased from 10.0% to 7.1% in the LGG-CaLG juice group and from 9.5% to 8.2% in the control juice group (baseline-adjusted OR 0.75; 95% CI 0.30 to 1.87; p=0.537). In the end of the study the percentage had decreased to 5.7% in the LGG-CaLG juice group and to 5.9% in the control juice group (baseline-adjusted OR 0.71; 0.27 to 1.87; p=0.490). The odds ratios indicate a non-significant risk reduction, 25% in the middle of the study and 29% in the end of the study, in favor of the LGG-CaLG group.

**TABLE 4. The number of healthy sextants at baseline and in the end of the study in the groups consuming either juice enriched with Lactobacillus GG and calcium lactate gluconate (LGG-CaLG juice group, n=197) or control juice (control juice group, n=197).**

<table>
<thead>
<tr>
<th>NUMBER</th>
<th>BASELINE</th>
<th>END</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LGG-CaLG juice group</td>
<td>Control juice group</td>
</tr>
<tr>
<td></td>
<td>LGG-CaLG juice group</td>
<td>Control juice group</td>
</tr>
<tr>
<td>0</td>
<td>0 (4.0%)</td>
<td>10 (5.1%)</td>
</tr>
<tr>
<td>1</td>
<td>0 (0.0%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>2</td>
<td>7 (3.5%)</td>
<td>5 (2.5%)</td>
</tr>
<tr>
<td>3</td>
<td>12 (6.0%)</td>
<td>18 (9.1%)</td>
</tr>
<tr>
<td>4</td>
<td>33 (17%)</td>
<td>35 (28%)</td>
</tr>
<tr>
<td>5</td>
<td>27 (14%)</td>
<td>30 (15%)</td>
</tr>
<tr>
<td>6</td>
<td>112 (56%)</td>
<td>99 (50%)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study we examined, for the first time, whether long-term consumption of a juice enriched with LGG and CaLG affects the development of caries in children. At the end of the 7-month study, the need for dental care was smaller and there were more children who had all the six sextants healthy (baseline-adjusted OR=1.69, 95% CI 0.99-2.89; p=0.056, Table 4).
Our positive post-treatment effect confirms earlier observations that LGG may inhibit the colonization of Streptococcus sobrinus (Meurman et al., 1995), a member of the mutans streptococci family. It has also been shown that LGG ferments sucrose very slowly indicating that this bacterium is not cariogenic (Saxelin, 1997; Haukioja et al., 2008). Some probiotic strains seem to inhibit S. mutans in human saliva (Näse et al., 2001; Caglar et al., 2005, 2006, 2008). There are also critical reports (Montalto et al., 2004) and some lactobacilli and bifidobacteria are actually cariogenic (Aas et al., 2008) which emphasizes that the effects are strain specific. In two recent studies LGG is found to ferment glucose (Haukioja et al., 2008; Hedberg et al. 2008). However, amount of glucose in saliva is rather low and LGG did not ferment or fermented only slowly sucrose and lactose in these studies. This characteristic could be one reason why we found stronger positive effects with probiotic milk (Näse et al., 2001) while only a trend with probiotic juice was seen in the present study.

Seven-month intervention is a short time for caries lesions to develop. Therefore we assessed the caries risk with both clinical and microbiological data (S. mutans counts) as has been found practical in our earlier study (Näse et al., 2001). The risk of dental caries was classified as low, moderate or high. The high caries risk decreased in both study groups while the moderate caries risk increased during the intervention. At the end of the study the combined moderate to high caries risk was 31% in the LGG-CaLG juice group and 37% in the control juice group which accords with earlier Finnish studies reporting caries prevalence of 28-41% in this age group (Alaluusua et al., 1983; Mattila et al. 1998). Nevertheless, in the end of the present study the need of dental care was smaller and there were more children who had all the six sextants healthy in the LGG-CaLG juice group compared to the control juice group.

The S. mutans counts from saliva were assessed from samples taken from the upper surface of the tongue as reported by Meurman and Rantonen (1994) from adult population and now found practical also in the present study with children. The moderate and high S. mutans levels decreased in both study groups, although no statistically significant difference was found between the groups. One explanation might be that there were only few children that had caries and S. mutans infection at all. At baseline, high S. mutans counts were detected in 11 out of 211 children (5.2%) in the LGG-CaLG juice group and only in 7 out of 220 children (3.2%) in the control juice group. Corresponding S. mutans counts in our earlier study were 13% in the LGG milk group and 8.6% in the placebo milk group (Näse et al., 2001). Our study juices contained sucrose because the taste of calcium needed to be covered with flavor and sugar is the natural ingredient of juice. The sugar content might be one reason why no statistically significant decrease in the S. mutans counts could be detected during the study.

The high intake of sucrose has been shown to increase the risk of dental caries in children (Ruottilen et al., 2004). In that study the most frequently used food rich in sugar was sweetened dairy products. On the other hand, it has been shown that brushing the teeth twice a day with fluoride toothpaste may have greater impact on caries in young children than restricting sugary foods (Gibson and Williams, 1999). In the present study we found that only 33% of the children brushed their teeth twice a day and 64% brushed once a day. According to a Finnish study only 18% of the 5-year-olds Finns brushes their teeth twice a day (Mattila et al., 1998) which is remarkable less than, for example in the UK, where about 50% of the children brushes their teeth more than once a day (Gibson and Williams, 1999).

In addition to probiotics, our study juice also contained calcium in the form of CaLG. This may explain some of the positive effects. The dissolution of mineral from enamel to the surrounding environment is proportionate to the concentration of calcium and phosphorus. Consequently, the addition of calcium and phosphate to a drink in amounts corresponding to the enamel content can prevent enamel from dissolving (Attin et al., 2005; Jensdottir et al., 2005; West et al., 2004).

The results of the present study showed that consumption of juice containing LGG and calcium might reduce the need for dental care in children. However, no statistically significant differences were observed between the study groups in the caries risk and salivary S. mutans counts. Nevertheless, in the LGG-CaLG juice group there were more children who had all the six sextants healthy, indicating a post-treatment effect that supplementation of juice with probiotics and calcium might be beneficial for dental health in children.

CONFLICT OF INTEREST

S. Pohjavuori, A.J. Ahola and R. Korpela were employees of Valio Ltd, sponsoring the study.

REFERENCES


