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Effect of Long-Term Consumption of a Probiotic Bacterium, *Lactobacillus rhamnosus* GG, in Milk on Dental Caries and Caries Risk in Children

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Key Words

Caries risk · Children · Day-care centres · Dental caries · *Lactobacillus* GG · Probiotic bacterium

Abstract

Lactobacillus rhamnosus GG, ATCC (LGG), has shown antagonism to many bacteria including mutans streptococci. This randomized, double-blind, placebo-controlled intervention study was designed to examine whether milk containing LGG has an effect on caries and the risk of caries in children when compared with normal milk. 594 children, 1–6 years old, from 18 municipal day-care centres were included. The children received the milk with meals from coded containers 5 days a week in the day-care centres for 7 months. The children's oral health was recorded at baseline and at the end, using WHO criteria. The caries risk was calculated based on clinical and microbiological data, comprising mutans streptococcus levels from dental plaque and saliva. The risk was classified as high if the child had a dmft/DMFT or initial caries score >0, and a mutans streptococcus count $\geq 10^5$ CFU/ml. The results showed less dental caries in the LGG group and lower mutans streptococcus counts at the end of the study. LGG was found to reduce the risk of caries significantly (OR = 0.56, p = 0.01; controlled for age and gender, OR = 0.51, p = 0.004). The effect was particularly clear in the 3- to 4-year-olds. Thus, milk containing the probiotic LGG bacteria may have beneficial effects on children's dental health.

Streptoccoci and lactobacilli, the most abundant of the acidogenic species resident in the oral cavity, are associated with the presence and onset of dental caries [Loesche, 1986]. A probiotic bacterium, *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG), isolated from healthy humans, has been shown to produce a substance with potent inhibitory activity on a wide range of bacterial species including *Streptococcus* spp. [Silva et al., 1987]. LGG has been shown to temporarily colonize the mouth and to inhibit in vitro a caries pathogen, *Streptococcus sobrinus* [Meurman et al., 1994; Meurman et al., 1995]. However, it cannot colonize tooth surfaces [Busscher et al., 1999].

LGG ferments sucrose very slowly, but cannot ferment lactose. In general, the beneficial effects of LGG in the human body are well documented, and it is used particularly in stabilizing the gastrointestinal microflora [Gorbach, 1990; Fuller, 1991; Salminen et al., 1993; Perdigon et al. 1995; Wagner et al., 1997; Miettinen et al., 1998; Pelto et al., 1998; Shida et al., 1998; Dunne et al., 1999; Gorbach, 2000; Guandalini et al., 2000]. LGG is now used in dairy products in many countries. This bacterium is thus different from the heterofermentative lactobacilli which have

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been shown to increase caries activity in monkeys [Bowen, 1967].

Milk and other dairy products contain calcium, which is the principal mineral in bones and teeth. Developing tooth tissues are affected by factors associated with the intake and metabolism of calcium and phosphorus. Milk on its own, as a complex colloidal fluid of organic and inorganic compounds, seems to be enamel-protective [Gedalia et al., 1991]. Milk also has 'cariostatic properties when ingested at the same time as a cariogenic challenge' [Bowen and Pearson, 1993]. Calcium lactate, also present in dairy products, has been shown to be anticariogenic [Kashket and Yaskell, 1997]. It can thus be expected that milk would be an ideal vehicle for administering enamel-protective agents for children.

Caries risk assessment is a complex issue. Researchers have been looking for the factors that would enable to predict the development of caries lesions. Many variables have been investigated for their association with the carious process. Risk indicators may include socio-economic factors such as income, psychosocial factors such as health attitudes, oral health habits, clinical variables such as the number of filled teeth, microbiological parameters such as mutans streptococcus and lactobacillus counts, and salivary calcium content [Hausen et al., 1996].

The present study was designed to assess whether the well-documented probiotic LGG bacterium might have beneficial effects also in the oral cavity and be used for long-term caries prevention in children. The specific aim of this placebo-controlled intervention study was to evaluate whether LGG in cow's milk can affect the development of caries and caries risk in day-care children. The hypothesis of the study was that LGG would affect oral microbiota in a positive way and that children receiving this bacterium in milk would develop less caries than children receiving normal milk.

Material and Methods

Outline of the Study

Eighteen municipal day-care centres, situated in equal socio-economical areas in the city of Helsinki, Finland, participated in this randomized, double-blind, placebo-controlled study. This study was part of a larger investigation conducted to examine the effects of long-term consumption of probiotic milk on children's health, especially on gastrointestinal and respiratory infections [Hatakka et al., 2001]. The sample size was estimated to show a 20% reduction in respiratory tract infections. A total of 594 children were randomized to the study or control groups according to a computer-generated blocked randomization list.

The children drank either LGG milk (Gefilus®, Valio Ltd., Riihimäki Dairy, Finland; pasteurized cow's milk containing 1% fat and live *Lactobacillus rhamnosus* GG, ATCC 53103, bacteria $5–10 \times 10^5$ CFU/ml) or control milk in the day-care centres 5 days a week for 7 months, from non-transparent colour-coded milk containers. The control milk came from the same dairy but contained no added lactobacilli. The study period was from the beginning of October 1998 to the end of April 1999. The day-care personnel, parents, children and investigators were unaware of which milk contained the LGG strain throughout the study. The randomization code was not broken until the intention-to-treat analyses were performed. The day-care personnel every day recorded the volume of milk each child drank. The parents of the children kept daily symptom diaries and recorded the children's respiratory and gastrointestinal symptoms, infections diagnosed by doctors and possible antibiotic treatments. The use of other products containing *Lactobacillus* or other pharmaceutical lactic acid bacteria was forbidden for 4 weeks prior to and throughout the intervention.

Clinical Examination, Questionnaire and Microbiological Methods

Experienced dentists in the Helsinki City Health Department examined the children's oral health according to the WHO criteria [WHO, 1987]. The study protocol was assessed in advance in co-operation with the dentists. The same examiner carried out the examination of the same children at baseline and at the end, without reference to the baseline data. The use of fluoride varnish was forbidden during the study, but necessary dental treatment was allowed. Information about the social background, health attitudes and dental care habits of the children was obtained using structured questionnaires filled in by the parents at baseline. The interview was also repeated at the end of the study.

In addition to the WHO guidelines, caries was recorded separately for occlusal, smooth (labial and oral) and approximal surfaces. The parameters studied were active caries (initial and decayed, dt/DT), cumulative caries (dmft/DMFT) and caries in occlusal, smooth and approximal surfaces.

Pooled plaque and saliva samples were taken by rotating a sterile cotton stick along the labial surfaces of the upper incisors, deciduous molars on the left buccal side under the prominence line of the teeth, the dorsal part of the tongue and at the bottom of the mouth, always in this order. The samples were taken in the day-care centres from all children always at the same time, i.e. 1 h after breakfast, at baseline, in the middle and at the end of the study. In addition, an unstimulated saliva sample was taken from the 5- to 6-year-old children with the free-flowing method. The pooled plaque and saliva samples were spread with cotton stick on Dentocult SM Strip mutans® slides (Orion Diagnostica, Espoo, Finland) and cultivated according to the manufacturer's instructions. The slides were counted under a stereomicroscope and scored as instructed by the manufacturer. The saliva samples were placed in Eppendorf tubes, chilled, centrifuged, deep-frozen and stored at –70 °C until further analyses of salivary proteins.

Compliance to the LGG intervention was measured by assessing the faecal colonization with LGG using standard laboratory techniques. Samples from 100 children at baseline and fom 60 children in the middle and at the end of the study were used.

Analysing Caries Risk

Caries risk was determined on the basis of combined clinical and microbiological results. Caries risk was classified into 'high risk' and 'moderate risk' groups. 'High risk' was recorded if the child had a dmft/DMFT or initial caries score >0 and a mutans streptococcus

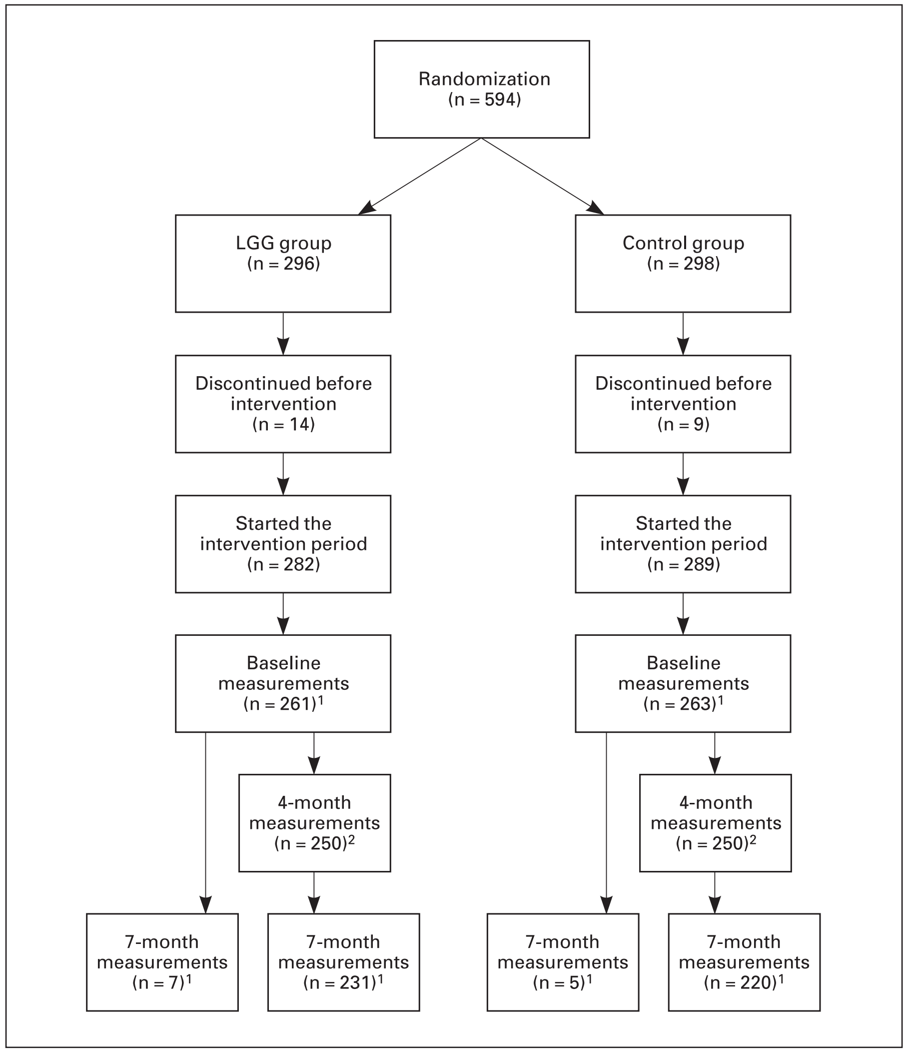

Fig. 1. Flow-chart indicating the progress of participants in the different phases of the 7-month study. [1] Clinical data and oral sample data, [2] oral sample data

count $\geq 10^5$ CFU/ml. 'Moderate risk' was recorded if either dmft/DMFT or initial caries score was >0 or the mutans streptococcus count was $\geq 10^5$ CFU/ml. Caries risk was recorded as 'low risk' if no caries was detected and the mutans streptococcus count was $< 10^5$ CFU/ml.

Statistical Methods

Logistic regression analyses were performed to compare the groups regarding caries and other binary response variables measured at the end of the study. The corresponding caries status at baseline was included as a grouping factor, because the children with increased caries risk factors at baseline developed more caries than those considered as 'low risk' children [Twetman et al., 1994]. Due to the minor differences between groups in age and sex distributions, age and sex were included as covariates when appropriate. The χ^2 test was used for unadjusted analysis.

In addition, age-stratified analyses were performed in order to control the possible confounding effect of age. The children were divided into three age groups: 1–2 years, 3–4 years and 5–6 years. All analyses were based on the intention-to-treat population. As the baseline-adjusted statistical analysis require complete data, the main results came from the children who participated in all study phases from baseline to the final examination. Statistical analyses were performed using the SPSS (Release 9.0) program.

Ethical Aspects

The study protocol had been approved by the Ethical Committee of the Helsinki City Health Department. The parents gave their informed consent on behalf of the children.

Results

The number of children investigated is given in the flow chart (fig. 1). Baseline characteristics and health histories of the children in the LGG and control groups are given in

Table 1. Characteristics of the LGG and control milk groups at baseline

Characteristic	LGG milk group n = 231	Control milk group n = 220
Mean age, years	4.6 (1.3–6.8)	4.4 (1.3–6.7)
1 year, %	6	6
2 years, %	12	13
3 years, %	14	22
4 years, %	23	17
5 years, %	23	27
6 years, %	23	14
Male/female, %	53/47	44/56
Mean number of siblings	1.2 (0–4)	1.0 (0–3)
Mean duration of breast-feeding, months	7.0 (0–32)	7.4 (0–30)
Mean duration of day-care, months	23 (0.5–60)	21 (0.2–66)
Mean house area, m^2	90 (48–250)	92 (34–250)
Smoking in the household, %	30	31
Health status during preceding 12 months		
Respiratory infections		
0–2, %	52	44
3–4, %	35	32
≥5, %	13 (0–13)	24 (0–13)
Gastrointestinal infections		
0–1, %	78	76
≥2, %	22 (0–3)	24 (0–10)
Antimicrobial treatments		
0–1, %	67	63
≥2, %	33 (0–11)	37 (0–9)
History of allergy, as diagnosed by a doctor		
Atopic diseases[a], %	21	22
Allergic eye infection, %	9	6
Food allergy, %	8	9

Figures are means or proportions (%). Figures in parentheses represent ranges.
[a] Allergic rhinitis, atopic eczema or asthma.

table 1. The oral health habits are given in table 2. There was no difference in the questionnaire results when the baseline and end-of-study data were compared.

The mean daily consumption of milk was 218 ml (range 76–312) in the age group of 1- to 2-year-old children in the control group and 248 ml (range 146–364) in the LGG group, 240 ml (range 42–584) in controls and 232 ml (range 65–104) in the LGG group in the age group of 3- to 4-year-old children, 269 ml (range 116–519) and 257 ml (range 105–498), respectively, in the age group of 5- to 6-year-old children. Apart from the age group of 1- to 2-year-olds, no difference was observed in milk consumption between the groups. There was no difference in the use of milk by caries-free and carious children (data not shown).

Results on faecal colonization with LGG showed good compliance. At baseline, LGG was detected in 4% of the controls compared with 12% of the LGG group. The respective percentages at the end of the study were 15 versus 91%.

There was no statistical difference between the groups in the number of antibiotic treatments during the study. 44.6% of the children in the LGG group and 50% in the control group had received antibiotics during the 7-month study period. When antibiotic treatments for upper respiratory tract and middle ear infections were considered, a significantly smaller need was found in the LGG group. These results have been published separately [Hatakka et al., 2001].

Mutans streptococcus levels of the pooled plaque and saliva samples from the different age groups are given in table 3. In the whole study population, the effects of LGG on caries were found to be positive, albeit not statistically significant. The baseline-adjusted odds ratios were: OR = 0.80 (95% CI 0.42–1.52) for $dt/DT > 0$; OR = 0.77

(0.40–1.46) for dmft/DMFT >0; OR = 0.81 (0.47–1.40) for initial caries development; OR = 0.72 (0.33–1.56) for approximal caries; OR = 0.79 (0.43–1.45) for occlusal caries, and OR = 0.61 (0.27–1.36) for smooth-surface caries development.

The age-stratified dental caries results in the LGG and control groups are given in table 4. In the age group of the 3- to 4-year-old children, LGG milk appeared to protect against caries, especially in occlusal surfaces (OR = 0.22, 95% CI 0.04–1.06, p = 0.059, baseline as a covariate). The baseline-adjusted odds ratios for the other caries variables used were: OR = 0.34 (95% CI 0.11–1.04) for dmft/DMFT >0, and OR = 0.34 (95% CI 0.12–1.03) for dt/DT >0. In the LGG group, the calculated caries risk decreased from 40 to 34% in all age groups combined. In the control group, an increase was observed from 39 to 43 %, respectively. Based on the results from the logistic regression analysis, the intervention with LGG was found to reduce the risk of caries statistically significantly: the baseline-adjusted odds ratio was 0.56 (95% CI 0.36–0.88, p = 0.01). When we controlled for age and gender, the corresponding odds ratio was 0.51 (95% CI 0.32–0.81, p = 0.004). The caries risk figures are given in more detail in figure 2a and b.

Table 2. Oral health habits of the children in LGG and control milk groups at baseline

	LGG milk group n = 231	Control milk group n = 220
Fluoride toothpaste, %	90	90
Once per day	71	66
Twice per day	29	34
Fluoride tablets, %	24	22
Once per day	95	93
Twice per day	5	7
Xylitol-products, %	93	91
Occasionally	48	53
1–2 times per day	44	35
>2 times per day	2	3
Drink when thirsty – water, %	36	32
Sugar snacks, %	98	97
Median times per week	3 (1–14)	4 (1–21)
Number of meals per day		
>5 times per day	11	15
Mean number	5 (2–8)	4 (3–8)
Brushing of teeth, %		
0–2 times per day	67	65
>2 times per day	33	35
Parental help with brushing, %	88	88

Figures in parentheses represent ranges.

Table 3. Distribution of children with respect to different mutans streptococcus scores during the study

Study	LGG group (n = 231)						Control group (n = 220)					
	class 0, 1		class 2		class 3		class 0, 1		class 2		class 3	
	n	%	n	%	n	%	n	%	n	%	n	%
1- to 2-year-old children												
Baseline	26	83.9	4	12.9	1	3.2	34	89.5	4	10.5	0	–
Middle	28	90.3	0	–	3	9.7	36	94.7	2	5.3	0	–
End	29	93.5	2	6.5	0	–	32	84.2	5	13.2	1	2.6
3- to 4-year-old children												
Baseline	67	80.7	7	8.4	9	10.9	59	72.9	10	12.3	12	14.8
Middle	72	86.8	7	8.4	4	4.8	70	86.4	5	6.2	6	7.4
End	72	86.8	8	9.6	3	3.6	66	81.5	6	7.4	9	11.1
5- to 6-year-old children												
Baseline	82	70.1	15	12.8	20	17.1	78	77.2	16	15.9	7	6.9
Middle	96	82.1	11	9.4	10	8.5	89	88.1	7	6.9	5	5.0
End	96	82.1	10	8.5	11	9.4	80	79.2	16	15.8	5	5.0

The mutans streptococcus scores were assessed by using Dentocult SM Strip mutans: Class 0 and 1 = <100,000 CFU/ml; Class 2 = >100,000–<1,000,000 CFU/ml; Class 3= ≥1,000,000 CFU/ml. 1- to 2-year-old children: LGG group, n = 31; controls, n = 38; 3- to 4-year-old children: LGG group, n = 83; controls, n = 81; 5- to 6-year-old children: LGG group, n = 117; control, n = 101.

Table 4. Dental caries in the LGG and control milk groups at baseline and end of the study

	1- to 2-year-old children					3- to 4-year-old children					5- to 6-year-old children				
	LGG (n = 31)		control (n = 38)		p value	LGG (n = 83)		control (n = 81)		p value	LGG (n = 117)		control (n = 101)		p value
	n	%	n	%		n	%	n	%		n	%	n	%	
dt/DT>0															
Baseline	0		0			3	3.6	4	4.9		10	8.5	10	9.9	
End	2	6.5	1	2.6	0.45	6	7.2	14	17	0.059	18	15	15	5	0.71
dmft/DMFT>0															
Baseline	0		0			3	3.6	7	8.6		16	14	17	17	
End	2	6.5	1	2.6	0.45	8	9.6	19	23	0.057	24	21	22	22	0.76
Initial caries															
Baseline	1	3.2	2	5.3		18	22	21	26		29	25	26	26	
End	2	6.5	4	10.5	0.64	17	20	23	28	0.30	33	28	30	30	0.85
Approximal caries															
Baseline	0		0			2	2.4	8	9.9		13	11	15	15	
End	0		0			6	7.2	12	15	0.72	16	14	20	20	0.37
Occlusal caries															
Baseline	0		2	5.3		15	18	16	20		20	17	20	20	
End	4	12.9	3	7.9	0.15	14	17	21	26	0.059	21	18	21	21	0.75
Smooth surface caries															
Baseline	1	3.2	0			8	9.6	7	8.6		20	17	6	5.9	
End	0		3	7.9	0.86	7	8.4	10	12	0.21	20	17	9	8.9	0.85

Discussion

Our results showed that the LGG milk had a beneficial effect on dental caries, especially in the 3- to 4-year-old children. Caries is known to correlate well with the number of oral lactobacilli in children [Granath et al., 1994]. In general, lactobacilli colonize acidic environments such as fissures and interdental spaces, and produce acids from fermentable sugars. Mutans streptococci and lactobacilli are often found together in carious lesions [Babaahmady et al., 1998]. However, in this study, occlusal, approximal and smooth-surface caries did not increase, even though lactobacilli were given to the children. This was assumed to be due to the inhibiting effect of LGG on mutans streptococci and other lactobacilli, as given in our study hypothesis. The hypothesis was confirmed by the present results.

This study was the first clinical long-term trial on the effects of a probiotic bacterium on dental caries and caries risk. Our definition of caries risk was based on clinical and/or microbiological examination. The method might be used for preschool children in clinical practice, too. A saliva sample positive for *S. mutans* correlates with clinical findings on caries [Splieth and Bernhardt, 1999]. Caries experience and/or mutans streptococcal levels in primary dentition may also be indicators of caries in permanent dentition [al-Shalan et al., 1997]. Plaque is an indicator of caries risk in 3-year-old children and therefore should be a key factor in health education [Mattila et al., 1998]. A pooled plaque and saliva sample produces more exact data about oral cariogenic bacteria than the mutans streptococcal level in saliva alone. It has also been shown that mutans streptococcal clones may selectively colonize specific hard-tissue sites [Grönroos and Alaluusua, 2000]. A modified chair-side test for determining mutans streptococcus levels, as we used in the present study, combined with clinical examination and structured questionnaires for parents might be recommended for the assessment of caries risk in day-care children.

The 7-month intervention time was short for caries to progress. In industrialized countries, where fluoride prophylaxis is commonly employed, caries progression is slow. Also, the number of mutans streptococci varies under different biochemical conditions [Bowden and Hamilton, 1998]. A change in the number of initial caries lesions of the 3- to 4-year-old children in the LGG group could indeed be due to an effect on *S. mutans*, because initial caries is known to correlate with mutans streptococcus counts [Babaahmady et al., 1998].

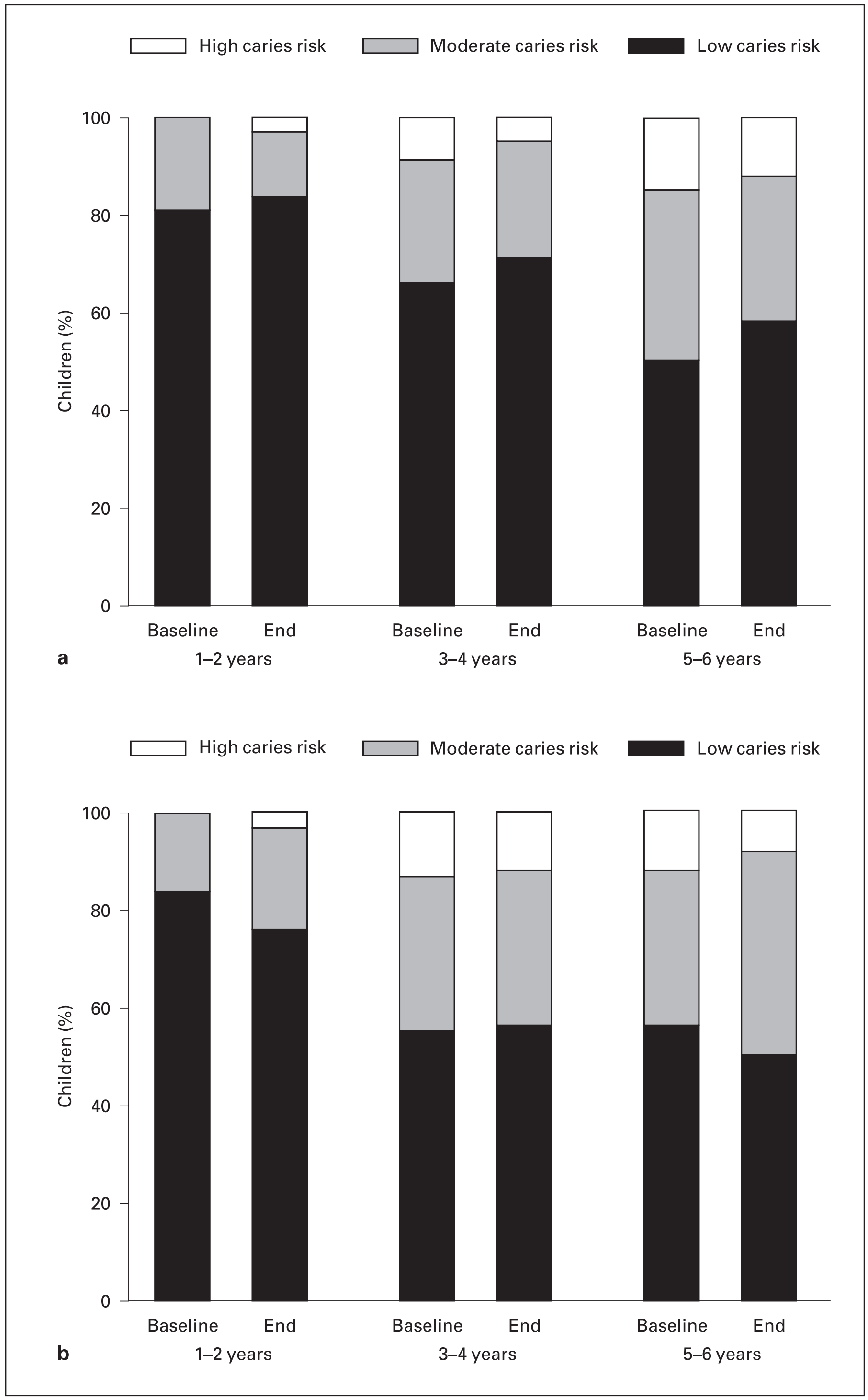

Fig. 2. Effects of LGG milk (**a**) and control milk (**b**) on caries risk in day-care children in three age groups, at baseline and at the end

Dairy products such as milk and cheese have been shown to be anticariogenic in humans by increasing the calcium content in plaque [Gedalia et al., 1991; Moynihan et al., 1999], but still, a clear difference was observed in caries development and caries risk calculations in the present study where both the test and control children drank milk. Thus the effect must have been due to the intervention of LGG and the unmodified milk could be considered as placebo in this respect.

LGG may also compete with other oral microorganisms by producing antimicrobial substances [Silva et al., 1987], such as pyroglutamic acid [Huttunen et al., 1995]. Mice that were infected with lactobacilli, but were free from streptococci and enterococci had a lower incidence of colonization by *S. gordonii*, according to Loach et al. [1994]. LGG has also been shown to increase humoral immunity [Perdigon et al., 1995], and antibodies against it have been detected in the saliva of experimental animals [Negretti et al., 1997]. Consequently, the mechanisms of action of a probiotic bacterium may be manifold also in the oral cavity.

LGG belongs to the homofermentative lactobacilli, which cannot ferment sucrose or lactose. It does not enhance caries and could therefore be safe for the teeth. Thus, LGG in milk could indeed be a promising preventive method for combating caries and reducing caries risk.

Conclusion

Milk containing the probiotic bacterium LGG was found to have a beneficial effect on children's dental health, especially at the age of 3-4 years. The consumption of LGG-containing milk might be considered as a new vehicle for improving the oral health of day-care children.

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