

ORIGINAL ARTICLE

## Caries-risk profile variations after short-term use of 5000 ppm fluoride toothpaste

ALAA MANNAA<sup>1,2</sup>, GUGLIELMO CAMPUS<sup>3</sup>, ANETTE CARLÉN<sup>4</sup> & PETER LINGSTRÖM<sup>1</sup>

<sup>1</sup>Department of Cariology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, <sup>2</sup>Department of Conservative Dental Sciences, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, <sup>3</sup>Department of Surgery, Microsurgery and Medicine Sciences – School of Dentistry, University of Sassari, Sassari, Italy, and <sup>4</sup>Department of Oral Microbiology and Immunology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

### Abstract

**Objective.** To assess the caries risk following 6 weeks' use of 5000 ppm F toothpaste using 'Cariogram' software. **Materials and methods.** A total of 34 participants, 17 mothers and their teenage children, were enrolled in a 6-week clinical trial in which they were given 5000 ppm F toothpaste. They were followed consecutively for 6 weeks with visits that were 2 weeks apart (four in total). A clinical examination was done at baseline and salivary chair-side tests to record the buffer capacity and mutans streptococci (MS) and lactobacilli (LB) counts were performed at each visit. Based on these data, seven caries-related variables were collected and inserted into the Cariogram software to calculate the actual chance of avoiding caries. **Results.** The use of 5000 ppm F toothpaste resulted in a statistically significant modification of the caries-risk profile, increasing the actual chance of avoiding caries in the future among the mothers and teenagers at each visit following baseline ( $p < 0.01$ ). The changes essentially related to the salivary parameters (buffer capacity, MS and LB counts). A statistically significant linear trend was observed for MS counts ( $p < 0.01$ ) and the number of subjects with a salivary concentration of  $MS < 10^3$  increased at each visit. The same trend was also observed for LB and buffer capacity scores ( $p = 0.04$  and  $p = 0.03$ , respectively). **Conclusions.** The short-term use of 5000 ppm F toothpaste is able to reduce the caries risk, which can be clearly demonstrated using 'Cariogram' software.

**Key Words:** Caries risk, cariogram, fluoride, toothpaste

### Introduction

Dental caries remains the single most prevalent and costly oral disease worldwide [1–3]. The World Health Organization (WHO) has highlighted the fact that, although there are huge improvements in the oral health of populations, large problems still persist [4].

The observed decline in dental caries in most industrialized countries over the past four decades has been largely attributed to the daily use of fluoridated (F) dentifrices [5–7]. This is mainly due to the role of fluoride in the de- and remineralization process [8]. An antimicrobial effect by the F compound is also known, but research has established that oral fluoride concentrations resulting from the use of  $\leq 1500$  ppm F toothpastes are insufficient to have a significant

antimicrobial effect on cariogenic bacteria [9]. Attention has therefore focused on developing novel toothpaste formulae, which may increase the oral bioavailability of F and in turn enhance its antimicrobial action. One adopted strategy has been to increase the F concentration in toothpaste, which could enhance its diffusion and deposition in the biofilm [10,11]. In general, toothpastes of this kind are recommended for high caries risk individuals above 10 years of age, such as subjects with dry mouth or root surface caries [12,13].

The majority of studies evaluating 5000 ppm F toothpastes have shown their ability to reduce plaque scores, increase plaque and saliva F concentrations, reduce biofilm acidogenicity and reduce caries [12–19]. Despite these positive findings, further

research is needed to explore the consequences of using 5000 ppm F toothpaste on caries-related plaque and salivary parameters and how this in turn may modulate the individual caries risk.

The 'Cariogram' is a free-share software that has been developed with the aim of (a) identifying those persons who will most probably develop caries and (b) providing these individuals with the appropriate preventive and treatment measures to arrest the disease [20]. The 'Cariogram' uses an algorithm with a 'weighted' analysis of specific caries-related factors developed to calculate the individual caries-risk profile [20–22]. The computer-based calculation is presented graphically in the form of a pie chart, which has five coloured sectors illustrating the contribution of the different caries-risk factors to the individual's caries-risk profile and the actual *chance of avoiding caries* in the future. The 'Cariogram' has been used extensively in several countries and has demonstrated fairly high efficacy and good reliability [22–24]. One possible way to evaluate the effectiveness of a high-F regimen would be to determine its ability to reduce the caries risk.

The hypothesis of this study was therefore that the short-term use of high-F toothpaste causes a variation in the individual caries risk. From this perspective, the aim of the present study was to assess consecutively the caries risk following 6 weeks' use of 5000 ppm F toothpaste using the 'Cariogram'.

## Materials and methods

Mothers and their teenage children (13–17 years of age) from families that had participated in a previous study were asked to volunteer. For details regarding the inclusion criteria and methods, please see Mannaa et al. [25]. Verbal information about the study was given to each volunteer and written informed consent in Arabic was obtained prior to the start of the study. Ethical approval for the study was obtained from the Faculty of Dentistry at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. The study was conducted in accordance with the Declaration of Helsinki [26].

Seventeen families, with a total of 34 participants, agreed to be enrolled in this clinical trial; 17 mothers ( $38.4 \pm 6.4$  years, mean  $\pm$  SD) and their 17 teenage children ( $14.5 \pm 1.2$  years). As previously described in Mannaa et al. [19], power analysis was performed.

The study was carried out over a period of 6 weeks, with a total of four visits 2 weeks apart: baseline, 2 weeks, 4 weeks and 6 weeks. The four visits were scheduled at the same time of the day for each individual.

Prior to each visit, the participants were asked to refrain from toothbrushing and all other oral hygiene measures for the last 24 h, as well as eating or drinking

anything except water for 1 h prior to the scheduled appointment.

Each volunteer was given one tube (113 g) of high-fluoridated toothpaste (Clinpro™ 5,000, 3M ESPE, St. Paul, MN) and a toothbrush with a coloured mark 2 cm from the bristle surface (Trisa®, TRISA AG, Switzerland). Oral hygiene instructions have been described in detail in a previous manuscript [19].

Clinical examinations and chair-side tests were performed under standardized conditions by the same investigator (AM) in the following order: (1) clinical assessment and (2) chair-side salivary testing for assessments of buffer capacity, as well as counts of cariogenic micro-organisms.

### Clinical assessment

Optimal artificial lighting, a tooth-drying device, a plain mirror and a WHO-CPI probe were used for the examination. Dental caries experience (DMFT/S) was recorded. Caries was diagnosed when there was a cavity at dentinal level [27]. Bite-wing radiographs were used for the diagnosis of proximal caries at baseline.

### Chair-side salivary assessments

The buffer capacity of stimulated saliva was determined using the CRT Buffer® strip (Ivoclar-Vivadent, Schaan, Liechtenstein). Stimulated saliva was collected while chewing on a piece of paraffin wax for 5 min. The test area of the buffer strip was wetted entirely with saliva using a pipette. After 5 min of reaction, a comparison was made with the coloured chart provided by the manufacturer and the buffer capacity was scored as low, medium or high. The CRT® Caries Risk Test (Ivoclar-Vivadent) was used to record the salivary counts of mutans streptococci (MS) and lactobacilli (LB). The agar surfaces were wetted with stimulated saliva and incubated at 37°C (99°F) for 48 h. The MS and LB counts were scored in four classes corresponding to the following CFU/ml: 0 = 0– $10^3$ , 1 =  $10^3$ – $10^4$ , 2 =  $10^5$ – $10^6$ , 3 =  $> 10^6$  CFU/ml for MS and 0 = 0– $10^2$ , 1 =  $10^2$ – $10^3$ , 2 =  $10^4$ – $10^5$ , 3 =  $> 10^5$  CFU/ml for LB.

### Risk assessment using the 'Cariogram'

The caries-risk profile was calculated for the study participants at each of the four visits using 'Cariogram' software [20]. For each subject, the following seven caries-related variables were put into the 'Cariogram' software: (1) caries experience, (2) related diseases, (3) diet content, (4) MS count, (5) fluoride programme, (6) salivary buffer capacity and (7) clinical judgement. Based on the entered variables, the chance to avoid caries in the future was calculated. Estimation of the dietary content

Table I. Results of the Cariogram analyses (chance of avoiding caries, circumstances, susceptibility, bacteria and diet) at each examination (baseline, 2 weeks, 4 weeks and 6 weeks). Mean  $\pm$  SE for 28 subjects at baseline, 2 and 4 weeks and 26 subjects at 6 weeks.

Cariogram sector	Mean $\pm$ SE				<i>p</i> -value <sup>a</sup>
	Baseline	2 weeks	4 weeks	6 weeks	
<i>Chance of avoiding caries</i>					
Total sample	29.5 $\pm$ 3.6	43.8 $\pm$ 3.8	48.7 $\pm$ 4.3	54.7 $\pm$ 2.6	< 0.01
Mothers	28.0 $\pm$ 4.8	39.4 $\pm$ 5.3	44.4 $\pm$ 5.9	53.7 $\pm$ 3.8	< 0.01
Children	31.0 $\pm$ 5.5	48.1 $\pm$ 5.4	52.9 $\pm$ 6.2	55.8 $\pm$ 3.6	< 0.01
<i>p</i> -value <sup>b</sup>	0.80	< 0.01	0.02	0.09	
<i>Circumstances</i>					
Total sample	20.9 $\pm$ 0.8	13.4 $\pm$ 0.8	12.0 $\pm$ 0.9	11.2 $\pm$ 0.7	< 0.01
Mothers	20.8 $\pm$ 1.1	14.6 $\pm$ 1.1	12.2 $\pm$ 1.4	11.7 $\pm$ 1.1	< 0.01
Children	20.8 $\pm$ 1.2	12.2 $\pm$ 1.1	11.7 $\pm$ 1.1	10.7 $\pm$ 1.0	< 0.01
<i>p</i> -value	0.16	0.08	0.13	0.27	
<i>Susceptibility</i>					
Total sample	8.6 $\pm$ 0.6	7.5 $\pm$ 0.6	7.2 $\pm$ 0.6	6.7 $\pm$ 0.5	< 0.01
Mothers	9.3 $\pm$ 0.7	8.6 $\pm$ 0.8	8.1 $\pm$ 0.8	7.3 $\pm$ 0.6	0.29
Children	7.9 $\pm$ 1.0	6.5 $\pm$ 0.9	6.4 $\pm$ 0.8	6.2 $\pm$ 0.8	0.51
<i>p</i> -value	0.39	0.90	0.48	0.62	
<i>Bacteria</i>					
Total sample	20.9 $\pm$ 1.6	18.2 $\pm$ 1.5	14.9 $\pm$ 1.6	12.6 $\pm$ 1.0	< 0.01
Mothers	20.0 $\pm$ 2.3	18.7 $\pm$ 2.1	16.4 $\pm$ 2.2	11.8 $\pm$ 1.3	< 0.01
Children	21.8 $\pm$ 2.3	17.6 $\pm$ 2.2	13.4 $\pm$ 2.2	13.3 $\pm$ 1.7	< 0.01
<i>p</i> -value	0.69	0.70	0.70	0.70	
<i>Diet</i>					
Total sample	20.2 $\pm$ 1.2	17.1 $\pm$ 1.4	17.5 $\pm$ 1.6	14.6 $\pm$ 0.9	0.03
Mothers	21.7 $\pm$ 1.5	18.9 $\pm$ 1.9	18.7 $\pm$ 2.1	15.3 $\pm$ 1.4	0.09
Children	18.8 $\pm$ 2.0	15.4 $\pm$ 1.9	16.4 $\pm$ 2.5	14.0 $\pm$ 1.2	0.10
<i>p</i> -value	0.44	0.84	0.30	0.85	

<sup>a</sup>Comparison between visits.<sup>b</sup>Comparison between mothers and children.

was made using the salivary LB counts (as a measure of the cariogenic diet) [28]. During the trial, none of the volunteers was exposed to any fluoride source except for the 5000 ppm F toothpaste. The fluoride variable was therefore scored '2' at baseline and '1' for all other examinations (2, 4 and 6 weeks). The saliva secretion rate was not included in the analysis since the secretion rate was only assessed at baseline. All individuals showed a normal salivary secretion rate. The same thing applies to the diet frequency and plaque amount.

### Statistical analyses

All the data were analysed using Stata SE<sup>®</sup> software v. 10.0. Analyses were made for all individuals and for mothers and children separately. All microbiological analyses and 'Cariogram' calculations were carried out coded. Descriptive statistics, including the means, standard deviations and frequencies (percentages), were calculated. To validate the hypothesis of the

study, only three variables (MS and LB scores and buffer capacity) were analysed in detail. The Cariogram variables included in the statistical analysis were: (1) the actual chance of avoiding caries, (2) circumstances (caries experience and related diseases), (3) susceptibility (fluoride programme and saliva buffer capacity), (4) bacteria (mutans streptococci) and (5) diet (dietary content). The data were analysed for statistically significant differences using repeated one-way measures analysis of variance (ANOVA) with the Cook-Weisberg post-hoc test. Linear trends in proportion were tested using the  $\chi^2$  test for trends. A mixed-design analysis of variance model was used to test for differences between mothers and children. A *p*-value of < 0.05 was considered statistically significant.

### Results

Thirteen families (26 subjects) completed the clinical trial. Of the 17 families (34 subjects) enrolled, three

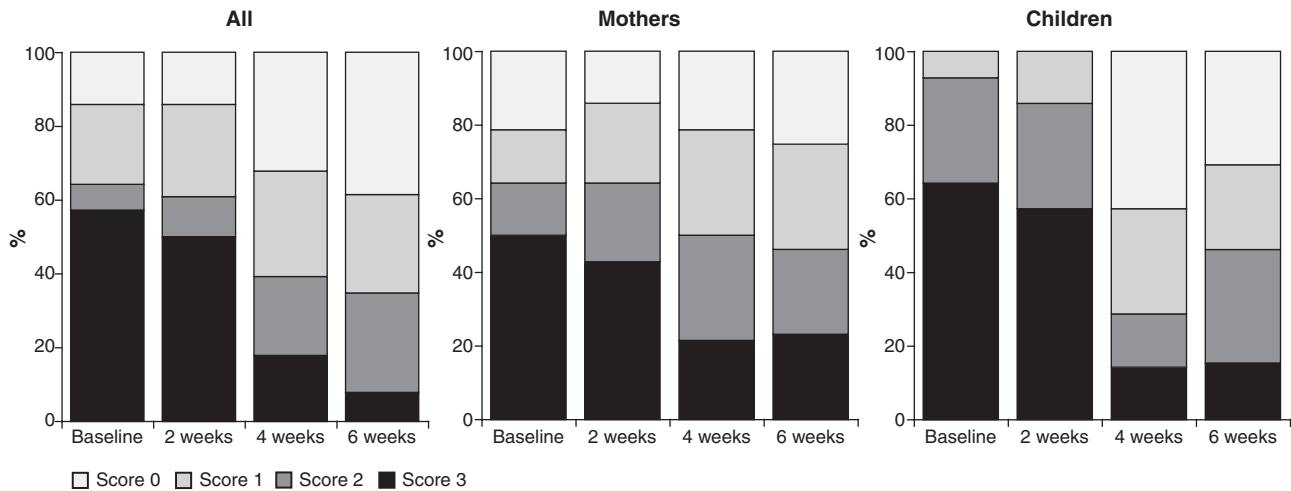


Figure 1. Sample distribution across the different recall examinations (baseline, 2, 4 and 6 weeks) for salivary mutans streptococci for all individuals and for mothers and children separately. The four scores correspond to the following values: 0 =  $0-10^3$ , 1 =  $10^3-10^4$ , 2 =  $10^5-10^6$ , 3 =  $>10^6$  CFU/ml ( $n = 28$  at baseline, 2 and 4 weeks and 26 subjects at 6 weeks).

families attended only the baseline visit and one family failed to show up for the final visit. As a result, data from 14 families were included in the statistical analysis (the 13 families which completed the study and the family which failed to attend the final visit). The total number of measurements included in each analysis was, therefore, 28 at baseline, 2 and 4 weeks and 26 at 6 weeks. Clinical caries data expressed as DMFS (mean  $\pm$  SD) were fairly high in the study sample:  $19.4 \pm 12.7$  for the mothers and  $8.6 \pm 8.7$  for the children.

Statistical analysis revealed that the use of 5000 ppm F toothpaste resulted in a significant modification of the caries-risk profile ('Cariogram' pie chart), thereby increasing the actual chance of avoiding caries in the future at each visit following baseline. Table I summarizes the weight (expressed in percentage) of the different sectors of the 'Cariogram' (chance of

avoiding new caries, circumstances, susceptibility, bacteria and diet) among the mothers and their teenage children throughout the trial.

The chance of avoiding caries increased significantly ( $p < 0.01$ ) during the trial for all individuals and for the two groups separately; from 28.0 at baseline to 53.7 at 6 weeks for the mothers and from 31.0 to 55.8 for the teenage children. At the end of the trial, a general improvement in the chance of avoiding caries was observed. A statistically significant difference between the mothers and their children was found at 2 and 4 weeks ( $p < 0.01$  and  $p < 0.05$ , respectively).

Changes in salivary MS, LB counts and buffer capacity levels across the different recall examination are shown in Figures 1,2,3. A statistical linear trend was observed for the MS figures ( $p < 0.01$ ), with an increasing number of subjects with a salivary count of

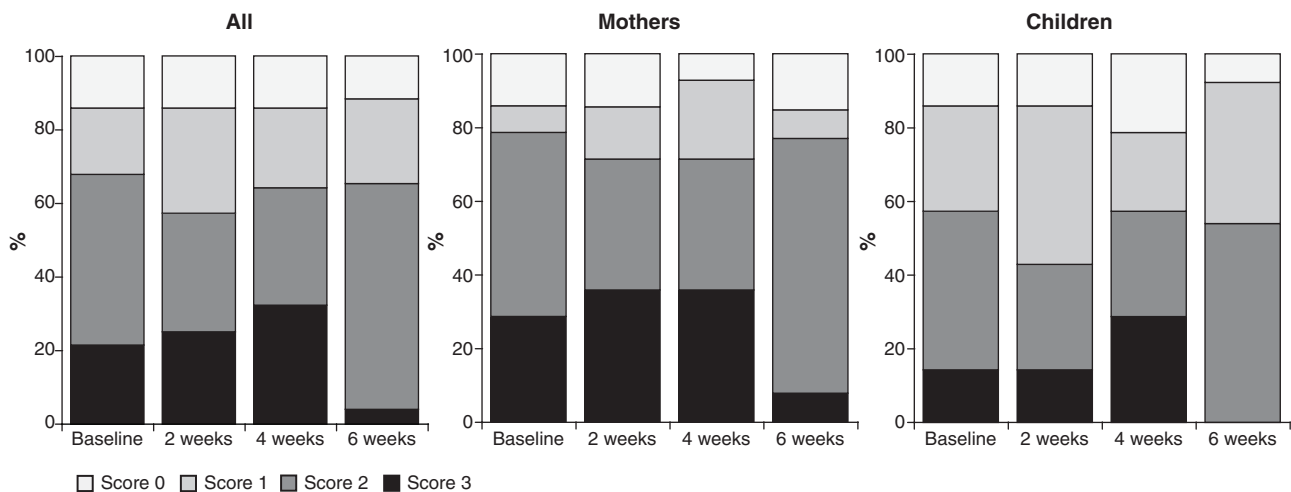


Figure 2. Sample distribution across the different recall examinations (baseline, 2, 4 and 6 weeks) for salivary lactobacilli counts for all individuals and for mothers and children separately. The four scores correspond to the following values: 0 =  $0-10^2$ , 1 =  $10^2-10^3$ , 2 =  $10^4-10^5$ , 3 =  $>10^5$  CFU/ml ( $n = 28$  at baseline, 2 and 4 weeks and 26 subjects at 6 weeks).

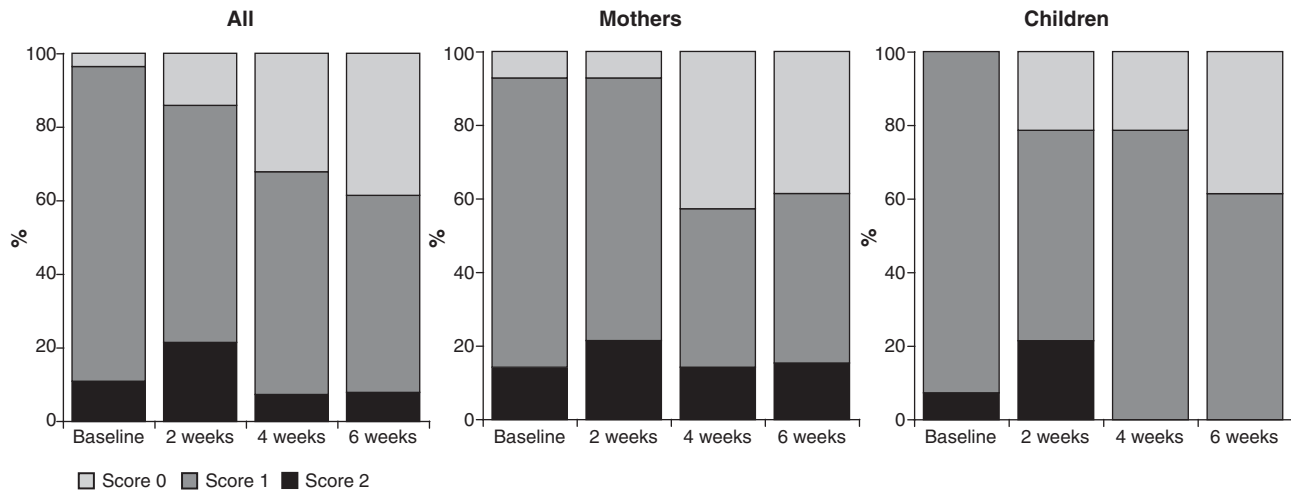


Figure 3. Sample distribution across the different recall examinations (baseline, 2, 4 and 6 weeks) for salivary buffer capacity levels assessed as low, medium or high for all individuals and for mothers and children separately. The three scores correspond to the following values: 0 = low, 1 = medium, 2 = high ( $n = 28$  at baseline, 2 and 4 weeks and 26 subjects at 6 weeks).

$MS < 10^3$ . The same trend was also observed for the LB scores and buffer capacity scores ( $p = 0.04$  and  $p = 0.03$ , respectively). A statistically significant difference was also found when comparing the mothers and their children for MS counts ( $\chi^2 = 5.25$ ,  $p < 0.05$ ) and LB counts ( $\chi^2 = 4.02$ ,  $p < 0.05$ ). A numerical but not significant trend was found for the buffer capacity ( $p = 0.25$ ). The chance of avoiding caries was not statistically significantly associated with the type of participant (mother or child), while all the other 'Cariogram' sectors were statistically significantly associated ( $p < 0.01$ ) with the chance of avoiding caries (Table II).

## Discussion

This study was designed to validate the hypothesis that the use of high-F toothpaste (5000 ppm) modifies the individual caries risk. The main result of the study confirms this hypothesis. A significant change in the caries risk as assessed by the 'Cariogram' software was

observed after 6 weeks' use of the high-fluoride regimen, both for all individuals and when evaluating mothers and children separately.

Although the 'Cariogram' was used to evaluate the change in caries-risk profile in relation to the use of high-F toothpaste (5000 ppm), all the standard variables were not included in the 'Cariogram' at the four different time points. This included the salivary secretion rate. Even though all the subjects displayed a normal secretion rate at baseline, it was decided to exclude this information since the secretion rate was not measured at all time points. As the participants were allowed to follow their usual dietary habits throughout the study period and no intake frequency was evaluated, this variable was not included in the 'Cariogram' analysis. The same thing also applies to the plaque amount variable.

It is interesting to note the gradual significant increase in the actual chance of avoiding caries, i.e. reduced caries risk, during the study period after the short-term use of a high-fluoride regimen from one third at baseline to more than 50% after 6 weeks for all individuals. Several reasons could account for this immediate response. Even though fluoride is known principally to act on the tooth surface favouring remineralization over demineralization, it also has an antimicrobial effect [8,29]. This is evident from the significant reduction in the MS count found in the present study.

A variation in the 'Bacteria sector' of the 'Cariogram' between the mothers and their children was found, with a tendency towards a faster change for the teenagers already occurring between baseline and 2 weeks, compared with 4–6 weeks for the mothers. It can be speculated that the teenage children have a less mature biofilm, which could benefit more readily from the high-F regimen. Moreover, there could be differences in the biofilm microbiology based on the

Table II. Mixed-design analysis of variance with fixed effect of sectors of the Cariogram (the actual chance of avoiding caries as the dependent variable) and the difference between mothers and children as the random effect.

	Coefficient (SE)	<i>p</i> -value	95%CI
Circumstances	-1.02 (0.06)	< 0.01	-1.14 to -0.90
Susceptibility	-1.04 (0.03)	< 0.01	-1.13 to -0.95
Bacteria	-0.96 (0.02)	< 0.01	-1.00 to -0.92
Diet	-1.01 (0.04)	< 0.01	-1.06 to -0.96
Participant (mother/teenage child)	0.19 (0.23)	0.40	-0.26 to -0.65

Number of observations = 110, number of groups = 4 (total number of visits),  $F = 5997.83$ ,  $p < 0.01$ .

contribution of the mode of bacterial transmission. Vertical transmission plays a predominant role in the acquisition of oral microflora in children, but horizontal transmission becomes important after the age of 5 years and this might further explain the differences in the bacterial changes observed between the mothers and their teenage children [30,31].

In the 'Cariogram', the fluoride program was set at '2' (fluoride toothpaste, no supplements) at baseline and '1' (additional F measures, infrequently) at 2, 4 and 6 weeks. This change in itself changes the weight among the different variables and increases the 'actual chance of avoiding caries'. One interesting finding was that the caries risk decreased even when the fluoride exposure was kept at '2' for all four visits. This indicates that the variables influenced by the high-F regimen (MS and LB counts and buffer capacity) accounted for this change in caries risk. This corresponds well with the reduced plaque acidogenicity, registered as changes in plaque pH, found after a sugar challenge for the same group presented in a previous study [19]. In addition, an increase in the retention of fluoride in dental plaque was found in the same study, which may explain the change in biofilm properties. Furthermore, the present data correspond well with previous findings in which an increase in fluoride in both interproximal saliva and plaque was found [16]. The effect of 2 years' daily use of a high-F toothpaste in comparison to a dentifrice containing 1450 ppm F on caries incidence and caries progression in adolescents has recently been studied [18]. A lower progression rate was found for those using the higher F regimen. One important finding was that the effect was strongest with poor compliance. All the individuals in the present study admitted that they had followed the given instructions. However, the possibility that some participants did not fully adhere to the instructions cannot be excluded.

In comparison with other methods of fluoride administration, toothpastes are regarded as a fairly easy vehicle for increasing the fluoride concentration. In the current study, the toothpaste was used by two different age groups, but this is thought to be a suitable method for any individual older than 12 years of age. Due to its high fluoride content, it is not recommended in younger children for toxicological reasons [32].

Although not evaluated in the present study, it can be anticipated that the plaque amount may have changed throughout the trial, as previously shown by the use of a high-fluoride regimen [17]. So, if this variable had been included in the analysis, an even greater increase in the 'actual chance of avoiding caries' would have been observed.

Even if statistically significant differences were obtained, the small number of subjects enrolled in this study might be seen as a shortcoming. Despite the

significant observations in the present study, further research is needed to assess the long-term effects of 5000 ppm F toothpaste on different biofilm properties determining caries initiation and progression.

### Acknowledgements

We would like to thank Dr Nadia Al-Hazmi, head of dental records, Dr Sahar Bukhary, head of the Oral Biology Division, and Dr Motaz Ghulman, vice dean of student affairs at the Faculty of Dentistry at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. We would also like to express our sincere appreciation to Dr Samer Aouad, 3M ESPE Scientific Marketing Supervisor in the United Arab Emirates.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### References

- [1] Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. *Am J Dent* 2009;22:3-8.
- [2] National Institutes of Health. Diagnosis and management of dental caries throughout life. NIH Consensus Statement 2001; 18:1-30.
- [3] Marsh PD. Are dental diseases examples of ecological catastrophes? *Microbiology* 2003;149:279-94.
- [4] Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral disease and risks to oral health. *Bull WHO* 2005;83:661-9.
- [5] Bratthall D, Hänsel-Petersson G, Sundberg H. Reasons for the caries decline: what do the experts believe? *Eur J Oral Sci* 1996;104:416-22.
- [6] Marinho VC, Higgins JP, Sheiham A, Logan S. Fluoride toothpastes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2003;1:CD002278.
- [7] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: a systematic review. *Acta Odontol Scand* 2003;61: 347-55.
- [8] Buzalaf MA, Pessan JP, Honorio HM, ten Cate JM. Mechanisms of action of fluoride for caries control. *Monogr Oral Sci* 2011;22:97-114.
- [9] Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. *Int Dent J* 2004;54(5 Suppl 1):304-9.
- [10] Zero DT, Raubertas RF, Fu J, Pedersen AM, Hayes AL, Featherstone JD. Fluoride concentrations in plaque, whole saliva, and ductal saliva after application of home-use topical fluorides. *J Dent Res* 1992;71:1768-75.
- [11] Davies R, Scully C, Preston AJ. Dentifrices - an update. *Med Oral Patol Oral Cir Bucal* 2010;15:976-82.
- [12] Baysan A, Lynch E, Ellwood R, Davies R, Petersson L, Borsboom P. Reversal of primary root caries using dentifrices containing 5,000 and 1,100 ppm fluoride. *Caries Res* 2001; 35:41-6.
- [13] Lynch E, Baysan A. Reversal of primary root caries using a dentifrice with a high fluoride content. *Caries Res* 2001;35: 60-4.

- [14] Schirmeister JF, Gebrande JP, Altenburger MJ, Monting JS, Hellwig E. Effect of dentifrice containing 5000 ppm fluoride on non-cavitated fissure carious lesions *in vivo* after 2 weeks. *Am J Dent* 2007;20:212–16.
- [15] Ekstrand K, Martignon S, Holm-Pedersen P. Development and evaluation of two root caries controlling programmes for home-based frail people older than 75 years. *Gerodontology* 2008;25:67–75.
- [16] Nordström A, Birkhed D. Fluoride retention in proximal plaque and saliva using two NaF dentifrices containing 5,000 and 1,450 ppm F with and without water rinsing. *Caries Res* 2009;43:64–9.
- [17] Nordström A, Mystikos C, Ramberg P, Birkhed D. Effect on de novo plaque formation of rinsing with toothpaste slurries and water solutions with a high fluoride concentration (5,000 ppm). *Eur J Oral Sci* 2009;117:563–7.
- [18] Nordström A, Birkhed D. Preventive effect of high-fluoride dentifrice (5,000 ppm) in caries-active adolescents: a 2-year clinical trial. *Caries Res* 2010;44:323–31.
- [19] Manna A, Carlén A, Zaura E, Buijs MJ, Bukhary S, Lingström P. Effects of high-fluoride dentifrice (5,000 ppm) on caries-related plaque and salivary variables. 2013; (submitted).
- [20] Bratthall D, Petersson GH. Cariogram - a multifactorial risk assessment model for a multifactorial disease. *Community Dent Oral Epidemiol* 2005;33:256–64.
- [21] Bratthall D. Dental caries: intervened - interrupted - interpreted. Concluding remarks and Cariography. *Eur J Oral Sci* 1996;104:486–91.
- [22] Hänsel Petersson G, Twetman S, Bratthall D. Evaluation of a computer program for caries risk assessment in schoolchildren. *Caries Res* 2002;36:327–40.
- [23] Campus G, Cagetti MG, Sacco G, Benedetti G, Strohmenger L, Lingström P. Caries risk profiles in Sardinian schoolchildren using Cariogram. *Acta Odontol Scand* 2009;67:146–52.
- [24] Campus C, Cagetti MG, Sale S, Carta G, Lingström P. Cariogram validity in schoolchildren: a two-year follow-up study. *Caries Res* 2012;46:16–22.
- [25] Manna A, Carlén A, Lingström P. Dental caries and associated factors in mothers and their preschool children - A cross-sectional study. *J Dent Sci* 2013;8:101–8.
- [26] World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull WHO* 2001;79:373–4.
- [27] Pitts NB. Modern concepts of caries measurement. *J Dent Res* 2004;83:C43–7.
- [28] Beighton D, Manji F, Baelum V, Fejerskov O, Johnson NW, Wilton JM. Associations between salivary levels of *Streptococcus mutans*, *Streptococcus sobrinus*, lactobacilli, and caries experience in Kenyan adolescents. *J Dent Res* 1989;68:1242–6.
- [29] Lussi A, Hellwig E, Klimek J. Fluorides - mode of action and recommendations for use. *Schweiz Monatsschr Zahnmed* 2012;122:1030–42.
- [30] Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. *J Clin Microbiol* 2001;39:2313–16.
- [31] van Loveren C, Buijs JF, ten Cate JM. Similarity of bacteriocin activity profiles of mutans streptococci within the family when the children acquire the strains after the age of 5. *Caries Res* 2000;34:481–5.
- [32] Levy SM. Review of fluoride exposures and ingestion. *Community Dent Oral Epidemiol* 1994;22:173–80.