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Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria

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SUMMARY Previous studies have suggested that probiotic supplements in dairy products may affect the oral microbial ecology, but the effect in orthodontic patients has not previously been reported. The aim of the present study was to examine whether short-term consumption of fruit yogurt containing probiotic bifidobacteria would affect the levels of salivary mutans streptococci and lactobacilli in patients with fixed orthodontic appliances.

A double-blind, randomized crossover study was performed and 24 healthy adolescents (12–16 years) undergoing orthodontic treatment were followed over four periods. During periods 2 and 4 (2 weeks each), the subjects ingested 200 g fruit yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010 (2×10^8 colony forming units/g) once daily or a control yogurt without viable bacteria. Periods 1 and 3 were run-in and wash-out periods of 1 and 6 weeks, respectively. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits before and after the yogurt consumption periods. Pre- and post-treatment values within each regimen were compared with a two-tailed marginal homogeneity test for categorical data.

A statistically significant reduction of salivary mutans streptococci was recorded after probiotic yogurt consumption ($P < 0.05$), which was in contrast to the control yogurt. No significant alterations of the salivary lactobacilli counts were observed.

Short-term daily consumption of fruit yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010 may reduce the levels of mutans streptococci in saliva during orthodontic treatment with fixed appliances.

Introduction

Fixed orthodontic appliances are considered to jeopardize dental health due to accumulation of microorganisms that may cause enamel demineralization, clinically visible as white spot lesions (Mitchell, 1992). Furthermore, the complex design of orthodontic bands and brackets may create an ecological environment that facilitates the establishment and growth of cariogenic mutans streptococci strains (Ahn *et al.*, 2007). White spot lesion formation can be seen as an imbalance between mineral loss and mineral gain (Featherstone, 2000) and recent systematic reviews have examined methods to prevent this side-effect of orthodontic treatment (Benson *et al.*, 2004; Derks *et al.*, 2004). Apart from fluoride exposure, very limited evidence was found and it was concluded that more high-quality clinical research would be needed to give evidence-based advice on the optimal caries-preventive strategy during orthodontic treatment.

Probiotic bacteria are live microbial food supplements that beneficially affect the host by improving its intestinal balance, and have emerged as an alternative way to combat infections (Rasić, 1983; Doron and Gorbach 2006). The key event is that harmless microorganisms, such as strains of lactobacilli or bifidobacteria, can occupy a space in a biofilm that otherwise would be colonized by a pathogen. Bifidobacteria are the predominant anaerobic bacteria within the intestinal lumen and play a critical role for maintaining equilibrium of the normal gut flora (Fuller, 1991). A number of probiotic-induced benefits on general health have been proposed, among them reduced susceptibility to infections, reduction of allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values (Reid *et al.*, 2003). The possible impact on oral health is, however, less explored and most studies have focused on installation or effect on oral microorganisms (Caglar *et al.*, 2005a). Clinical studies with probiotic strains such as *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, *Bifidobacterium* DN-173 010, or a lactobacilli mix have consistently displayed a reduced prevalence of caries-associated mutans streptococci in saliva (Meurman and Stamatova, 2007).

The possible effect of probiotics on the salivary microflora of orthodontic patients does not appear to have been previously reported. The aim of the present study was

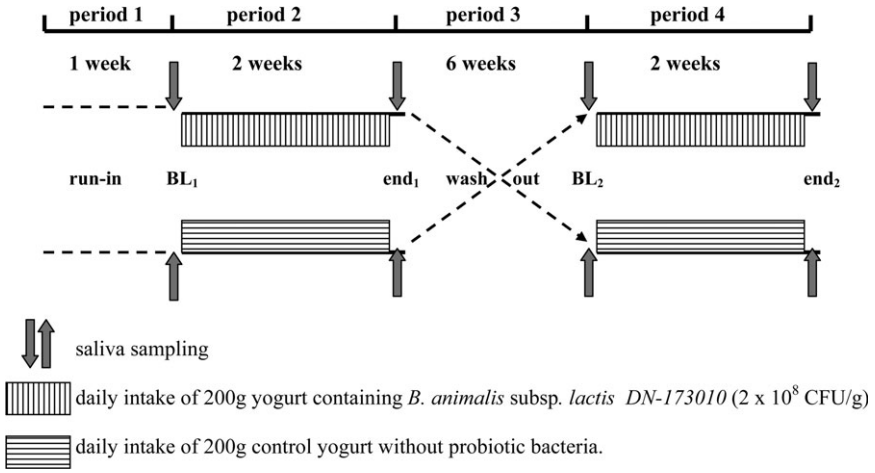

Figure 1 Outline of the crossover study design with run-in and wash-out periods. BL denotes the pre-treatment baseline sampling and end represents the post-treatment sampling.

therefore to examine whether short-term consumption of fruit yogurt containing bifidobacteria would affect the levels of salivary mutans streptococci and lactobacilli in patients with fixed appliances. The null hypothesis was that the probiotic fruit yogurt would not alter the bacterial levels.

Subjects and methods

Study group

The material comprised 26 healthy non-medicating adolescents (18 females, 8 males), 12–16 years of age (mean: 14 ± 1.2 years), who had been undergoing bimaxillary treatment with fixed orthodontic appliances for approximately 3 months. The patients volunteered after verbal and written information, and informed consent was obtained from their parents. Habitual consumers of xylitol chewing gums and subjects with systemic antibiotic or topical fluoride treatments within 4 weeks were not included. All subjects had good dental health with no active untreated carious lesions and reported daily tooth brushing habit using fluoridated toothpaste. During period 4, two girls did not wish to continue their participation; therefore, the entire protocol was completed by 24 subjects.

Study design

The study was a double-blind, randomized crossover design and consisted of four consecutive time periods as shown in Figure 1. The protocol was in accordance with the Helsinki Declaration of Human Rights and approved by the Ethical Committee at the School of Dentistry, University of Yeditepe, Istanbul, Turkey. During periods 2 and 4 (2 weeks each), the subjects were instructed to consume 200 g fruit yogurt per day containing either bifidobacteria or a control yogurt without probiotic bacteria. Periods 1 and 3 were run-in and wash-out periods of 1 and 6 weeks, respectively, in which the subjects were requested to refrain from all yogurt consumption. The subjects were, however, encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth.

Test and control yogurts

The probiotic strawberry fruit yogurt (Activia Çilekli Meyveli, Danone, Istanbul, Turkey) contained *Bifidobacterium animalis* subsp. *lactis* DN-173010, while the control yogurt (Çilek Meyveli) was without viable bacteria. The probiotic strain was originally isolated from a healthy human and added to the yogurt at a concentration of 2×10^8 colony forming units (CFU)/g by the manufacturer. The daily intake was 200 g and the subjects were advised to eat the yogurt at dinnertime. No tooth brushing was allowed for at least 1 hour after yogurt consumption. The test and the control yogurts, given in a randomized order, had a similar taste and consistency and were delivered in white cups marked 'A' or 'B'. The content was unknown to either the test subjects or the clinician responsible for the samplings and the code was not revealed until after the statistical calculations.

Saliva sampling and microbial evaluation

Sampling of stimulated whole saliva was carried out immediately before and after periods 2 and 4. After thorough rinsing with water, the subjects were asked to chew on a piece of paraffin wax for 5 minutes and the saliva was collected directly into a graded test tube. The secretion rate was calculated as millilitre/minute. The counts of salivary mutans streptococci and lactobacilli were estimated with a chair-side test (CRT, Ivoclar Vivadent AG, Schaan, Liechtenstein) according to the manufacturer's instructions. In brief, saliva was inoculated on a dip-slide with selective agar media for mutans streptococci and lactobacilli. After

adding a $NaHCO_3$ tablet to the tube, the dip-slides were immediately cultivated at 37°C for 48 hours. The colonies were identified by morphology with the aid of a stereomicroscope with ×10 magnification and the density of the CFU (CFU/ml) was visually compared with the aid of a chart provided by the manufacturer. The scores used for classification are shown in Tables 1 and 2.

Statistical analysis

The data were processed with the Statistical Package for Social Sciences (version 14.0, SPSS Inc., Chicago, Illinois, USA). Pre- and post-treatment values within each regimen were compared with a two-tailed marginal homogeneity test for categorical data. Statistical significance level was established at $P < 0.05$.

Results

The stimulated saliva secretion rate exceeded 1.4 ml/minute for all subjects. The pre- and post-treatment levels of salivary mutans streptococci and lactobacilli are given in Tables 1 and 2. All subjects exhibited detectable levels of salivary mutans streptococci and lactobacilli at baseline and over 60 per cent had levels $\geq 10^5$ CFU. There was no significant difference between the baseline values obtained at the start of periods 2 and 4. A statistically significant ($P < 0.05$) reduction of salivary mutans streptococci was registered after 2 weeks' consumption of the test yogurt, while no alterations were found in the control group. During the test periods with the probiotic test yogurt, the number of subjects with high mutans streptococci count decreased from 63 to 21 per cent. Seventeen subjects exhibited decreased scores, six had unchanged scores, while one displayed an increased score. During the control period, five subjects displayed a decreased score while 19 had an unchanged score.

Regarding salivary lactobacilli, no statistically significant changes were observed between the pre- and post -consumption samples, either during the test yogurt or control yogurt regimen periods. During both periods, 15 subjects had unchanged scores, two showed an increase and seven a decrease.

Table 1 Distribution of salivary mutans streptococci in adolescents with fixed orthodontic appliances at baseline and after 2 weeks' consumption of probiotic and control fruit yogurt. Values denote the number of subjects ($n = 24$).

Time	*Mutans streptococci* score, CFU/ml			
	$<10^3$	$10^3 < 10^5$	10^5–10^6	$>10^6$
Probiotic fruit yogurt				
Baseline (pre-consumption)	0	9	10	5
2 weeks (post-consumption)*	12	7	4	1
Control fruit yogurt				
Baseline (pre-consumption)	0	10	12	2
2 weeks (post-consumption)	1	11	12	0

*Post-consumption distribution statistically different from baseline, $P < 0.05$.

Table 2 Distribution of salivary lactobacilli in adolescents with fixed orthodontic appliances at baseline and after 2 weeks' consumption of probiotic and control fruit yogurt. Values denote the number of subjects ($n = 24$).

Time	Lactobacilli score, CFU/ml			
	$\leq 10^3$	10^4	10^5	$\geq 10^6$
Probiotic fruit yogurt				
Baseline (pre-consumption)	0	8	15	1
2 weeks (post-consumption)	0	11	13	0
Control fruit yogurt				
Baseline (pre-consumption)	0	6	17	1
2 weeks (post-consumption)	0	8	16	0

Discussion

The present study was undertaken to investigate the effect of fruit yogurt containing probiotic bacteria on caries-associated microorganisms in saliva. The novel approach used in the present research included subjects with increased caries risk. It is well established that the prevalence of mutans streptococci and lactobacilli is increased in plaque and saliva during treatment with fixed appliances which may lead to white spot lesion formation (Scheie *et al.*, 1984; Rosenbloom and Tinanoff, 1991). In the present study, the intervention started 3 months after the insertion of the appliances to avoid the confounding effect of an immediate decrease in bacterial counts that may take place at appliance insertion (Scheie *et al.*, 1984). The counts of salivary mutans streptococci and lactobacilli were estimated using a simple chair-side test. Previous studies have shown that the method correlates well with conventional laboratory methods ($r = 0.76$; Twetman *et al.*, 2000; Karjalainen *et al.*, 2004; Tanabe *et al.*, 2006). Fruit yogurt was chosen as the vehicle for the probiotic supplement because of its high buffer capacity and non-erosive and low cariogenic potential (Mundorff *et al.*, 1990; Moynihan *et al.*, 1996; Caglar *et al.*, 2006a; Kargul *et al.*, 2007). Moreover, yogurt is commonly preferred by adolescents and no compliance problems were experienced. It should, however, be noted that one subject who reported a possible allergy to strawberries was provided with a plum fruit yogurt. No side or adverse effects were registered during the trial.

The results showed that daily consumption of probiotic yogurt for 2 weeks decreased the mutans streptococci counts in saliva and reinforced previous findings with bifidobacteria- and lactobacilli-derived probiotics (Caglar *et al.*, 2005b, 2006b, 2007, 2008). Thus, the null hypothesis concerning

mutans streptococci was rejected. In accordance with previous studies, no effects on the levels of salivary lactobacilli were noted. The duration of the reduced streptococci counts is not known but apparently, it was less than 6 weeks as indicated by the period 4 baseline sampling after the wash-out period. The reason for the bacteria-hampering effect is not fully known but may be explained by a combination of local and systemic immune response as well as non-immunologic defence mechanisms (Meurman, 2005). The principal health promoting effects are ascribed to enhancement of the mucosal immune defence and macrophage activity as well as elevations of the numbers of killer cells, T-cells, and interferon (Fuller and Gibson, 1997). Bifidobacteria are gram-positive, aciduric and lactic acid-producing species of particular importance since they represent 3–7 per cent of the total population of the intestinal tract (Biavati and Mattarelli, 2001). In the oral cavity, bifidobacteria may play a role in deep dentine caries progression but not in enamel demineralization (Becker *et al.*, 2002). The ecological niche and microbial profile adjacent to an orthodontic bracket may offer access for direct bacterial replacement in which *S. mutans* species are displaced by less harmful bifidobacteria. This local event has been elucidated *in vitro* and it was shown that bifidobacteria could survive in saliva and bind to *Fusobacterium nucleatum*-covered hydroxyapatite (Haukioja *et al.*, 2006).

Probiotic bacteria do not permanently colonize the oral cavity, or the intestines, and must be taken in sufficient amounts on a daily basis (Meurman, 2005; Yli-Knuuttila *et al.*, 2006). While a recent study demonstrated recovery of viable *Bifidobacterium DN-173010* cells in faeces during administration of fermented milk (Collado *et al*., 2006), no attempt was made to detect the strain in the oral cavity. The reason for the demonstrated difference between the reduction seen in the streptococci and the lack of reduction in lactobacilli is not clear. Apart from strain-specific differences, a possible explanation is that mutans streptococci normally grow on exposed surfaces, easy accessible for the probiotic substrate, while lactobacilli are recovered in shed retentive areas with limited contact with the ingested yoghurt.

The findings must, for a number of reasons, be interpreted with caution. First, the sample size was limited and caries-associated bacteria in saliva should be regarded as an intermediate end point for caries. It remains unclear whether or not this really is beneficial for patients. Furthermore, the semi-quantitative nature of the microbial estimations was a limitation. Second, there are no long-term studies available on the effect of probiotic bacteria on the oral microflora, and third, the optimal daily dose is not yet established. Finally, head-to-head tests of different probiotic strains are still lacking and it is possible that a combination of probiotic strains could be even more effective. In any case, it seems obvious that adolescent patients with fixed orthodontic appliances constitute a very suitable group for further studies of risk patients concerning enamel mineralization and probiotic supplements. If successful, the probiotic home-care intervention may be a cost-effective alternative to a professional topical programme for white spot lesion prevention during orthodontic treatment.

Conclusion

The present study demonstrated that daily consumption of fruit yogurt with *Bifidobacterium animalis* subsp. *lactis* DN-173010 could reduce the salivary levels of mutans streptococci in orthodontic patients with fixed appliances. Further studies are needed to clarify if this approach is an alternative strategy for the prevention of demineralization and white spot lesion formation during orthodontic treatment.

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