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Clinical monitoring of the effect of fluorides on long-existing white spot lesions

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Abstract
The aim of this examiner-blind, clinical monitoring was to observe long-existing white spot lesions over a period of 6 months and to evaluate the effectiveness of fluoridated toothpastes on these lesions. Thirty-nine subjects with at least one lesion participated in the study. They were randomly divided into two groups ($n=20$ and $n=19$) and instructed to brush their teeth either with toothpaste containing sodium fluoride (1500 ppm) (group 1) or with toothpaste containing amine fluoride (1250 ppm) (group 2). Lesions were measured with quantitative light-induced fluorescence (QLF) and alterations were quantified by ΔQ as fluorescence loss integrated over the lesion area (% $\times$mm^2). All QLF images were taken by one examiner at baseline (ΔQ1) and follow-up visits after 2, 4, 6, 8, 10, and 12 weeks, and after 4, 5, and 6 months (ΔQ10). The results showed a slight but not significant progression of ΔQ (% $\times$mm^2) in group 1 (($p>0.05$; t-test), ΔQ1 -20.31 ($\pm$ 41.06); ΔQ10 -26.2 ($\pm$47.69)) and in group 2 (($p>0.05$), ΔQ1 -22.28 ($\pm$43.86); ΔQ10 -26.39 ($\pm$46.4)). The results indicated no significant differences regarding the influence of the fluoridated toothpastes ($p>0.05$). It can be concluded that within 6 months the long-existing white spot lesions are stable concerning fluorescence loss over the lesion area. Moreover, fluoride does not seem to have any effect on long-existing white spot lesions.

Key Words: *Cariostatic agents, dental caries, toothpaste*

Introduction

In 1966, Backer Dirks [1] was the first to describe the (possible) remineralization of *in vivo* formed natural enamel lesions, so-called white spot lesions, as normal occurrence in the oral environment. In the following decades, a great number of *in situ* and *in vitro* investigations have shown that remineralization occurs in newly formed artificially enamel lesions [2–4], as well as in lesions formed under plaque *in situ* [5] and *in vivo* [6]. A more recent study [7] has demonstrated that both newly formed white spot lesions and *in vitro* formed lesions obviously remineralize similarly *in vitro* with respect to the baseline mineral loss.

Most of the dentifrices sold in the industrialized world contain fluorides as a remineralization agent. In the United States, for example, more than 95% of consumers purchase and use fluoride-containing formulations. The most commonly employed species of fluoride ions are sodium monofluorphosphate, sodium fluoride, and amine fluoride. The relative efficacy of these agents has been the subject of continuing debate for a number of years [8]. Several studies have shown that it is possible to promote remineralization of white spot lesions by topical exposure to agents such as fluorides in various delivery vehicles [9]. However, all these clinical trials have used newly formed incipient white spot lesions [9–11]. It seems generally accepted that the effect of fluorides on long-existing white spot lesions is questionable, and thus these lesions are generally considered to be stable concerning demineralization and remineralization [12].

From the foregoing, it is surprising that no clinical study is available from the literature that has measured or monitored remineralization and demineralization processes on long-existing white spot lesions and the influence of fluoride containing

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dentifrices on these lesions. The aim of this study was therefore to monitor long-existing white spot lesions *in vivo* weekly and monthly, respectively, and to evaluate the effectiveness of fluoride toothpastes on the remineralization and demineralization process of these lesions over a short period of 6 months.

Patients and methods

Protocol

Forty-four subjects with at least one long-existing white spot lesion were selected to participate in this examiner-blind, single-center clinical monitoring using a parallel comparison design. All study personnel were blinded to the kind of toothpaste used for the duration of the study. Only the study statisticians saw unblinded data, but none had any contact with the study. The research protocol was approved by the Ethics Committee of the Freie Universität Berlin (vote number 214–27). All subjects received detailed particulars (verbal and written) on the principles of treatment and the purpose of the study and signed appropriate informed consent forms. For subjects between 12 and 18 years of age the signed consent forms were obtained from the subjects and also from a parent or guardian. The subjects were also asked to contact the doctor in attendance (examiner) in the advent of adverse reactions. The examiner of this study was a dentist and experienced to the diagnosis of long-existing white spot lesions.

Selection of patients

The volunteers selected for this clinical study were students attending the University School of Dental Medicine, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, as well as patients requesting dental treatment in the Department of Operative Dentistry and Periodontology of the University School of Dental Medicine. The candidates for inclusion in this study were at least 12 years of age, had good general health, good oral hygiene (approximal plaque index $<25\%$; [13]), and at least one tooth with a visible, not newly formed long-existing white spot lesion on their dentition (defined as a crescent-shaped spot on the buccal surface being more than 1 mm above the gingival margin with a chalky and shiny appearance after 1 s of air drying). Concerning subjects between 12 and 18 years of age only first molars have been included in the study to verify that the lesions are long-existing white spot lesions. Thus, in the present study "long-existing white spot lesions" can be defined as lesions with a relative age of at least 5 years; estimation of the age of the lesions based on age of the patients, estimated age of the teeth, and location of the lesions on the teeth. The volunteers were not admitted to the study if any of the following criteria was present: (1) a known allergy to any ingredients of the toothpastes used, (2) continuous intake of analgesic medication, (3) an antibiotic therapy within the previous 6 months or other therapies producing xerostomia, (4) one or more caries lesions, (5) one or more of the following chronicle diseases: epilepsy, cystic fibrosis, diabetes mellitus, xerostomia, renal insufficiency, leukemia, (6) radiotherapy in the past or in the near future in the head/neck area, (7) patients who would use other oral hygiene products containing fluoride during the study, (8) hereditary brown enamel, (9) dentinogenesis imperfecta, (10) tetracycline discoloration, (11) gingivitis or periodontitis, (12) pregnant or breast-feeding women, or (13) residence outside the city, insufficient address for the follow-up visits, or unwillingness to return for follow-up visits.

Treatment regime

All teeth were thoroughly examined by one trained examiner in order to exclude caries lesions with cavitation. If necessary, radiographic investigation was performed. All white spots on vestibular surfaces being at least 1 mm above the gingival margin and crescent-shaped were investigated using air for 1 s in order to dry the white spot and to exclude other reasons for discoloration (e.g. fluorosis). The air was directed perpendicular to the surface from a distance of 10 mm to the white spots using only air syringes with identical diameters. Following baseline data collection, all teeth corresponding to the including criteria were selected for the study.

Determination of whether the subject used toothpaste containing sodium fluoride or amine fluoride was made by referring to a randomization list created in the Department of Medical Biometry and Clinical Epidemiology (Charité-Campus Benjamin Franklin); details of the randomization list were known to the person who gave the toothpaste to the subjects, but unknown to the examiner. In group 1, the subjects used a toothpaste containing sodium fluoride (Crest Cavity Protection, 1500 ppm; Procter & Gamble, Cincinnati, Oh., USA), while the participants in group 2 used a toothpaste containing amine fluoride (Elmex, 1250 ppm; GABA, Münchenstein, Switzerland). The subjects were instructed by the examiner to brush their teeth 3 times a day for 3 min with an electric toothbrush (Crest Spin Brush Pro; Procter & Gamble). Additionally, the panelists were allowed to use other oral hygiene products without fluoride, such as dental floss and toothpicks. New toothpastes and electric toothbrushes were supplied every 4 weeks. Product use was unsupervised, but every 4 weeks subjects returned their dentifrice tubes and toothbrushes so that compliance could be determined collecting the empty toothpaste tubes and used toothbrushes.

Following assignment, before taking a photograph and before each measurement, the teeth with long-existing white spot lesions were professionally cleaned using prophylaxis paste without fluorides (Hawe Cleanic, art. no. 3230; Hawe-Neos Dental, Bioggio, Switzerland) and a polishing cup (Hawe Pro-Cup, art. no. 990/120; Hawe-Neos Dental). The polishing paste was removed using water spray.

Measurements

Long-existing white spot lesions were photographed (Nikon D 100; Nikon, Tokyo, Japan) after air drying for 1 s. The photographs were taken for documentation of surface texture and color of all selected lesions. Subsequently, the fluorescence of the lesions was monitored with quantitative light-induced fluorescence (QLF\Clin fluorescence intra-oral camera, 2001; Inspektor Research Systems, Amsterdam, The Netherlands, and the software QLF Patient 3.0.3.2, 2001; Inspektor Research Systems) as described previously [14]. All pictures were taken in the same room without any ambient light. Post-baseline examinations of the used lesions were performed after 2, 4, 6, 8, 10, and 12 weeks, as well as after 4, 5, and 6 months using the digital replace frame created by the used software. All QLF images were taken by the one blinded, trained examiner at baseline, and all follow-up examinations were taken by the same examiner.

The analysis of the taken images was done by the blinded, trained examiner using the evaluation software Qlf 2.00f, (2001; Inspektor Research Systems). The threshold used to determine whether areas were sound or carious was -5%. The maximum fluorescence loss in the lesion ΔF (%) and the area of the lesion (mm^2) were measured as described previously [14]. The best representation of clinical lesion progression or regression is given by the longitudinal changes in ΔQ, where ΔQ is defined as the fluorescence loss integrated over the lesion area ($\% \times mm^2$).

Statistical analysis

Subject size had been calculated referring to previous clinical studies evaluating the effect of fluoridated toothpaste on newly formed white spot lesions, since there is no clinical study available in the dental literature evaluating long-existing white spot lesions. However, no changes concerning re- and demineralization were expected.

The statistical unit was the subject. Thus, results obtained from different teeth of the same subject were averaged. Values of ΔF (%), lesion area (mm^2), and ΔQ ($\% \times mm^2$) were logarithmically transformed to obtain normally distributed values. Groups were compared using the t-test for independent samples. Comparisons versus baseline within groups used the t-test for dependent samples. No adjustment for multiple testing was used. The level of significance was 0.05 (two-sided). All analyses were performed using commercially available software (SPSS for Windows, release 11.5; SPSS, Chicago, Ill., USA).

Results

With the sample size of $n=20$ and $n=19$ a difference of 0.93 standard deviations could have been detected with a power of 86% and a level of significance of 0.05 (two-sided).

Concerning the demographic characteristics the 44 subjects selected for this study were 17 males and 27 females ranging from 12 to 38 years in age with a mean age of 27 (± 4.7) years. The subjects were normally distributed regarding their age. Concerning the clinical characteristics, the distribution of the selected 73 long-existing white spot lesions was as follows: 6 incisors (2 incisors in group 1 and 4 in group 2), 6 premolars (3 premolars in group 1 and 3 in group 2), and 61 molars (30 molars in group 1 and 31 in group 2). A photograph of a representative long-existing white spot lesion is given in Figure 1a. When using the QLF, the lesion appears as a dark region, as shown on the fluorescence image in Figure 1b. Concerning the surface texture and the color of the observed lesions, no changes have been observed within the 6 months of the study. No side effects have been observed during the period of the study. The flow of the subjects through each stage of the study is shown in Figure 2.

At baseline, the mean fluorescence loss ΔF (%) of the long-existing white spot lesions in group 1 (-14.41 (± 5.03)) was comparable to the mean fluorescence loss (ΔF %) of the lesions in group 2 (-14.43 (± 2.95)). At the subsequent observation times, the mean fluorescence loss (ΔF%) showed slightly but obviously higher values in group 1 compared to group 2, except visit three; the values in group 1 varied between $-14.18 \pm 4.9\%$ and $-15.93 \pm 4.97\%$ and in group 2 between $-14.17 \pm 3.08\%$ and $-15.01 \pm 4.52\%$. The measurements of the size of the lesion areas (mm^2) varied between 0.63 ± 1.05 mm^2 and 1.17 ± 1.8 mm^2 in group 1 and between 0.98 ± 1.59 mm^2 and 1.25 ± 2.13 mm^2 in group 2 during the 10 measurements. The areas of the lesions in group 2 were slightly larger or comparable to group 1 except for visits 7, 8, and 9. At these visits the areas were slightly larger in group 1. For ΔQ ($\% \times mm^2$) the results showed slightly higher or comparable values in group 2 compared to group 1 in 6 visits; the values in group 1 varied between $-12.45 \pm 25.09\%$ and $-26.2 \pm 47.69\%$ and in group 2 between $-20.51 \pm 39.79\%$ and $-28.03 \pm 45.42\%$. For visits 4, 7, 8, and 9, slightly higher values have been revealed for group 1. The statistical analysis revealed no significant differences ($p > 0.05$) between groups for

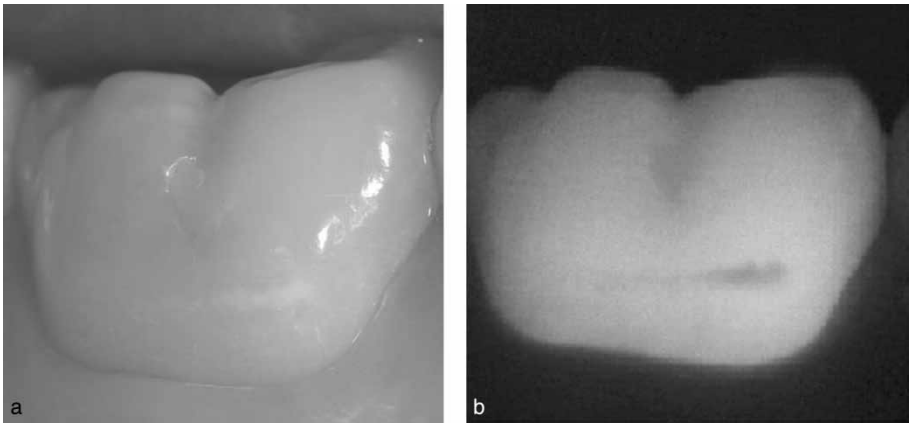

Figure 1. (a) Clinical image of a long-existing white spot lesion on the buccal surface of a mandibular molar. Note the spot's distance to the gingival, verifying the long-existing type of white spot lesion. (b) Fluorescence image of the same white spot lesion. Note that the lesion appears dark on the QLF image (comparable to newly formed lesions).

ΔF (%), lesion area (mm^2), and ΔQ (% $\times mm^2$) except visit 7 (ΔF (%), $p = 0.02$; (mm^2), $p = 0.04$; ΔQ (% $\times mm^2$), $p = 0.04$).

In Figure 3a–c the differences for all 10 visits versus baseline for ΔF (%), lesion area (mm^2), and ΔQ (% $\times mm^2$) are given for both groups. For the 2nd visit up to visit 6 the differences comparing each visit of both groups with baseline measurements revealed no obvious changes in fluorescence loss ΔF (%), lesion area (mm^2), and ΔQ (% $\times mm^2$) for the long-existing white spots lesions. At the subsequent observation times, the differences comparing each visit of both groups with baseline measurements revealed a slight increase for fluorescence loss ΔF (%; Figure 3a), lesion area (mm^2; Figure 3b) and ΔQ (% $\times mm^2$; Figure 3c) in group 1 and no changes in group 2. Statistical analysis revealed no significant changes concerning alterations of long-existing white spot lesions compared to baseline except in group 1 for visit 7 for ΔF (%; $p = 0.03$) and ΔQ (% $\times mm^2$; $p = 0.04$) and for visit 10 for ΔF (%; $p = 0.02$).

Separate analysis for molars only revealed comparable results as for the whole sample, except for ΔF (%), visit 7, which was not significant for molars only ($p = 0.56$).

Discussion

Owing to their age, long-existing white spot lesions could have an inaccessible surface for the remineralization and demineralization process. It is commonly accepted that newly erupted surfaces, being freely accessible to the fluids of the mouth, are well protected by fluoridated drinking water, whereas teeth that have erupted 3 years or more before water fluoridation started showing progressively less protection [1]. It can be speculated that the hard mineral surface and the reduction of lesion porosity decreases the ability of the observed white spot lesions to remineralize and to demineralize *in vivo* [16]. Another possible explanation concerning the missing alteration of long-existing white spot lesions may be the fact that *in vivo* arrested lesions contain a high concentration of organic material [17]. This could also block further pathways of fluoride penetration through the surface of white spots.

Weatherell et al. [18] have emphasized that the general remineralization effect of fluorides might be minimal. Measurement of fluoride concentration in rat molars indicates that several areas of the dentition might be inaccessible to extraneous ions. Furthermore, a meta-analysis has not provided any definitive evidence in support of a major role of fluoride toothpaste in the caries decline during the past 20 to 25 years [8]. Moreover, it can be assumed that initially demineralized enamel does not completely remineralize [19]. Nevertheless, it is well known that early carious enamel is highly reactive to topical fluorides, and that fluoride concentration within the early carious enamel will rapidly reach levels observed within the surface layers of incipient caries, even after only a few topical exposures [19]. In order to understand quantitatively the mechanisms of topically applied fluorides' cariostatic effects, systematic studies of fluoride reactivity with various lesion stages under dynamic cycling conditions might be necessary [20]. This may be particularly important for elucidating the cariostatic effect promoted by fluoride dentifrices and rinses, where the relatively short duration and high concentrations of fluoride exposure may impart special benefits at certain stages of lesion development [19]. While the fluoride reactivity of early lesions has a cariostatic effect, long-term benefits remain unclear and should not be overestimated [19]. The age of the used white spots may have crucially influenced the results of this study, but, again, no effects had been expected.

This concept is supported by the results of numerous studies using newly formed white spot lesions and QLF [9–11,21] which have found that areas of the lesions decreased, and the loss of enamel fluorescence was partly regained indicating remineralization, or, at least, regression of the lesion. Moreover, it seems that newly formed white spot

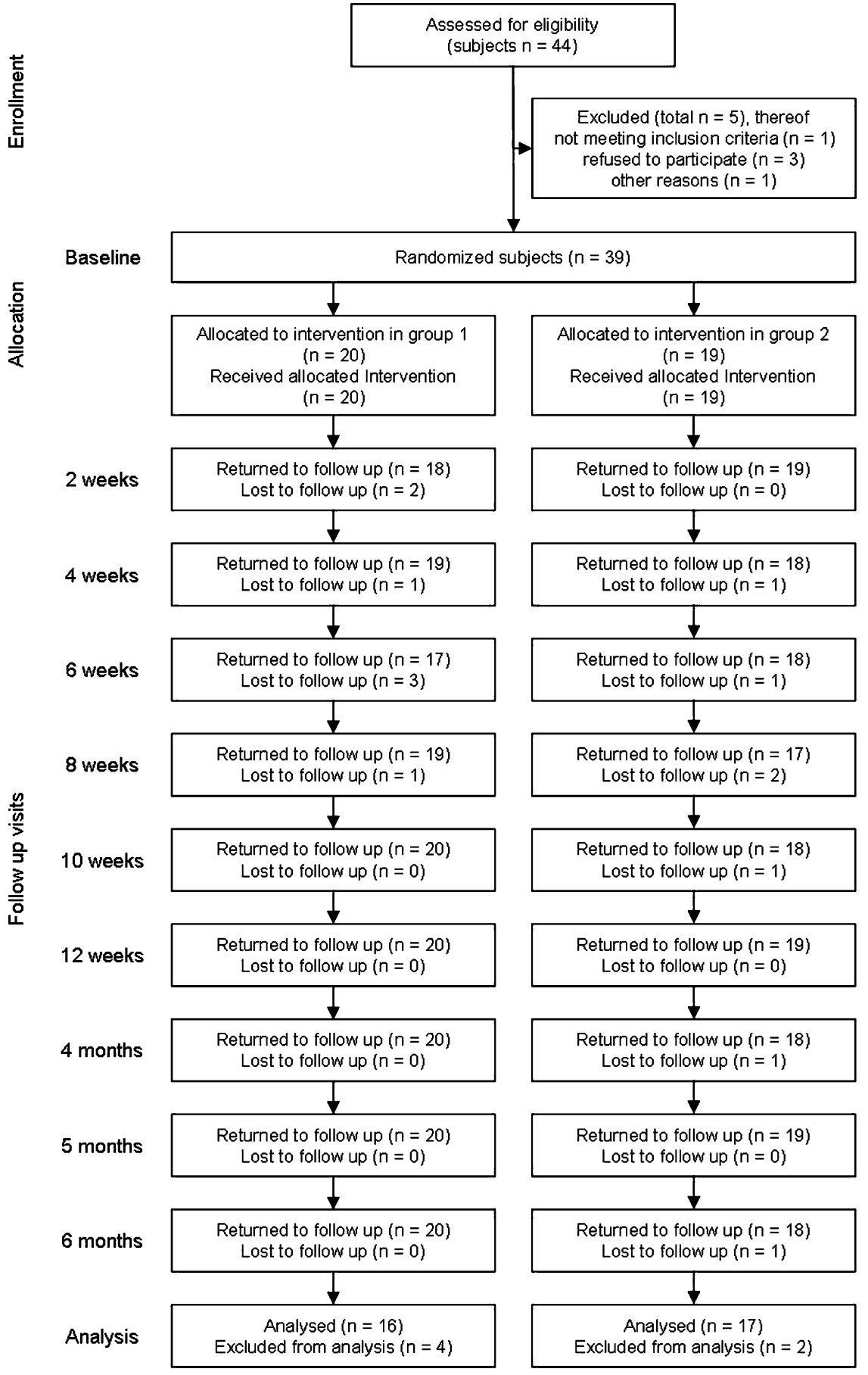

Figure 2. Diagram showing the flow of subjects through each stage of the trial.

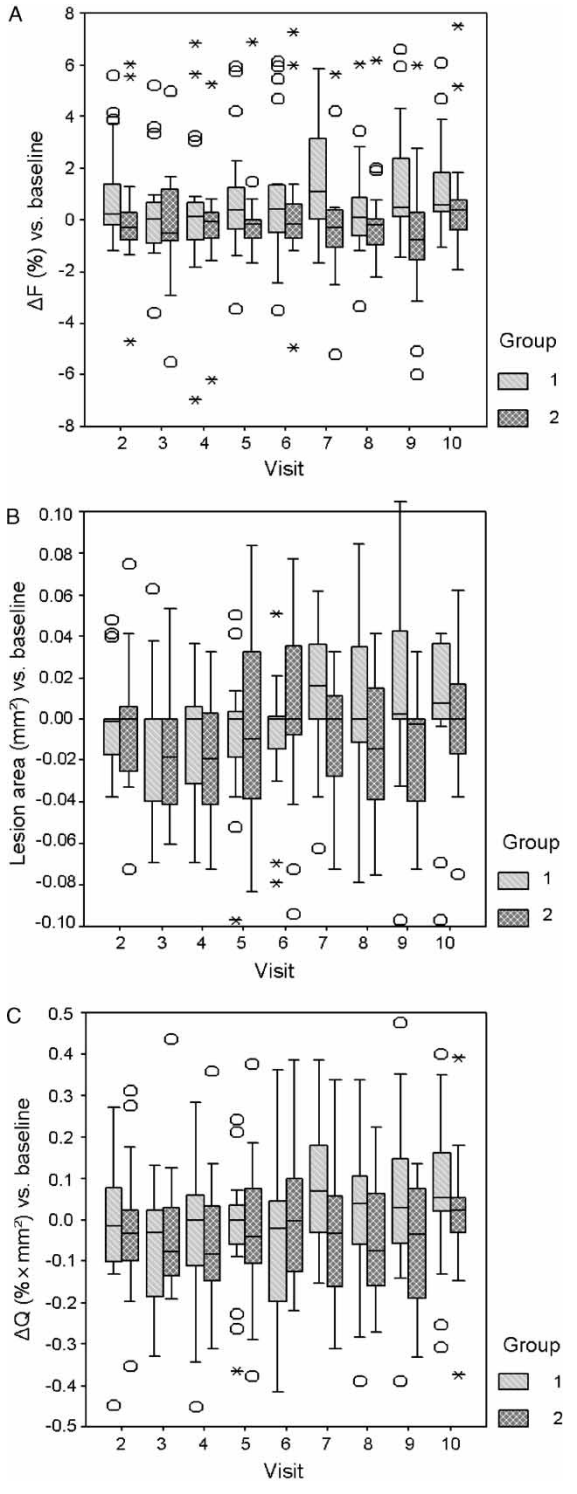

Figure 3. (a–c) Box-and-whisker plots of the differences for all 10 visits versus baseline for ΔF (%) (a), lesion area (mm^2) (b) and ΔQ (% $\times mm^2$) (c) for both groups. The solid bars indicate the median of the observations. The upper and lower limitations of the boxes indicate the first (25%) and third (75%) quartile of the observations, i.e. $q_{0.25}$ and $q_{0.75}$. The box length is thus defined as $d = q_{0.75} - q_{0.25}$. The upper antennae terminate at the maximum of observations within the range $q_{0.75} + 1.5d$. The lower antennae are defined analogously. All observations smaller than $q_{0.25} - 1.5$ d or larger than $q_{0.75} + 1.5d$ are indicated as circles or asterisks. Observations indicated as circles (outliers) are those within the range $q_{0.25} - 3d$, $q_{0.75} + 3d$, observations indicated as asterisks (extreme values) are those outside this range.

lesions with smooth surfaces can disappear [22,23], while long-existing white spots and proximal carious lesions obviously do not [1]. Toothbrushing on smooth, newly formed surfaces might contribute to lesion regression, as found in the studies mentioned above. The present data therefore suggest that the pseudo intact surface layer of white spot lesions existing *in vivo* for a long period of time possesses a greater hardness (and thickness) than the newly performed lesions. Thus, in the case of the present study, toothbrushing merely created any abrasion and in consequence no decrease of the lesion area could be observed.

The widespread use of fluoride toothpaste in industrialized countries makes it difficult or impossible to resolve the issue through analytical studies, because all available teeth for *in vivo* studies should have been excessively exposed to fluoride long before the study. Also the fluoride history of the teeth used in this study remains unclear. Thus, clinical studies concerning the fate and possible alterations of long-existing white spot lesions *in vivo* are limited to a monitoring over a short period of time, while experimental studies over a long period of time seem to be unethical because it would be necessary to withhold fluoride toothpaste from regular users [8]. Thus, in the present study, only the short-term effect of fluoridated toothpaste within 2 weeks or 1 month could be monitored.

The present results show large standard deviations and these are a reflection of the variable nature of the teeth used in the study. Moreover, the biological variations of the teeth are affected by their fluoride history, by conditions within the oral environment as well as by the varying age of the white spot lesions [16]. Therefore, the number of remineralization experiments that quantify mineral contents using *in vivo* formed white spots (and corresponding fluoride levels) is limited, due to the above-mentioned variables being difficult to standardize. With regard to *in vitro* studies, no difference should exist in remineralization efficacy between lesions formed *in vivo* and *in vitro* [7]. Nevertheless, teeth remineralize at different rates and to different extents *in vitro*, and the standard deviations are similar to those observed *in vivo* [16]. Thus, and for the reasons mentioned above concerning the reduced porosity, *in vivo* long-existing white spot lesions were determined to be best suited for the present monitoring of the immediate effect of fluoride toothpaste on long-existing white spot lesions. However, there were no separate analyses for premolars and incisors because the statistical power was estimated as too low and no significant differences were expected. Only molars were evaluated separately.

The scoring methodology used to quantify the alteration has been shown to be reliable and reproducible [21,24,25]. ΔQ is comparable to the total mineral loss of the lesions (ΔZ) as measured by

transversal microradiography (TMR [15]), the current gold standard for *in vitro* and *in situ* demineralization and remineralization studies. QLF has been validated against TMR in permanent teeth and has shown excellent agreement [10]. The *in vivo* repeatability and reproducibility of QLF are excellent for the quantification of smooth surface caries and the QLF method has been applied already in a few clinical studies [26]. However, there are several operational characteristics of QLF that must be adhered to in order to obtain images of consistent quality. While *in vitro* studies demonstrated that the degree of dehydration of tooth tissue influences the fluorescence intensity, this effect is much smaller under clinical conditions. However, for longitudinal measurements, QLF recordings should be performed under standardized conditions regarding the hydration status of the tooth. Lesions which are not limited to one surface but are situated mesiobuccally or distobuccally (e.g. circular lesions) cannot be recorded because the optical axis of the QLF device has to be oriented perpendicularly to the tooth surface [11]. Plaque and extrinsic stain should be removed before examination, since this will fluorescence on QLF images and complicate analysis. Moreover, ambient light should be reduced as much as possible and should be consistent between visits [27]. These factors have been considered in the present study using standardized conditions for measurements. Therefore, it can be assumed that the method of monitoring did not influence the present results. In total, this study documents very well the repeatability of measuring long-existing white spot lesions with the QLF. However, the combination of other methods in similar follow-up studies could shed even more light on these theories.

It can be concluded that within 6 months long-existing white spot lesions seem definitively to be stable. Moreover, fluoride does not seem to have any effect on long-existing white spot lesions. From the results of the present study it can be assumed that fluorides have no further remineralizing effect on these (already arrested) lesions; on the other hand it should be taken into account that the long-existing white spot lesions did not show any further demineralization effects. Since long-existing white spot lesions seem to be stable, it could also be mentioned that it is of importance that clinicians make careful decisions whether a lesion is active or arrested. Unnecessarily "fluoride exhausted" patients are not to wish for, i.e. from an environmental as well as from a behavioral point of view.

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