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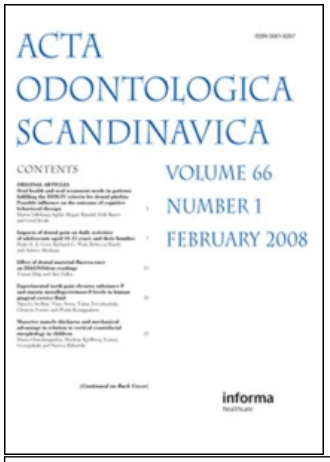

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Esber Çaglar [a]; Ozgur Onder Kuscu [a]; Senem Selvi Kuvvetli [a]; Sule Kavaloglu Cildir [a]; Nuket Sandalli [a]; Svante Twetman [b]
[a] Department of Paediatric Dentistry, Dental School, Yeditepe University, Istanbul, Turkey
[b] Department of Cariology and Endodontics, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

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ORIGINAL ARTICLE

Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and lactobacilli

ESBER ÇAGLAR[1], OZGUR ONDER KUSCU[1], SENEM SELVI KUVVETLI[1], SULE KAVALOGLU CILDIR[1], NUKET SANDALLI[1] & SVANTE TWETMAN[2]

[1]*Department of Paediatric Dentistry, Dental School, Yeditepe University, Istanbul, Turkey and* [2]*Department of Cariology and Endodontics, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark*

Abstract

Objective. Probiotic bacteria are thought to reduce the risk of some infectious diseases. The aim of the present study was to examine whether or not short-term consumption of ice-cream containing bifidobacteria can affect the salivary levels of mutans streptococci and lactobacilli in young adults. ***Material and methods***. A double-blind, randomized crossover study was performed and 24 healthy subjects (mean age 20 years) were followed over 4 periods. During periods 2 and 4 (10 days each), they ingested 100 ml (53 g) ice-cream containing *Bifidobacterium lactis* Bb-12 once daily or a control ice-cream without viable bacteria. Periods 1 and 3 were run-in and washout periods, respectively. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits at baseline and immediately after the intervention period. ***Results.*** A statistically significant reduction ($p < 0.05$) of salivary mutans streptococci was recorded after consumption of the probiotic ice-cream. A decline of high mutans streptococci counts was also seen after intake of the control ice-cream, but the difference compared to baseline was not statistically significant. The salivary lactobacilli levels were unaltered after both regimes. ***Conclusions***. Daily consumtion of ice-cream containing probiotic bifidobacteria may reduce the salivary levels of mutans streptococci in young adults.

Key Words: *Bacteria, caries prevention, probiotics, saliva*

Introduction

Probiotic bacteria are live microbial food supplements that beneficially affect the host by improving its intestinal balance [1,2]. The key event is that non-pathogenic microorganisms, such as strains of lactobacilli or bifidobacteria, can occupy a space in a human biofilm that otherwise would be colonized by a pathogen. Bifidobacteria are naturally occurring in the oral cavity [3] and among the predominant anerobic bacteria within the intestinal lumen. A body of evidence suggests that bifidobacteria play a critical role maintaining equilibrium of the normal gut flora [4], and a number of probiotic-induced benefits on general health have been proposed, such as reduced susceptibility to infections, reduction of allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values [5]. The possible impact on oral health is less explored, however, with most studies focusing on installation or effect on oral microorganisms [6–8]. In a previous study, our group demonstrated a reduced prevalence of caries-associated mutans streptococci in the saliva of young adults after daily intake of yogurt containing the probiotic strain *Bifidobacterium* DN-173010 [9].

Although dairy foods such as yogurt, milk, and cheese are considered useful vehicles for probiotic delivery, the best way of administration has yet to be identified [7]. Ideally, the delivery should be suitable for all ages and especially for very young children, since it has been suggested that exposure early in life may facilitate a permanent installation of health-promoting strains [10–12]. In this context, ice-cream could be an interesting carrier of probiotic food, with reported advantagous properties [13,14]. Moreover, the clinical effects may vary significantly from one probiotic strain to the other, and, to our knowledge, the possible effect of *Bifidobacterium lactis* Bb-12 on the oral microflora has not been reported. The aim

Correspondence: Svante Twetman, Department of Cariology and Endodontics, Faculty of Health Sciences, University of Copenhagen, Nørre Allé 20, DK-2200 Copenhagen N, Denmark. Tel: +45 3532 6810. Fax: +45 3532 6505. E-mail: stw@odont.ku.dk

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of the present study was therefore to examine whether short-term consumption of ice-cream containing *Bifidobacterium lactis* Bb-12 can affect the levels of salivary mutans streptococci and lactobacilli in young adults. The null hypothesis was that the probiotic ice-cream would not alter the bacterial levels.

Material and methods

Subjects and study design

The study group comprised 24 healthy non-medicating subjects (10 F and 14 M) aged 20 years who volunteered after giving informed consent. To be considered for invitation, subjects had to have good oral health with no untreated caries lesions and daily toothbrushing habits using fluoride toothpaste. The exclusion criteria were: (i) habitual consumption of probiotics or xylitol chewing gums, (ii) systemic antibiotic medication within 6 weeks, and (iii) recent repeated topical fluoride treatments. Prior to baseline, the subjects received both oral and written information about the study and signed a consent form. The sample size was estimated from a power calculation ($\alpha = 0.05$ and $\beta = 0.20$) based on our previous findings using categorized bacterial scores. One male subject failed to complete the full study protocol and dropped out during period 3 as a result of illness.

The study had a double-blind, randomized, cross-over design and the experimental period comprised four consecutive time periods. Periods 1 and 3 were run-in and washout periods of 1 and 2 weeks, respectively, in which the subjects were asked to refrain from intake of milk-based, probiotic and xylitol-containing products. During periods 2 and 4, each of 10 days' duration, the subjects were instructed to eat a cup of ice-cream per day containing either probiotic bifodobacteria or a control ice-cream without viable bacteria. The subjects were encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth twice a day during all four periods. The study protocol was approved by the School of Dentistry Ethics Committee at the University of Yeditepe.

Intervention

The probiotic ice-cream, Max Star Cup® (Algida Türkiye, Çorlu, Turkey), contained *Bifidobacterium lactis* Bb-12® bacteria (1×10^7 colony forming units per gram), while the control ice-cream, Max Star Cup® (Algida Türkiye, Çorlu, Turkey), was without viable bacteria. Daily intake was 100 ml (53 g) on a single occasion and the subjects were requested to eat the ice-cream at noon. No toothbrushing was allowed for 1 h after intake. The test and the control products were given in random order; they had similar taste (vanilla choclate) and consistency and were delivered in white cups marked "A" or "B". The content was unknown to the test subjects and to the clinician responsible for the samplings. The code was not unveiled until after the statistical calculations.

Microbial evaluation

Samplings of paraffin-stimulated whole saliva were carried out immediately before (baseline) and after (follow-up) periods 2 and 4. After a thorough rinse in water, the saliva was collected, over the course of 5 min, directly into a graded test tube. The counts of salivary mutans streptococci and lactobacilli were evaluated using the CRT® test (Ivoclar Vivadent AG, Schaan, Liechtenstein) in accordance with the manufacturer's manual. Briefly, saliva was inoculated on a dip-slide with selective agar media for mutans streptococci and lactobacilli. After adding a $NaHCO_3$ tablet, the slides were cultivated at 37°C for 48 h. The colonies were identified by morphology with the aid of a stereomicroscope with $10\times$ magnification, and the density of the colony forming units (CFUs) was visually compared against a chart provided by the manufacturer. The scores used for mutans streptococci and lactobacilli growth are given in Tables I and II.

Statistical method

The data were processed with the SPSS® software (version 14.5, Chicago, Ill., USA). Comparisons of bacterial scores between and within the regimes (follow-up versus baseline) were performed with a two-tailed chi-square test for categorical data. A p-value <0.05 was considered statistically significant.

Results

The baseline and follow-up levels of salivary mutans streptococci and lactobacilli are given in Tables I and II. All except four subjects exhibited detectable levels of salivary mutans streptococci at baseline and around 60% exhibited levels $\geq 10^5$ CFU. The baseline mutans streptococci scores were slightly lower before the control regime compared to the test period, but the difference was not statistically significant ($p > 0.05$). A statistically significant ($p < 0.05$) reduction of salivary mutans streptococci was registered after the 10-day consumption of probiotic ice-cream. A certain decline of high mutans streptococci counts ($\geq 10^5$) was also evident after intake of the control ice-cream, but the difference compared to baseline was not statistically significant. During the period of test product consumption, the number of subjects with high mutans streptococci counts ($\geq 10^5$ CFU) decreased from 56% to zero. Seventeen exhibited decreased scores

Table I. Salivary mutans streptococci score at baseline and at follow-up after intake of probiotic ice-cream or control. Values in table denote the number of subjects.

	Mutans streptococci score, CFU/ml			
Time	No growth	$<10^5$	10^5–10^6	$>10^6$
Probiotic ice-cream ($n=23$)				
Baseline	0	10	10	3
Follow-up	8	15	0	0
Control ice-cream ($n=24$)				
Baseline	4	8	8	4
Follow-up	9	9	6	0

Chi-square test for probiotic ice-cream (follow-up vs. baseline): $p<0.0005$; contingency coefficient 0.569.
Chi-square test for control ice-cream (follow-up vs. baseline): $p=0.09$ (NS); contingency coefficient 0.340.

(1–3 steps) and 6 had an unchanged score. During the control regime, 13 subjects displayed a 1–2 step decrease, 9 had unchanged scores, while 2 were registered with an increased score.

Regarding salivary lactobacilli, all participants displayed detectable growth at baseline, but the majority had moderate scores (10^3–$<10^5$ CFU). The baseline levels were similar prior to the two intervention periods and no statistically significant changes appeared between the baseline and follow-up samples, i.e. not during the period of active ice-cream intake and not during the control period. No side or adverse effects were reported during the course of the study.

Discussion

Bifidobacteria have a wide commercial use and are generally regarded as safe (GRAS) for use as ingredients in milk-based infant formulas [15]. Studies have been performed to validate the survival and positive effects of *Bifidobacterium lactis* Bb-12 within the human body, including immune response and gastrointestinal health in young children [15, 16], but the research concerning probiotics and dental health is more limited. It should be noted that the present study group comprised healthy young adults without dental caries problems. For ethical reasons, it was decided not to include legally incompetent subjects (such as a (minor) child) in reasearch at this early step, but the encouraging findings may call for an intervention study in the most interesting target group, namely caries-prone children. The counts of salivary mutans streptococci and lactobacilli were estimated using a simple chair-side test that correlates well with conventional laboratory methods with selective media [17,18].

To our knowledge, the present study was the first to examine the possible effects of *Bifidobacterium lactis* Bb-12 on caries-associated microorganisms in the oral cavity. The results were in full harmony with our previous findings concerning short-term daily administration of a similar strain, *Bifidobacterium animalis* subsp. *lactis* DN-173010, in yogurt [9]. Thus, the null-hypothesis concerning salivary mutans streptococci was rejected. Interestingly, however, the significant mutans streptococci reduction was obtained with a low amount of probiotic bacteria in the present study. Here, only 53 g of the ice-cream with 1×10^7 CFU per gram was ingested daily compared to 200 g containing 2×10^8 CFU per gram in our former study [9]. This clearly illustrates the fact that the optimal dose needed for bacterial suppression in the oral environment is still to be determined. The data must be interpreted with some care, however. First, the sample size was limited, and, second, mutans streptococci in saliva can only be regarded as an intermediate endpoint for caries; it remains to be investigated whether or not this really

Table II. Salivary lactobacilli score at baseline and follow-up after intake of probiotic ice-cream or control. Values in table denote the number of subjects.

	Lactobacilli score, CFU/ml			
Time	$<10^3$	10^3–$<10^5$	10^5–$>10^6$	$\geq10^6$
Probiotic ice-cream ($n=23$)				
Baseline	3	10	9	1
Follow-up	5	13	5	0
Control ice-cream ($n=24$)				
Baseline	3	13	7	1
Follow-up	6	11	7	0

Chi-square test for probiotic ice-cream (follow-up vs. baseline): $p=0.38$ (NS); contingency coefficient 0.249.
Chi-square test for control ice-cream (follow-up vs. baseline): $p=0.54$ (NS); contingency coefficient 0.208.

is beneficial for the patients. Third, there are no long-term studies available on the effect of bifidobacteria on the oral microflora. In this respect, our finding that the mutans streptococci levels were insignificantly lower at the control baseline may indicate that the washout period was too short and should be extended to exceed 2 weeks in future crossover trials.

The mechanism of probiotic action in the oral cavity is not fully understood, but commonly explained by a combination of local and systemic immune response as well as non-immunologic defense mechanisms [6,7]. The principal health-promoting effects are ascribed to enhancement of mucosal immune defense and macrophage activity as well as elevations of the numbers of killer cells, T-cells, and interferon [4]. In the oral cavity, bifidobacteria is quite acidogenic and may play a role in deep dentine caries progression rather than in the early enamel demineralization [3]. To be effective against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as part of the biofilm and compete with the growth of dental pathogens [19]. This local event was elucidated by Haukioja et al. [20] in a recent *in vitro* study. It was shown that bifidobacteria could survive in saliva and bind to *Fusobacterium nucleatum*-covered hydroxyapatite, stressing the importance of other oral bacteria in the potential action of probiotic strains.

In daily routines, the administration of probiotics to small children may be difficult. A previous study has suggested that a pacifier with slow-releasing probiotic lozenges could be an option [21]. The present finding that ice-cream could serve as a vehicle for probiotics was also interesting. Ice-cream, with its natural content of casein, calcium and phosphorus, is a dairy product that can be stored for a long time without any significant decrease in the number of viable probiotic cells [13,22]. For example, the shelf life of the present ice-cream was 24 months. The product was well accepted by all participants and no one in the study group could detect the difference between the active and the control ice-cream. Thus, there are reasons for believing that probiotic ice-cream could be a suitable product also for young children.

In conclusion, the daily intake of ice-cream containing probiotic bifidobacteria may reduce the levels of caries-associated mutans streptococci in saliva. Being an intermediate endpoint, further studies are needed to clarify whether this approach is an alternative strategy for the prevention of enamel demineralization.

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