Intra-familial Cariological Studies on a Saudi Population

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Abstract

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Objectives: The aims of this thesis were to: 1) describe the caries experience and caries-related factors in mothers and their preschool and school children, 2) correlate quantified supragingival plaque bacteria between mothers and their children and identify possible microbial associations, 3) examine whether bacteria in pooled supragingival plaque samples, quantified using a "checkerboard DNA-DNA hybridisation"-based panel of caries-related bacteria, could reflect the caries experience in a manner similar to saliva samples analysed using chair-side methods, 4) measure the effects of six weeks' use of a 5,000 ppm fluoride toothpaste on caries-related factors in dental plaque and saliva and 5) consecutively assess the caries risk following six weeks' use of 5,000 ppm fluoride toothpaste using the "Cariogram". **Materials and methods:** A total of 258 individuals (86 mothers and two of their children, 4-6 and 12-16 years old) were examined cross-sectionally (Studies I, II & III) out of which 17 families were enrolled (mothers and 13- to 17-year-old children) a year later in a longitudinal six weeks trial (Studies IV & V) in which 5,000 ppm fluoride toothpaste was administered. In Study I, anamnestic data were collected, and clinical oral examinations and chair-side tests were performed. In Studies II and III, pooled interproximal supragingival plaque samples were analysed for their content of bacterial strains using the checkerboard DNA-DNA hybridisation technique. In Study II, microbial associations for all three age groups together were sought using cluster analysis, while principal components analysis (PCA) was used for each of the three age groups separately. In Study III, relationships between the bacterial scores and the caries experience (DMFT/dmft and D/d groups) were assessed. In Study IV, the participants were assessed on four (two weeks apart) visits. Sampling of approximal fluid for fluoride analysis and approximal plaque for organic acid analysis was performed. Chair-side tests were performed to register the lactic acid production rate on the tongue, approximal plaque pH, salivary buffer capacity and counts of cariogenic microorganisms. In Study V, caries-risk assessment following the use of 5,000 ppm fluoride toothpaste was performed consecutively on each of the four visits using the "Cariogram" software. **Results:** In Study I, the mean caries experience (DMFT/dmft) was high in the mothers and their younger and older children (12.4 ± 5.3, 9.0 ± 5.0 and 5.8 ± 4.1, respectively). The DMFT/dmft increased with higher salivary mutans streptococci counts in all age groups ($p<0.05$). The caries experiences of the children were positively correlated with those of their mothers ($R^2_{4-6}=0.12$,

$R^2_{12\text{-}16}$=0.18, p<0.01). A positive association between mothers and both children was evident for toothbrushing habits, snacking frequency and gingival health (p<0.05). An association between plaque scores, salivary buffer capacity and mutans streptococci (MS) counts was found between mothers and older children (p<0.05). In Study II, three complexes were formed from the dendrogram. PCA results were similar in all three groups. The correlation analyses of bacterial counts between mothers and their children showed a significant association for most of the bacterial strains (p<0.05 or 0.01). In Study III, no significant relationships were found between the bacterial scores and DMFT/dmft or D/d groups. In Study IV, the six weeks' use of 5,000 ppm fluoride toothpaste significantly increased the approximal fluid fluoride concentration, and salivary buffer capacity. It also decreased the lactic acid production rate, plaque acideogenicity ($AUC_{5.7}$, $AUC_{6.2}$, maximum pH fall) and salivary mutans streptococci counts. In Study V, the use of 5,000 ppm fluoride 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 lactobacilli (LB) counts]. A statistically significant linear trend was observed for MS counts (p<0.01) and the number of individuals with a salivary concentration of MS < 10^3 increased on each visit. The same trend was also observed for LB and buffer capacity scores (p=0.04 and p=0.03 respectively). **Conclusions:** The caries experience in Saudi mothers and their children is high, with similar contributory caries-related factors. Supragingival plaque microbiota are correlated between the mothers and their children with similar supragingival plaque microbial associations present in all three family members. The analysed pooled plaque samples did not reveal any significant relationships between the bacterial counts and the caries experience in any of the family members. The 5,000 ppm fluoride toothpaste has the ability to reduce the cariogenic potential of dental plaque and saliva, as well as the caries-risk profile.

Key words: Dental Caries, Cariogram, Families, Fluoride, Microbiology, Plaque, Risk Assessment

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Contents

Original papers

This thesis is based on the following five original papers, which are referred to by their Roman numerals in the text:

I. Mannaa A, Carlén A, Lingström P. Dental caries and associated factors in mothers and their preschool and school children – A cross-sectional study. J Dent Sci 2013; http://dx.doi.org/10.1016/j.jds.2012.12.009

II. Mannaa A, Carlén A, Dahlén G, Lingström P. Intra-familial comparison of supragingival dental plaque microflora using the checkerboard DNA-DNA hybridization technique. Arch Oral Biol 2012; 57: 1644-1650.

III. Mannaa A, Carlén A, Campus G, Lingström P. Supragingival plaque microbial analysis in reflection to caries experience. BMC Oral Health 2013; 13: 1-5.

IV. Mannaa 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. (*Submitted)*

V. Mannaa A, Campus G, Carlén A, Lingström P. Caries-risk profile variations after short-term use of 5,000 ppm fluoride toothpaste. (*Submitted*)

Introduction

Although a great deal of knowledge has been acquired about various diseases, they remain jigsaw puzzles, pieces of which are known, while others are still missing. Dental caries is a chronic disease that fits this portrayal. Our understanding of this disease is intricate due to its multifactorial nature and the fact that it is induced by a three-dimensional dynamic multi-bacterial ecological niche, namely the dental plaque biofilm. Nevertheless, cariology has advanced over the past 50 years with numerous advances in terms of the pathogenesis, transmission, diagnosis, prevention and management of caries. All of this has led to a shift in the manner dentistry is practised and, in the 21st century, contemporary dentistry has become governed by the concept of minimal intervention and invasiveness. However, this concept has not been fully grasped by dental practitioners in either the developed or the developing parts of the world. In addition, it seems that the "drill and fill" concept still persists.

Despite the availability of free dental services in several countries around the world, caries is still a major public health problem. In developing countries, dental professionals are aware of this fact. However, research is still in its infancy and more determination is needed to conduct large-scale studies to identify the reasons behind the high caries prevalence in these countries. The majority of scientific reports are published locally and international publications are very limited. The caries experience is usually expressed in terms of prevalence or incidence. For this reason, the nationwide adoption of the discipline of research and the comprehension of its value is indispensable to the aim of raising the standards of dental treatment in any developing country. This in turn will encourage the adoption of the medical model of caries management rather than the restorative model. Moreover, it will provide the building blocks required to structure community-based preventive programmes and ensure that they target the high-risk groups. This will also facilitate the adoption and practice of minimally invasive dentistry.

In order to elucidate the picture of dental caries in any population, different age groups of interest should be studied. In this context, targeting families would facilitate both the reporting of age-related data and the study of various caries-related, parent-child associations as well as the identification of age groups at risk of caries development.

Background

Scientific and global views of dental caries

Dental caries is the localised destruction of dental hard tissues by the bacterial fermentation of dietary carbohydrates (Marsh and Martin, 2009a). The disease begins with microbiological shifts within the dental biofilm. It is also affected by salivary flow and composition, exposure to fluoride, dietary consumption patterns and different preventive behaviours (Selwitz et al., 2007). It is generally a slowly progressing chronic disease (Mejàre et al., 1999; Selwitz et al., 2007). In terms of location, it can affect the crown (coronal caries) and/or the root (root caries) portions of primary and permanent teeth and affect smooth (facial, lingual/palatal and proximal) and occlusal surfaces (pit and fissures). Caries can be confined to the enamel or spread to affect the dentine and/or cementum (Selwitz et al., 2007). The pathogenesis of caries is determined by the occurrence of an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms (Fejerskov, 2004; Scheie and Peterson, 2004).

In recent decades the common consensus from worldwide reports is that dental caries appears to be in decline (Marthaler, 2004; Baelum et al., 2007). However, dental caries continues to be the single most prevalent and costly oral disease worldwide, despite the availability of different fluoride products (National Institutes of Health, 2001; Marsh, 2003; Jeon et al., 2011). The World Health Organisation (WHO) has concluded that despite huge improvements in the oral health of populations, problems still persist (Petersen et al., 2005). This is especially the case among underprivileged groups in societies ranging from low to high incomes but more specifically in lower socio-economic groups, immigrants and children (Petersen et al., 2005; Bagramian et al., 2009). In most industrialised countries, dental caries remains a major public health problem, with the vast majority of adults and 60-90% of schoolchildren being affected (Petersen and Lennon, 2004).

Familial aspects related to dental caries

Dental caries is a disease whose development and progression are affected by biological and non-biological determinants (Holst et al., 2001). In any given society, unlike the biological determinants (plaque, saliva, bacteria), the non-biological determinants (behaviour, attitude, education, social class, and income) are not the same and vary between

different societies around the world (Holst et al., 2001).

In this context, it is believed that the family structure (for example, birth rank, family size) may play an important role in the development of childhood caries (Wellappuli and Amarasena, 2012). The study of caries in families is not alien to dental research and has been the focus of both earlier and more recent investigations. The literature available on this topic has included a diversity of family-related aspects linked to caries. Some studies have discussed caries aggregation/clustering in families, the vertical transmission of cariogenic bacteria from parents to their children and the horizontal transmission of cariogenic strains between spouses (Garn et al., 1976; Emanuelsson et al., 1998; Redmo Emanuelsson and Wang, 1998; Berkowitz, 2003; Lindquist and Emilson, 2004; Strickland and Markowitz, 2011). The influence of early transmission of mutans streptococci from mothers to their children on the development of caries in their children has also been described (Köhler and Andréen, 2012). Other studies have investigated the influence of parental oral health knowledge, attitudes, behaviours and oral hygiene practices on their children's current and future oral health and caries risk, correlating parental/caregiver bacterial counts with those of their children (Mattila et al., 2000; 2005a; 2005b; Tanner et al., 2002a; Skeie et al., 2008; 2010; Tuli and Singh, 2010; Li et al., 2011; Holst and Schuller, 2012; Hooley et al., 2012). In addition, various caries prediction models in children have been proposed taking different parent-related factors and genetic susceptibility/resistance to dental decay into consideration (Werneck et al., 2010; Fontana et al., 2011; Shearer et al., 2011; Werneck et al., 2011). Moreover, there is a paucity of studies describing the influence of the family structure on the caries experience of children. This is particularly relevant in developing countries where the oral health status of both the children and adults may be considered to be worse than that of their counterparts in the developed world.

In the Kingdom of Saudi Arabia (KSA), rare attempts have been made to study correlations between mothers and their children regarding different caries-related variables. One study compared the levels of *S. mutans* and *Lactobacilli* in caries-free children and children with Severe Early Childhood Caries (SECC) with that of their corresponding mothers, and found a significant relationship in the mother-child pair in the SECC group with respect to salivary levels of *S. mutans* (Al-Shukairy et al., 2006). Another study assessed the level of salivary secretory immunoglobulin A (sIgA) in caries-free children and children with SECC and their corresponding mothers and found a positive high correlation for sIgA between mothers and children in both groups (Al-Amoudi et al., 2007).

Oral health status and caries management in the Kingdom of Saudi Arabia

There is a general awareness among dental professionals in KSA about the high caries experience in the Saudi population. Unfortunately, no oral health-related national data registry exists for KSA. Some oral health related epidemiological surveys limited to one of the major cities or provinces in KSA are available but nothing at a national level. The majority of these surveys report the caries status in terms of prevalence with data mainly from one gender usually males. Table 1 provides a quick glance at the data available on caries for different age groups in KSA.

The WHO goals for caries by the year 2020 are to increase the proportion of caries-free six-year-olds, to reduce the DMFT particularly the D component at age 12 years and to reduce the number of teeth extracted due to dental caries at the ages of 18, 35-44 and 65-74 (Hobdell et al., 2003). Compared with the western societies and taking account of these goals, it appears that there is a high caries experience in KSA especially in children. The caries pattern in Saudi children is characterised by bilateral occurrence in both primary and permanent dentitions, where the majority of children have both posterior and anterior caries, the mandibular second molars are the most commonly affected posterior teeth and the maxillary central incisors are most affected among the anterior teeth (Baghdadi, 2011). Contrary to the developed countries, the caries experience appears to be higher in Saudi children with a high socio-economic status (Al-Mohammadi et al., 1997; Baghdadi, 2011). In addition, the difference in caries experience in urban and rural areas often reported for developing countries does not appear to apply to KSA (Al-Shammery, 1999; Baghdadi, 2011).

It is believed that Saudi children are vulnerable to tooth decay due to several reasons. They can be grouped into factors related to the oral health conception in Saudi society and factors related to the standards of professional dental services in KSA. The society-related factors include a general lack of knowledge and awareness of oral health care and hygiene practices, lack of parental guidance with the late introduction of oral health care, a minimal interest in regular dental visits and the seeking of dental treatment restricted to emergency situations and pain relief, little attention devoted to the importance of dental preventive measures, poor dietary habits with the excessive consumption of sweets and junk food and parents' overindulgence of their children with this kind of cariogenic food. Factors related to the level of professional dental treatment include the lack of a dental recall system as an accepted norm in dental practice, deficiency in the implementation of the "early oral heath care concept"

and the fixation of dentists and university dental curricula on the restorative model of caries management. The latter is contrary to the medical model, which emphasises the importance of caries prevention and minimally invasive dentistry.

Table 1. Review of the caries experience (mean DMFT/dmft) in KSA (1979-2006).

Source	Age	DMFT/dmft (mean)
Al-Mohammadi et al., 1997	2 years	0.8
Wyne et al., 2001		6.7
Wyne et al., 2001	3 years	6.9
Al-Mohammadi et al., 1997	4 years	5.8
Wyne et al., 2001		8.5
Wyne et al., 2001	5 years	9.2
Paul, 2003		7.1
Al-Malik et al., 2003	2-5 years	4.8
Salem and Holm, 1985	3-5 years	1.2
Wyne, 2008		6.1
Al-Mohammadi et al., 1997	6 years	3.9
Al-Tamimi and Petersen, 1998		6.4
Wyne et al., 2001		9.3
Al-Dosari et al., 2004	6-7 years	6.4
Al-Wazzan, 2004		7.3
Al-Malik and Rehbini, 2006		8.1
Barmes and Zahran, WHO assignment report 1979	12 years	2.0*
Hussein, 1985		2.0*
Leous, WHO Assignment Report, 1992		2.1*
Leous, WHO Assignment Report, 1995		1.7*
Al-Tamimi and Petersen, 1998		2.9
Al-Shammery, 1999	12-13 years	2.7
Al-Dosari et al., 2004		4.8
Al-Sadhan, 2006	12-14 years	5.9
Leous, WHO Assignment Report, 1992	15 years	1.7-5.9*
Petersen, 1994	35-44 years	8.7*

* Source: http://www.mah.se/CAPP/Country-Oral-Health-Profiles/EMRO/Saudi-Arabia/Oral-Diseases1/Dental-Caries/

Despite the previously mentioned high caries prevalence in KSA, there has been little recognition of the issue. Not many efforts have been made to discuss the current situation, educate and motivate the general population and adopt preventive and modern minimally invasive dental strategies (Baghdadi, 2011).

Dental plaque as a biofilm

Since the early pioneering investigations into dental plaque in the 17th century, its microbial composition has captivated dental researchers. These early studies lead to the recognition of dental caries as a plaque-mediated oral disease. Concurrently, two schools of thought emerged: the first (non-specific plaque hypothesis) attributed oral diseases to the mere presence of plaque, while the second (specific plaque hypothesis) stated that only a few species from the diverse collection of organisms comprising the plaque microflora are actively involved in the disease (Loesche, 1979; Theilade, 1986). Based on this, caries research focused on the study of specific bacteria isolated from planktonic cultures and descriptions of single-species implication in the disease process. Further studies viewed dental plaque as an organised consortium of different bacteria.

In 1991, the ecological plaque hypothesis was formulated, bridging the gap between the two earlier theories (Marsh, 1991). The hypothesis stated that disease occurs as a result of a shift in the balance of the resident microflora due to ecological perturbations in the local environment (Marsh, 1991). The current concept of biofilm structure based on the revolutionary biofilm engineering studies has supported this hypothesis and has led to a renaissance in our understanding of dental plaque and plaque-mediated diseases such as dental caries and periodontitis (Costerton et al., 1995; Russell, 2009). A new era in dentistry thus emerged in the late 20th century, portrayed by the view of dental plaque as a dynamic and complex microbial ecosystem comprising an assortment of micro-niches, metabolic functions and intra-species interactions (Bowden, 2000; Marsh, 2003; Aas et al., 2005; Beighton, 2005; Jenkinson and Lamont, 2005; Socransky and Hafajee, 2005; Kolenbrander et al., 2006; Marsh and Percival, 2006; Kuramitsu et al., 2007; Haffajee et al., 2008; Filoche et al., 2010)

These developments have driven scientists to study the complex nature of the oral biofilm and its role in disease development, progression and prevention. Dental caries was once believed to be a simple disease with *Streptococcus mutans* as the sole etiological agent (Kleinberg, 2002). However, evidence has pointed to the existence of microbial

succession within the oral biofilm consisting of a shift away from the early colonisers such as the streptococci (*S. oralis*, *S. mitis*, *S. gordonii* and *S. sanguinis*) to late colonisers such as *Prevotella intermedia* with the presence of bacteria acting as a bridge between the early and late colonisers like *Fusobacterium nucleatum* (Marsh and Martin, 2009b). Consequently, there has been a transition from the traditional focus on the acidogenic and aciduric mutans streptococci and *lactobacilli* to other cariogenic bacteria such as low pH non-mutans streptococci, *Rothia*, *Bifidobacterium* spp and *Veillonella* spp (Marsh and Martin, 2009b; Filoche et al., 2010). As a result, the oral microflora are now studied in the context of a biofilm and the balance between homeostasis and shifts in this microbial community is what defines the nature of dental plaque and dictates whether health or disease prevails.

Oral microbiological analyses

The human body is composed of more than 10^{14} cells, of which only 10% are mammalian (Marsh et al., 2011). The human oral microbiome includes hundreds of microorganisms, which as mentioned previously colonise the oral surfaces and grow as the biofilm – dental plaque (Filoche et al., 2010). The majority of these microorganisms are bacteria originally identified and characterised using culture-dependant methods (Marsh et al., 2011). Recent culture-independent approaches have enhanced our knowledge of the complexity of the oral microflora. At present and based on these techniques, the human oral microbiota consists of more than 700 species, each composed of strains with different phenotypes and genotypes (Beighton, 2009). Oral infections are distinctive in the sense that the bacteria commonly present in the resident oral flora are key players in disease initiation and progression (ten Cate, 2006).

Fewer than 50% of the resident oral microflora can be cultivated, rendering culture-based analyses unsuitable for holistic studies (Marsh et al., 2011; Pozhitkov et al., 2011). Microbiological analysis has witnessed a burst of culture-independent molecular technologies ranging from clone counting and sequencing (16S ribosomal RNA analysis), fingerprinting of amplified polymerase chain reaction (PCR) products (a technique called "terminal restriction fragment length polymorphism"), quantitative PCR, pyrosequencing to high-throughput microarrays and metagenomic and metatranscriptomic approaches (Sakamoto et al., 2005; Pozhitkov et al., 2011, Nyvad et al., 2013). Among the nucleic acid-based technologies that have revealed the complex microbiology of dental plaque, is the checkerboard DNA-DNA hybridisation technique (Socransky et al.,

1994). This technique uses whole genomic DNA probes and gives a simultaneous and quantitative analysis of up to 28 plaque samples against 40 key microbial species (Socransky et al., 1994). For the analysis, 28 alkali lysates of dental plaque, and two DNA standards representing 10^5 and 10^6 cells per target species are fixed on a membrane in thin lanes. They are then simultaneously cross-hybridised with digoxigenin-labeled whole genome probes (Wall-Manning et al., 2002). The technique is called "checkerboard" because the genomic probes are hybridised at right angles to the DNA of multiple oral samples, and the processed images of the hybridisations resemble a checkerboard (Pozhitkov et al., 2011).

The checkerboard DNA-DNA hybridisation technique can be performed using one of three probe types, whole genomic probes, oligonucleotide probes (16S rRNA gene-based probes) or multiple displacement amplification-based probes (Socransky et al., 1994; Paster et al., 1998; Brito et al., 2007). The technique offers ample advantages as it is rapid, sensitive, relatively inexpensive and permits the enumeration of a large number of species in large-scale studies with numerous samples (Socransky et al., 1994; Sakamoto et al., 2005; Socransky and Haffajee, 2005; Nyvad et al., 2013). In addition, it overcomes many of the limitations of culture-based techniques, primarily the loss of viability of organisms during transport, as it requires preserved bacterial DNA and not viable bacteria (Do Nascimento et al., 2006; Sassone et al., 2007). Moreover, the entire sample is employed without dilution or amplification, overcoming quantification problems caused by serial dilution or PCR amplification procedures (Do Nascimento et al., 2006). Furthermore, the membranes can be stripped and re-probed with a new set of different DNA probes or in the event of technical errors (Do Nascimento et al., 2006). On the other hand, the technique has some limitations: high-quality DNA is required for the preparation of the probes and standards, which in turn calls for the careful evaluation of probe specificity, whole genomic probes are prepared using the entire genome of a bacterial species as the target, thereby increasing the probability of cross-reactions/cross-hybridisation between species because of common regions of DNA among closely related species (Do Nascimento et al., 2006; Gellen et al., 2007, Nyvad et al., 2013). The technique is not an open-ended approach, as it can only detect species for which probes have been made (Do Nascimento et al., 2006; Nyvad et al., 2013). Furthermore, the technique has a high cut-off limit (10^4) for bacterial quantification, making the detection of bacteria in low counts problematic (Socransky et al., 1998).

Dental plaque acidogenicity

Cariogenic microorganisms are characterised by being acidogenic and aciduric with mutans streptococci, lactobacilli and *Bifidobacterium* surpassing non-mutans streptococci and *Actinomyces* in such properties (Takahashi and Nyvad, 2011). Plaque acidogenicity is a collective term, which reflects changes in dental plaque pH and the acidic *milieu* produced by cariogenic bacteria. The plaque pH curve based on changes *in vivo* in response to sugar exposure, was first described by Stephan in 1940. Since then, there has been a debate about the relevance of these pH changes for caries development (Stephan, 1940; Dong et al., 1999, Bowen, 2013). On theoretical grounds, acid production in plaque has been believed to be crucial for caries development because enamel solubility is pH dependent (Leach, 1959; Larsen and Jensen, 1989). In 1944, Stephan reported that plaque-pH falls following sugar exposure were greater in caries-active than in caries-inactive subjects (Stephan, 1944). Later on, human, animal, *in situ* and *in vitro* studies supported a positive relationship between plaque sugar-fermenting activity and dental caries (Charlton et al., 1971; Agus et al., 1980; Bodden et al., 1983; van Houte et al., 1996; Lingström et al., 2000).

Plaque pH can be assessed clinically by means of sampling, microtouch or telemetric methods (Lingström et al., 1993). In plaque samples collected prior to and after rinsing with a 10% sucrose solution, plaque acidogenicity can also be laboratory assessed by determining the anion concentrations of organic acids, mainly sucrose-induced lactate (Damen et al., 2002). However these methods are too complicated for use in the clinic. Researchers have therefore been striving to develop simplified methods to clinically measure plaque acid production and pH. Recently, two chair-side methods have been developed. The first is the pH "strip" method, which is used to record approximal plaque pH (Carlén et al., 2010). In this method, commercially available pH-indicator strips measuring pH in the range of 4.0-7.0 are inserted into the area of measurements. The second is the Clinpro™ Cario L-Pop™, an indicator swab, which reflects the rate of lactic acid production in the tongue biofilm (Bretz et al., 2007). Its performance is based on the enzymatic oxidation of lactic acid by lactate dehydrogenase, coupled to a cascade of redox indicators that generate the colour signal (Bretz et al., 2007).

Minimal intervention dentistry

Dentistry in the 21st century has witnessed a burst of developments in caries diagnosis and management. These advances have enabled a more conservative and highly precise caries treatment but, more importantly,

superior caries prevention and control. There has therefore been a revival of the concept of minimal intervention dentistry. This approach integrates concepts of prevention, control and treatment including early lesion detection, risk assessment, the implementation of preventive strategies, patient education and conservative restorative practices (Featherstone and Doméjean, 2012). Patient care is based on biological rather than restorative solutions. In other words, the concept deals with the causes of the disease and not just the symptoms (Featherstone, 2000; Sheiham, 2002; Mount, 2007; Featherstone and Doméjean, 2012).

Caries-risk assessment

The cornerstones of modern caries management are risk-based prevention and disease management (Fontana and Gonzalez-Cabezas, 2012). Caries-risk assessment is essential for the correct prevention and management of dental caries and should be incorporated into daily practice in order to: (1) determine the activity of the carious lesions, (2) estimate the degree of risk in order to customise the intensity of the treatment, (3) identify the main aetiological agents contributing to the current disease that might be targeted in the management of the disease, (4) establish the need for additional diagnostic procedures, (5) formulate the best restorative treatment plan for the patient, (6) enhance the overall prognosis of the patient and (7) evaluate the efficacy of the caries management plan (Twetman and Fontana, 2009; Fontana and Gonzalez-Cabezas, 2012).

Since the caries-risk concept was introduced, different caries-risk models have been developed (Krasse, 1985). Nowadays, the two mostly used models are (a) the "Cariogram" by Bratthall and (b) CAMBRA (caries management by risk assessment) by Featherstone (Bratthall, 1996; Featherstone et al., 2003; Featherstone, 2004). The "Cariogram" is a caries-risk prediction software developed to describe and calculate the individual caries-risk profile, expressing graphically the chance an individual has of avoiding caries in the near future (Bratthall, 1996). The program takes account of several risk factors involved in the caries etiology and illustrates the strength of these factors in a particular individual using an algorithm with a "weighted" analysis of the data entered.

The graphical presentation is in the form of a pie chart (Figure 1) with five coloured sectors: the dark-blue sector (diet) is based on a combination of dietary content (salivary lactobacilli [LB] count) and diet frequency, the red sector (bacteria) is based on a combination of the amount of plaque and the mutans streptococci (MS) count, the light-blue sector (susceptibility) is based on a combination of fluoride regimen, saliva secretion and saliva buffer capacity, the yellow sector

(circumstances) is based on a combination of caries experience and related diseases and the green sector shows an estimation of the chance of avoiding caries and is what is calculated after each of the above sectors takes its share.

The "Cariogram" is a useful tool that can be used regularly to make appropriate targeted preventive care decisions and recall assessments (Petersson et al., 2010a). CAMBRA is an extension of the concept proposed by Krasse, and is a model based on the "Caries Balance Theory" (Featherstone, 2004). In addition to a caries-risk assessment tool, the "Cariogram" can be used as a pedagogic model to inform and educate patients about their dental health, caries status and risk.

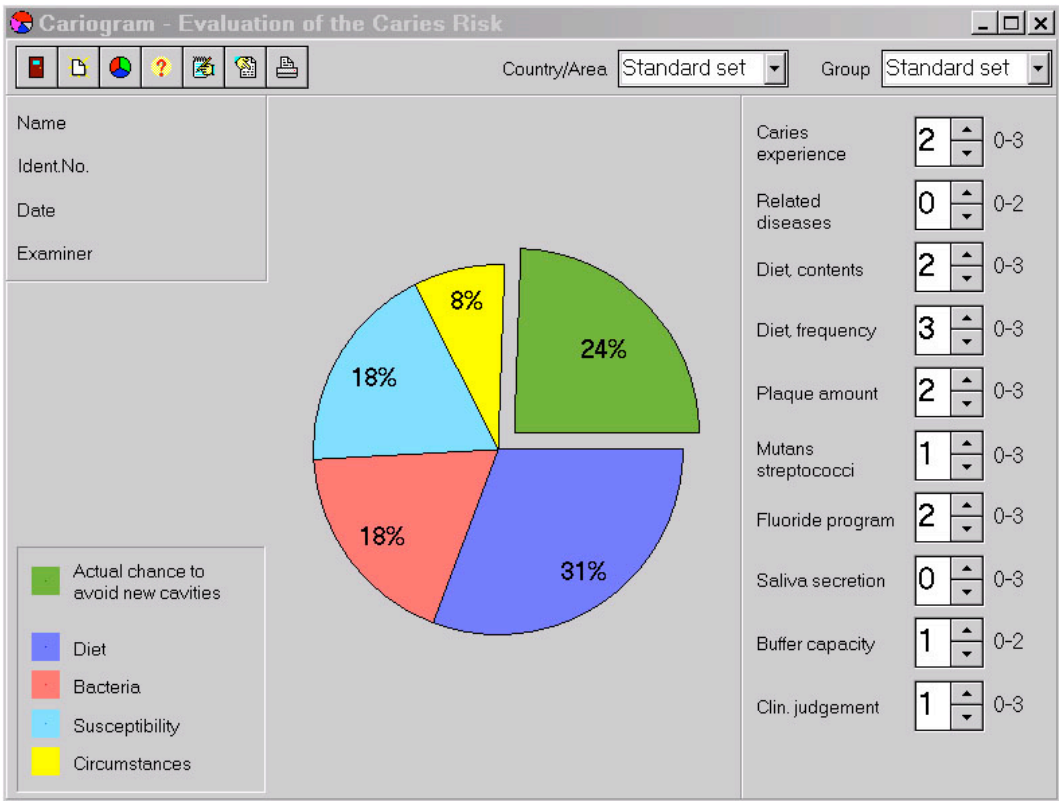


Figure 1. The different variables included in the Cariogram software and the different sectors of its pie chart.

Caries prevention

Dental caries is a chronic, infectious and transmissible disease (Fitzgerald and Keyes, 1960; Caufield and Griffen, 2000). Once infected, an individual is at risk of developing the disease throughout life. To put it in another way, the disease can be prevented but not cured or eradicated. Traditional preventive options against caries are differentiated into three classical categories: primary, secondary and tertiary prevention

(Longbottom et al., 2009). Primary prevention includes those measures that prevent disease initiation. Secondary prevention focuses on disease treatment in its early stages in order to arrest or reverse the disease process after the clinical signs are initiated. Tertiary prevention includes measures which remove and replace irreversibly damaged dental tissue in order to prevent further progression of the disease. In addition, certain disease scenarios call for a "hybrid" interaction of non-operative and operative procedures (Longbottom et al., 2009).

To the present day, strategies to control or prevent dental caries include a single or a combination of patient or professionally applied options: pit and fissure sealants, dietary assessment/modification, fluoride applications, enhancement of remineralisation and oral hygiene instructions (Longbottom et al., 2009). For a long time, the practice has been to control caries by acting on biofilm formation/maturation (mechanical/chemical plaque control), by modifying the kinetics of apatite solution (use of fluoride, dietary control, control of salivary flow) or by a combination of both (Llena Puy and Forner Navarro, 2008). However, recent strategies advocate the modification/disruption of the oral biofilm as a key to modern caries management (Longbottom et al., 2009; Twetman, 2010; Rodrigues et al., 2011). The goal is therefore to alter the dental biofilm ecology in order to counteract rapid pH decreases in the dental biofilm and maintain a neutral environment, which supports microbial homeostasis (Twetman, 2010).

Although these solutions may sound simple, the lack of effective implementation could lead to a further deterioration in the future oral health of the international community, with a subsequent strain on the dental profession and an escalation in the cost of dental services (Bagramian et al., 2009). The emerging public health issues are related to disparities in the prevalence and treatment of dental caries. The WHO therefore continues to emphasise that efforts to improve the overall situation are still needed (WHO, 2001; Moynihan and Petersen, 2004). Recently, experts have become inclined towards a return to and renovation of the basics of prevention. Moreover, the WHO has underlined the need to integrate oral disease prevention with national and community health programmes (WHO, 2003; Bagramian et al., 2009).

Fluoride - the dental "Janus"

Research on the oral health benefits of fluoride spans 100 years and it is now recognised as the principal reason for the worldwide decline in caries prevalence (Buzalaf et al., 2011). The precise mechanisms behind the anti-caries actions of fluoride are still the subject of debate

(Duckworth and Morgan, 1991; Marsh and Martin, 2009a). In general, fluoride exerts its main effect by reducing demineralisation and enhancing remineralisation (Koo, 2008; Buzalaf et al., 2011). The local action of fluoride is based on the fluoridation of the surfaces of the hydroxyapatite crystals (Shellis and Duckworth, 1994; Marquis, 1995). The resulting fluorapatite is thermodynamically more stable and resistant to acid dissolution than hydroxyapatite (Marsh and Martin, 2009a, Gazzano et al., 2010). Since fluoride has a lower solubility, its precipitation is enhanced by contact with solutions containing calcium and phosphate (Gazzano et al., 2010). This accounts for the effect fluoride has on the dissolution of enamel and dentin during demineralisation and precipitation during remineralisation (ten Cate, 1999).

In addition, fluoride has an antimicrobial action dependent on inhibiting the metabolism of plaque bacteria, especially cariogenic streptococci (Hamilton, 1990; Marquis, 1995; Van Loveren, 2001; Koo, 2008; Marsh and Martin, 2009a; Buzalaf et al., 2011). Under acidic conditions, fluoride in the form of HF easily crosses the bacterial membranes due to its lipophilic nature (Marsh and Martin, 2009a). Since the intracellular pH of bacteria is alkaline with respect to the extracellular pH, once inside the cell, HF dissociates into H^+ and F^- (Hamilton, 1990; Marsh and Martin, 2009a). On the one hand, H^+ will acidify the cytoplasm inhibiting various enzymes and reducing the transmembrane pH gradient thereby affecting the bacterial uptake and secretion processes (Marsh and Martin, 2009a; Buzalaf et al., 2011). On the other hand, F^- will reduce glycolysis via the direct inhibition of enolase, indirectly inhibit sugar transport and hinder the synthesis of intracellular storage compounds such as glycogen (Marsh and Martin, 2009a; Buzalaf et al., 2011). Consequently, fluoride ends up reducing the bacterial acid tolerance, stabilising the composition of the plaque microflora and lowering plaque cariogenicity (Marquis, 1995; Marsh and Martin, 2009a; Buzalaf et al., 2011).

The current models for increasing the anti-caries effects of fluoride agents necessitate the maintenance of a cariostatic concentration of F in oral fluids (Vogel, 2011). Fluoride is bioavailable orally in the form of two calcium-bound reservoirs: mineral deposits of fluoride as CaF_2 in saliva, the fluid phase of dental plaque and fluorapatite, and Ca-F deposits bound to proteins and mucosal tissue (biologically bound) as well as to bacteria (bacterially bound) in dental plaque (Vogel, 2011). Since all these reservoirs are mediated by calcium, their formation is limited by the low concentrations of calcium in oral fluids (Vogel, 2011). Novel procedures are needed to overcome this problem in order to increase the formation of fluoride reservoirs, especially after its topical

application (Vogel, 2011).

In the past, the golden standard for caries prevention was water fluoridation, emphasising its pre-eruptive importance (Buzalaf et al., 2011). The focus then shifted towards its topical post-eruptive effects with a burst in the availability of fluoridated products including primarily toothpastes, but also other products such as mouthrinses, gels, varnishes, and toothpicks (Buzalaf et al., 2011). However, there are those who are still in favour of the systemic methods of fluoride delivery other than water fluoridation such as dietary fluoridated supplements in the form of salt or milk (Sampaio and Levy, 2011).

Despite the availability of different fluoride products on the market and the huge variation in expert opinion regarding the reason behind caries decline, there is clear agreement regarding the beneficial effects of fluoride toothpaste (Bratthall et al., 1996). Fluoride dentifrices are regarded as the most important artillery in the fight against caries (Bratthall et al., 1996; Marinho et al., 2003). However, Lynch et al. (2004) concluded that low levels of plaque and salivary fluoride resulting from the use of 1,500 ppm fluoride toothpastes, are insufficient to have a significant antimicrobial effect on plaque bacteria. Scientists are therefore striving to develop novel toothpaste formulas, which can increase the oral bioavailability of fluoride and in turn enhance its antimicrobial action. One recent strategy has been to increase the fluoride concentration in toothpastes, since this would be coupled with a greater driving force for its diffusion through the biofilm towards the tooth surface, as well as an increased deposition in the biofilm (Zero et al., 1992). Commercially available fluoridated toothpastes have a maximum concentration of 1,500 ppm fluoride. In Europe and the United States, dentifrices containing fluorides in concentrations of > 1,500 ppm are available on the market, with or without a professional prescription (Colgate Duraphat® 2800 ppm, Colgate Duraphat® 5000 ppm, Colgate® PreviDent® 5000 Plus®, 3M Clinpro™ 5000 ppm, R.O.C.S.® Medical 5000 ppm, Dentsply Sensodyne® NUPRO® 5000 ppm, Sultan Topex® ReNew™). In general, these toothpastes are recommended for high caries risk individuals above the age of 10 years such as those with xerostomia or root surface caries (Davies and Davies, 2008; Davies et al., 2010).

Several studies evaluating 5,000 ppm fluoride toothpastes have shown their ability to reduce plaque scores and prevent or reverse caries (Baysan et al., 2001; Lynch and Baysan, 2001; Schirrmeister et al., 2007; Ekstrand et al., 2008; Nordström et al., 2009; Nordström and Birkhed, 2010). One recent study has shown that two weeks use of 5,000 ppm fluoride toothpaste without post-brushing rinsing results in higher plaque and saliva F concentrations as compared to 1,450 ppm fluoride toothpastes (Nordström and Birkhed, 2009). Despite these positive

effects, further research is needed to explore the consequences of the use of 5,000 ppm fluoride toothpaste on caries-related plaque and salivary parameters.

Apart from the fluoride concentration, optimal caries protection from dentifrice is dependent on an efficient fluoride delivery (Chesters et al., 1992; Chestnutt et al., 1998; Ashley et al., 1999; Zero et al., 2010). In order to maximise fluoride benefits, the frequency of brushing, quantity of paste used, duration of brushing and thoroughness of rinsing are all brushing behaviour factors to consider (Zero et al., 2010). Sjögren (1995) proposed the "modified toothpaste technique" and showed that the use of a toothpaste slurry, along with no post-brushing water rinsing and the avoidance of post-brushing eating and drinking, enhanced fluoride accumulation in saliva and approximal plaque. In 2010, Zero and co-workers suggested both brushing time and dentifrice quantity as important determinants of oral fluoride retention (Zero at al., 2010). In their study, they compared brushing times of 30 to 180 seconds, as well as brushing with 0.5 g versus 1.5 g dentifrice. They concluded that prolonging brushing time and using 1.5 g dentifrice significantly increased the fluoride recovered in saliva after brushing.

Fluoride continues to reign over the world of preventive dentistry and remains the most effective anti-caries agent.

Hypotheses and aims

1. The hypothesis of *Study I* was that children have caries experiences and caries-related habits and behaviours similar to those of their mothers and the aim was to describe the caries experience and caries-related factors in mothers and their preschool and school children.

2. The hypothesis of *Study II* was that the dental plaque microbiology of children is similar to that of their mothers and the aim was to correlate quantified supragingival plaque bacteria between mothers and their children and identify possible microbial associations.

3. The hypothesis of *Study III* was that higher caries experiences are associated with higher counts of caries-related bacteria in supragingival plaque, and the aim was to examine whether bacteria in pooled supragingival plaque samples quantified using a "checkerboard DNA-DNA hybridisation"-based panel of caries-related bacteria, were able to reflect the caries experience in a manner similar to saliva samples analysed using chair-side methods.

4. The hypothesis of *Study IV* was that regular brushing with 5,000 ppm fluoride dentifrice can lead to a reduction in the cariogenic potential of plaque and saliva and the aim was to measure the effects of six weeks' use of a 5,000 ppm F toothpaste on caries-related factors in dental plaque and saliva.

5. The hypothesis of *Study V* was that short-term use of high fluoride toothpaste causes a reduction in the individual caries risk and the aim was to consecutively assess the caries risk following six weeks' use of 5,000 ppm fluoride toothpaste using the "Cariogram".

Materials and methods

Study design and participants

The study design, participants and investigations for each of the five studies included in the thesis are summarised the table below.

Table 2. The design, participants and investigations for each of the five studies included in the thesis.

Study	Design	Participants	Data collected
Study I	Cross-sectional	86 families (258 participants): 86 mothers + 86 children (4-6 yrs old) + 86 children (12-16 yrs old)	Caries experience (DMFT/deft) Plaque amount (PlI) Gingival index (GI) Salivary chair-side tests Questionnaire
Study II	Cross-sectional	Same as in Study I	"Checkerboard DNA-DNA Hybridisation" analysis scores
Study III	Cross-sectional	Same as in Study I	Caries experience (DMFT/deft) "Checkerboard DNA-DNA Hybridisation" analysis scores
Study IV	Longitudinal (6 weeks)	17 families (34 participants): 17 mothers + 17 children (13-17 yrs old)	Approximal fluoride concentration pH measurement Organic acid analysis Tongue lactic acid production rate Salivary chair-side tests Date collection at baseline, 2-, 4- and 6-weeks recall visits
Study V	Longitudinal (6 weeks)	Same as in Study IV	Caries risk assessment using the "Cariogram" at baseline, 2-, 4- and 6-week recall visits

The dental records at King Abdulaziz University Hospital (KAUH) in Jeddah, Kingdom of Saudi Arabia, were screened to identify suitable family candidates. Of 6,705 dental records examined, 142 candidate families met the inclusion criteria, which were having Saudi nationality, having at least two siblings in the family aged 4-6 and 12-16 years and all family members being healthy. The families were contacted by telephone and a total of 86 families agreed to participate. From each family, the mother and two of her children, aged 4-6 years (C_{4-6}) and 12-16 years (C_{12-16}), volunteered, making a total of 258 participants (Studies I-III). For Studies IV and V, 17 of the original 86 families participated including only the mothers and teenagers (34 participants). Apart from the inclusion criteria of having Saudi nationality and being healthy, Studies IV and V had the following exclusion criteria: regular use of chewing sticks (Miswak), pregnancy of the mothers and smoking habits.

Clinical data sampling and recording

All clinical data collection and sampling for the five studies was conducted by the principal investigator (AM) at the Dental Health Clinic at KAUH, Jeddah, Kingdom of Saudi Arabia. The laboratory analyses were also performed by AM with assistance from the personnel at the laboratories at the Departments of Cariology as well as Oral Microbiology and Immunology at the University of Gothenburg, Gothenburg, Sweden, and the Department of Preventive Dentistry at the Academic Centre for Dentistry Amsterdam, University of Amsterdam and VU University, Amsterdam, The Netherlands.

Study I

Interview/questionnaires

In Study I, a structured interview in Arabic language was conducted to obtain information about the socio-economic status of the family, individual oral hygiene habits and dietary habits (Table 3).

Caries registration

The dentition status/caries experience was expressed using the DMFT (Decayed, Missing and Filled Teeth) index for permanent teeth and dmft for primary teeth. Caries was registered clinically and radiographically (bitewings to record approximal decay) at the D_3 level (Pitts, 2004). All

the approximal surfaces in the dentition from the mesial surface of the first premolars/primary molars to the distal surface of the second molars/primary molars were included.

Table 3. The different questionnaire components included in the structured interview.

Socioeconomic status of the family	Individual oral hygiene habits	Individual dietary habits
Mother's education: Illiterate Lower education (elementary, intermediate or secondary schooling) Higher education (bachelor, master's or doctor's degree) **Family income per month:** No income Low (< 4000 SR*) Moderate (4000 - < 10000 SR) High (≥ 10000 SR) Don't know **Total number of children per family:** 2-4 5-7 8-10	**Regular brushing:** No Yes **Toothbrushing frequency:** Once or a few times a week Once a day Twice a day More than twice a day Other **Toothbrushing tool:** Manual toothbrush (B) Miswak (M) Electric brush Combination (B+M) Combination (all) **Interdental cleaning:** Dental floss (F) Toothpick (TP) Interdental brush Combination (F+TP) Nothing used **Toothpaste use:** No Yes **Type of toothpaste:** Non-fluoridated Fluoridated Don't know	**Intake of between-meal snacks:** No Yes **Intake frequency of nine commonly consumed snacks**:** No intake Once a month Once a week 2-3 times a week Once or more a day

*: In local currency: Saudi riyals (SR), 1 euro ≅ 5.05 SR

**: Drinks with sugar added, dates, soft drinks, sweets/chocolates, buns/biscuits/cakes, ice cream, sweetened flakes, chocolate drinks and chips

Plaque amount and gingival health

The plaque index (PlI) by Silness and Löe (1964) and the gingival index (GI) by Löe and Silness (1963) were used to record plaque amount and gingival health. The facial/buccal, lingual/palatal and approximal surfaces of six teeth (16, 12, 24, 36, 32 and 44) were examined in the mothers and their older children. Teeth 55, 52, 64, 75, 72 and 84 were examined in the younger children. In the case of mixed dentition, the permanent molars and incisors were used (16, 12, 36, 32), while the primary molars (64, 84) were used if the permanent premolars (24, 44) had not yet erupted.

For both PlI and GI, each of the four surfaces of the teeth (buccal, lingual/palatal, mesial and distal) was given a score from 0-3. The scores from the four surfaces of the tooth were added and divided by four in order to give the index for the tooth. The index for the patient was obtained by adding up the indices for all six teeth divided by six, for each index respectively. For GI, an interpretation ranging from no to severe inflammation was recorded, based on the calculated average index.

Salivary analyses

The CRT® caries risk test (Ivoclar-Vivadent, Schaan, Liechtenstein) was used to record the salivary buffer capacity, mutans streptococci (MS) and lactobacilli (LB) counts. The buffer capacity of stimulated saliva was determined using a colored chart provided by the manufacturer (low: yellow, medium: green, high: blue). The MS and LB counts were scored in two classes: low ($<10^5$) and high ($\geq 10^5$) according to the manufacturer.

Studies II and III

Oral microbiological analysis using the "Checkerboard DNA-DNA Hybridisation Technique"

Pooled supragingival plaque was sampled according to Gellen et al. (2007) from the approximal sites between the 2nd premolar and 1st molar in the mothers and older children, and the approximal sites between the primary molars in the younger children using sterile Gracey curettes. The analysis of bacterial species was performed using the checkerboard DNA-DNA hybridisation method at the laboratory of the Department of Oral Microbiology and Immunology, University of Gothenburg, Sweden according to Wall-Manning et al. (2002). The technique is summarised in Figure 2. The panel included the following 18 bacterial strains:

Streptococcus mutans, Streptococcus sobrinus, *Streptococcus mitis*, *Streptococcus gordonii*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Actinomyces odontolyticus*, *Actinomyces oris*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Veillonella parvula*, *Rothia dentocariosa*, *Bifidobacterium dentium*, *Parvimonas micra*, *and Enterococcus faecalis*. In Study III, the 15 caries-related bacteria, from the above 18 strains, were included.

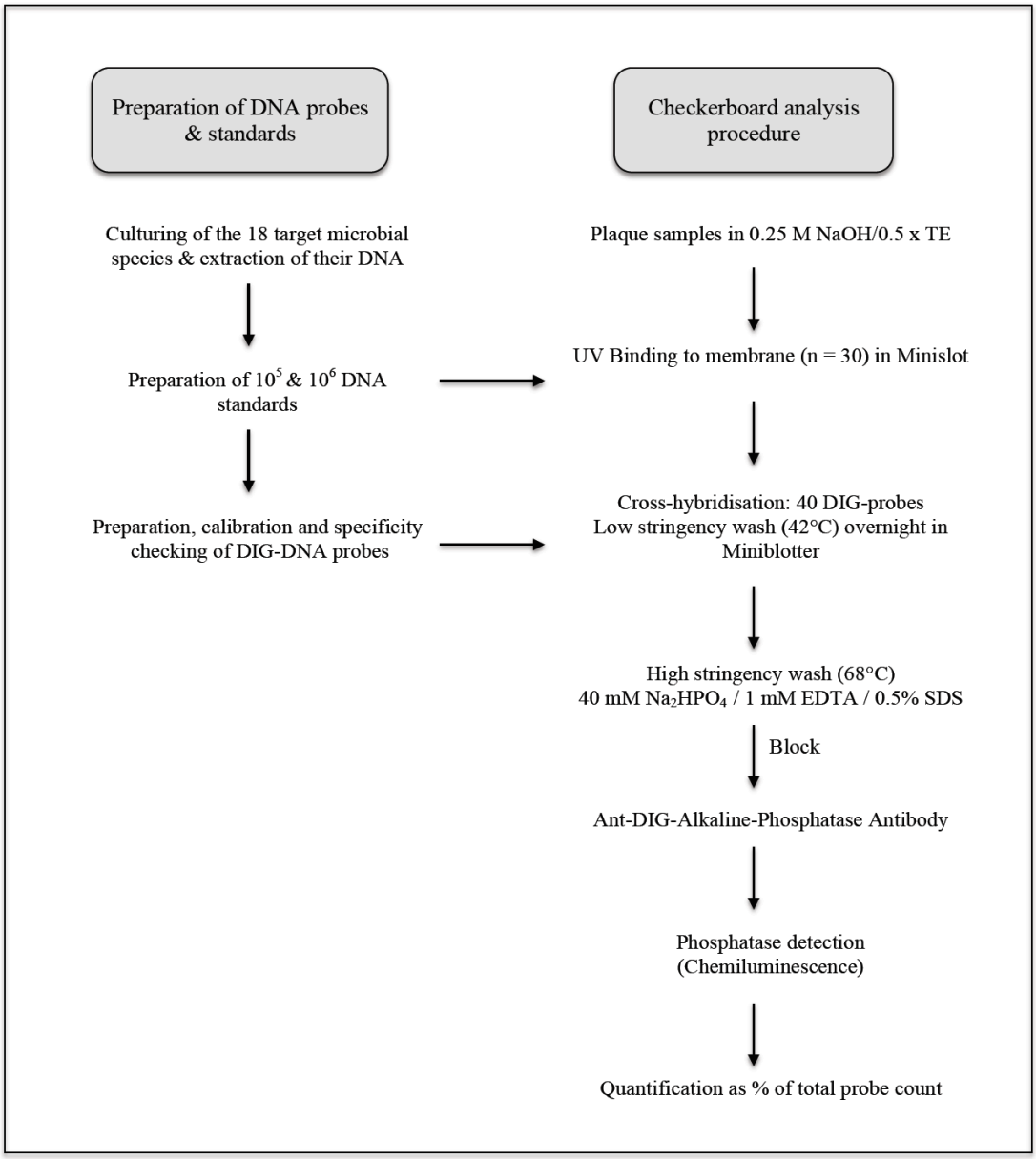


Figure 2. Summary of the "Checkerboard DNA-DNA Hybridisation Technique".

Studies IV and V

Time span of the studies

Both Studies IV and V are based on a clinical evaluation of 5,000 ppm fluoride toothpaste. Both studies were performed simultaneously and carried out over a period of six weeks, with a total of four visits: first visit (baseline), second visit (2 weeks), third visit (4 weeks) and final visit (6 weeks). The visits were scheduled for each individual at the same time of the day. All the mother-child pairs came together to the visits at the clinic.

High fluoridated toothpaste

At baseline, the participants were each given one tube of 113 g high-fluoridated toothpaste (Clinpro™ 5000, 3M ESPE, St. Paul, U.S.A) and a toothbrush with a coloured mark 2 cm along the length of the bristle surface (Trisa®, TRISA AG, Switzerland) to be used throughout the whole study. The toothpaste contains 1.1% sodium fluoride and an innovative tri-calcium phosphate ingredient. The participants were instructed to brush their teeth with the toothpaste twice a day using a modified version of the technique described by Sjögren et al. (1995). The compliance of the participants with the instructions regarding the toothbrushing technique was assessed using a questionnaire in an interview setting at each visit following baseline. Prior to each visit, the volunteers were asked to avoid toothbrushing and all other oral hygiene measures 24 hours before each visit to the clinic and not to eat or drink anything but water for one hour.

Approximal fluid fluoride analysis (Study IV)

Approximal fluid was sampled using a standardised triangular-shaped paper point (1.5 × 5 mm), cut from Munktell filter paper No. 1600 (Grycksbo Pappersbruk, Sweden) and frozen according to Kashani et al. (1998). The fluoride concentration was analyzed using an ionspecific electrode (Orion 720A) and expressed as m*M*. All the samples were analysed once. The fluoride detection level was 0.02.

pH measurements (Study IV)

For plaque-pH registration, the pH "strip" method developed by Carlén et al. (2010) was used. The pH-indicator strips used measure a pH value in the range of 4.0 to 7.0 (Spezialindikator, Merck, Darmstadt, Germany). Plaque pH was measured before (baseline; 0 min) and at 5, 10, 15 and 20

min after a 1-min mouthrinse with 10 ml of a 10% sucrose solution. The pH-related measurements (area under the curve at pH 5.7 [$AUC_{5.7}$] and pH 6.2 [$AUC_{6.2}$]) were determined using a computer program (Larsen and Pearce, 1997).

Organic acids analysis (Study IV)

Two plaque samples (resting and fermenting) were collected for the protein analysis and capillary ion electrophoresis using a Gracey curette. The resting plaque sample was taken from site 25/26 prior to rinsing with a 10% sucrose solution. The participants then rinsed for one min with the sucrose solution. Fermenting plaque was collected 10 minutes after the start of the sucrose rinse from site 46/45. This was done in parallel with the pH strip measurements. The plaque samples were transferred to a pre-cooled tube, spun down by centrifugation, and put on ice until they were further processed within one hour. After that, the samples were heated in order to kill the bacteria and to release all the acids, and cooled on ice. The samples were sent on dry ice to the Academic Centre for Dentistry Amsterdam in The Netherlands, for further processing and analyses. The plaque samples were processed by centrifugation and the supernatants were used to determine organic acids as their anions by capillary ion electrophoresis on a Beckman P/ACE MDQ system (Beckman Coulter, Inc., USA). The plaque pellets remaining after centrifugation, were used for protein analysis according to Bradford (1976). The protein analysis was done to normalise the acid data so that the concentrations of acetic, butyric, formic, lactic, propionic and succinic acids were expressed as μmol/mg of plaque protein. The Bradford assay is a colorimetric protein assay based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 which under acidic conditions is converted from its red to a bluer form to bind to the protein being assayed. The absorbance at 595 nm was analysed using the Spectramax® Plus plate reader and Softmax® Pro software (Molecular Devices Corporation, USA).

Tongue lactic acid production rate (Study IV)

The rate of production of lactic acid from a biofilm sampled from the dorsum of the tongue was assessed using Clinpro™ Cario L-Pop™ (3M ESPE, Seefeld, Germany) and scored on a scale of 1-9 according to the manufacturer instructions. The 9 possible scores were divided into three risk categories, indicating a low (scores 1-3), medium (scores 4-6) or high (scores 7-9) level of lactic acid metabolism.

Salivary analysis (Study IV)

The salivary buffer capacity, MS and LB counts were determined as described in Study I. For MS the following classes were used: 1) 0-< 10^3, 2) 10^3-10^4, 3) 10^5-10^6 and 4) > 10^6 CFU/ml. The corresponding classes for LB were 1) 0 -< 10^2, 2) 10^2 -10^3, 3) 10^4-10^5 and 4) >10^5 CFU/ml.

Caries-risk assessment (Study V)

A reduced "Cariogram" model was used to evaluate the efficacy of the 5,000 ppm fluoride toothpaste in reducing the caries risk. The variables included in the model were: caries experience, related diseases, dietary content, MS count, fluoride programme, saliva buffer capacity and clinical judgement. For each participant, the seven variables were scored at each visit as shown in Table 4 except for the caries experience, which was recorded only at baseline. Four "Cariogram" pie charts were therefore produced for each participant (at baseline, 2, 4 and 6 weeks). For each visit, the mean actual chance of avoiding caries, bacteria, diet and susceptibility obtained by the "Cariogram" were calculated.

Ethical considerations

The studies included in the present thesis were granted ethical approval by the Faculty of Dentistry at King Abdulaziz University and conducted in accordance with the Declaration of Helsinki. Verbal information about the studies was given to the participants and written informed consent was obtained. All the participants were coded when entering the individual studies.

Statistical methods

All the data in Studies I-IV were analysed using the IBM® SPSS® statistical package (PASW versions 18.0, 19.0 and 20.0 IBM®, Chicago, Ill, USA). The data in Study V were analysed using Stata SE® software v. 10.0. Descriptive statistics, including the means, standard deviations, and frequencies, were calculated. Table 5 summarises the variables included in each of the five studies and the statistical analyses that were performed.

For the snacking frequency variable in Study I, no intake or an intake of once a month or once a week was considered low (code 1), while a frequency of two-three times a week and once or more a day was high (code 2). The snacking frequency was calculated by adding the codes for all nine snacking items (total score: minimum=9, maximum=18). The variable was dichotomised and a cut-off point of 14

Table 4. **"**Cariogram" variables used in Study V with their corresponding scores.

Cariogram variable	Score
Caries experience	0: caries free/no fillings 1: better than normal 2: normal for age group 3: worse than normal
Related diseases	"0" meaning no diseases for all participants since they were all healthy
Dietary content	Based on salivary LB counts (determined by the CRT Bacteria® test): 0: lowest count 1: low count 2: moderate count 3: highest count
Mutans Streptococci	Determined by the CRT Bacteria® test: 0: very low count 1: low count 2: high count 3: very high count
Fluoride programme	0: maximum fluoride programme/fluoride toothpaste + additional measure 1: fluoride toothpaste + some additional measures 2: fluoride toothpaste/no supplements 3: no fluoride toothpaste **Note:** For all participants, the baseline score was set at "2" and the 2-, 4- and 6-week recall visit scores were set at "1"
Saliva buffer capacity	Based on the CRT Buffer® test: 0: adequate (blue) 1: reduced (green) 2: low (yellow)
Clinical judgement	0: more positive than what the Cariogram shows based on the scores entered 1: normal setting, risk according to the other values entered 2: worse than what the Cariogram shows based on the scores entered 3: very high caries risk, examiner is convinced that caries will develop, irrespective of what the Cariogram shows based on the scores entered **Note:** For all participants and all four visits, the score was set at "1"

was used, where participants with a total code of < 14 were considered to have a low snacking frequency and those with ≥ 14 a high snacking frequency. The PlI variable in Study I was also divided into two groups (low: ≤ 1, high: PlI > 1). Due to the absence of records corresponding to the lowest and highest levels of GI in Study I, it was re-categorised into two levels (no-mild inflammation and moderate-severe inflammation) instead of four (no inflammation, mild, moderate and severe inflammation).

In Study II, microbial associations were sought using cluster analysis for all three age groups together, using an average linkage (between groups) sort (UPGMA) providing a dendrogram of microbial associations. In the dendrogram, observed distances are scaled to fall into the range of 0-25. Stepwise community ordination was performed using principal components analysis (PCA) for the three age groups separately. Since the visualisation of data in 18 dimensional plots is difficult, the dimensionality was reduced by the analyses to provide two-dimensional plots in which strains that were frequently encountered together were in close proximity in the plots (dispersion plots).

Regarding the checkerboard scores in Study III, only a few signal "0" data were recorded and they were therefore combined with signal "1" data into "≤1".

Table 5. The variables and statistical analyses included in each of the five studies.

	Variables	Statistical analyses
Study I	Caries experience (DMFT/dmft) Mother's education Family income Total number of children per family Regular toothbrushing Snacking frequency Plaque index Gingival index Salivary buffer capacity, MS and LB counts	One-way ANOVA (differences between means) Pearson's correlation, X^2 or Fisher's exact test (mother/child relationships) Cohen's kappa (intra-examiner reliability of caries registration) Significance level: 5%

Study II	Checkerboard scores for 18 bacterial strains in each age group	Spearman's test (mother/child microbial data correlations) Cluster analysis (microbial associations for all age groups together) Community ordination/principal component analysis (microbial associations for each age group separately)
Study III	Caries experience: DMFT/dmft D/d Checkerboard scores for 15 bacterial strains in each age group	One-way ANOVA (differences in the caries experience in relation to the bacterial scores) Fisher's exact test (association between D/d groups and bacterial scores) Significance level: 5%
Study IV	Fluoride concentration CCLP score pH-related measurements: $AUC_{5.7}$, $AUC_{6.2}$, maximum pH fall and minimum pH Organic acid concentration Salivary buffer capacity, MS and LB counts	Power analysis * General linear model (repeated measures) with Dunnett's post-hoc t-test for the fluoride concentration, pH-related measurements and organic acid concentrations Friedman's test with post-hoc pairwise comparisons using a Wilcoxon test and controlling for type I error across the comparisons using Bonferroni adjustment for CCLP-based lactic acid formation rate and the salivary buffer capacity, MS & LB counts Significance level: 5%

Study V	Cariogram sectors: Actual chance of avoiding caries Bacteria Diet Susceptibility Salivary buffer capacity, MS and LB counts	One-way ANOVA with Cook-Weisberg post-hoc test Linear trends in proportion using $\chi 2$ test for trend Mixed-design analysis of variance Significance level: 5%

* Using G*Power (version 3.1 for Macintosh) given an effect size of 0.25, a power of 0.80 with one group and a total of four measurements. The calculated sample size was 24 subjects with an actual power of 0.82 and F = 2.74.

Results

Study I: caries experience and caries-related factors in mothers and their children

Interview results (socio-economic status, oral hygiene and dietary habits)

The results from the interview showed that the majority of the mothers had a lower educational level. Around 52% of the families had an income of < 10,000 SR, 45% had an income of > 10,000 SR and 3% had no regular income or were unable to recall how much it was. Oral hygiene habits are summarised in Table 6. Regarding the start of oral hygiene practices in their children, the majority of the mothers delayed dental care until signs of tooth decay were noted, as soon as the child was old enough to hold a toothbrush or at a later time point. A large majority of the mothers and their children consumed snacks between main meals. The most frequently consumed snacks were dates and chocolates/sweets by the mothers and chips, in addition to chocolates/sweets, by both the younger and older children. Based on the calculated snacking frequency variable, most of the mothers had a low snacking frequency, while more than half the younger and older children had a high snacking frequency.

Clinical data

Plaque amount and gingival health

Regarding plaque deposits, 31% of the mothers, 50% of the younger children and 46% of the older children had a high plaque scores (PlI >1). As for gingival health, 64% of the mothers, 43% of the younger children and 71% of the older children had moderate to severe gingival inflammation.

Caries experience

Four per cent of the mothers, 9% of the younger children and 12% of the older children were caries free (DMFT/dmft = 0). There was no family in which all three members together were caries-free. There were no statistically significant differences in caries experience with respect to

gender in the children. The caries experiences (DMFT/dmft) for the mothers and children are shown in Table 7.

Table 6. Oral hygiene (%) in the mothers, younger (C_{4-6}) and older children (C_{12-16}).

Factors	%		
	Mothers (n =86)	**C_{4-6} (n =86)**	**C_{12-16} (n =86)**
Oral hygiene habits			
Do you brush your teeth regularly?			
No	18.6	38.4	52.3
Yes	81.4	61.6	47.7
Toothbrushing frequency			
Once or a few times a week	1.4	0	7.3
Once a day	17.1	52.8	41.5
Twice a day	57.2	37.7	43.9
More than twice a day	24.3	9.5	7.3
Other	0	0	0
Toothbrushing tool			
Manual toothbrush (B)	46.5	100	84.8
Miswak (M)	1.2	0	0
Electric brush (E)	0	0	0
Combination (B+M)	51.1	0	15.2
Combination (all)	1.2	0	0
Interdental cleaning			
Dental floss (F)	13.9	0	0
Toothpick (TP)	0	0	2.3
Interdental brush	0	0	0
Combination (F+TP)	2.3	0	0
Nothing used	83.8	100	97.7
Toothpaste use			
No	3.5	3.5	3.5
Yes	96.5	96.5	96.5
Type of toothpaste			
Non-fluoridated	0	0	0
Fluoridated	42.2	41	24.1
Don’t know	57.8	59	75.9

Table 7. Descriptive statistics of the caries experience expressed as DMFT/dmft (mean ± SD) in the mothers, younger (C_{4-6}) and older children (C_{12-16}).

	Mean ± SD		
	Mothers (n =86)	**C_{4-6}** (n =86)	**C_{12-16}** (n =86)
Decayed (D/d)	5.5 ± 3.9	8.0 ± 5.1	4.5 ± 3.7
Missing teeth (M/m)	2.7 ± 3.0	0.4 ± 1.4	0.2 ± 0.5
Filled teeth (F/f)	4.3 ± 3.4	0.6 ± 1.6	1.2 ± 1.8
DMFT/dmft	12.4 ± 5.3	9.0 ± 5.0	5.8 ± 4.1

Salivary parameters

Fifty-eight per cent of the mothers, 51% of the younger children and 49% of the older children had a low buffer capacity**.** The MS counts were high ($\geq 10^5$ CFU/ml saliva) in 55% of the mothers and 62% of both the younger and older children**.** The LB counts were high ($\geq 10^5$ CFU/ml saliva) in 66% of the mothers, 51% of the younger children and 42% of the older children.

Caries experience and related factors

A lower DMFT/dmft with higher maternal education was noted and it was only statistically significant in the mothers ($p<0.05$)**.** A lower caries experience was also observed in relation to higher family income, but this was only statistically significant in the older children ($p<0.05$). Lower DMDT/dmft values were seen in relation to regular toothbrusing and they were only statistically significant in the mothers and younger children ($p<0.05$). Higher DMFT/dmft values with larger numbers of children per family and a higher snacking frequency were observed (ns)**.** Clear trends between PlI, GI and salivary buffer capacity, MS and LB counts and the caries experience were observed. However, these relationships did not always reach statistical significance in all three family members**.** The MS count was the only factor that was significantly related to the caries experience in all three family members ($p<0.05$)**.** The caries experiences of the children were positively correlated to those of their mothers (Figure 3, $p<0.01$). Regarding the mother-child relationships, significant

relationships were found more frequently between the mothers and their older children ($p<0.05$) than between the mothers and their younger children, except for regular toothbrushing, snacking frequency and GI, which were statistically significant between the mothers and both of their children ($p<0.05$).

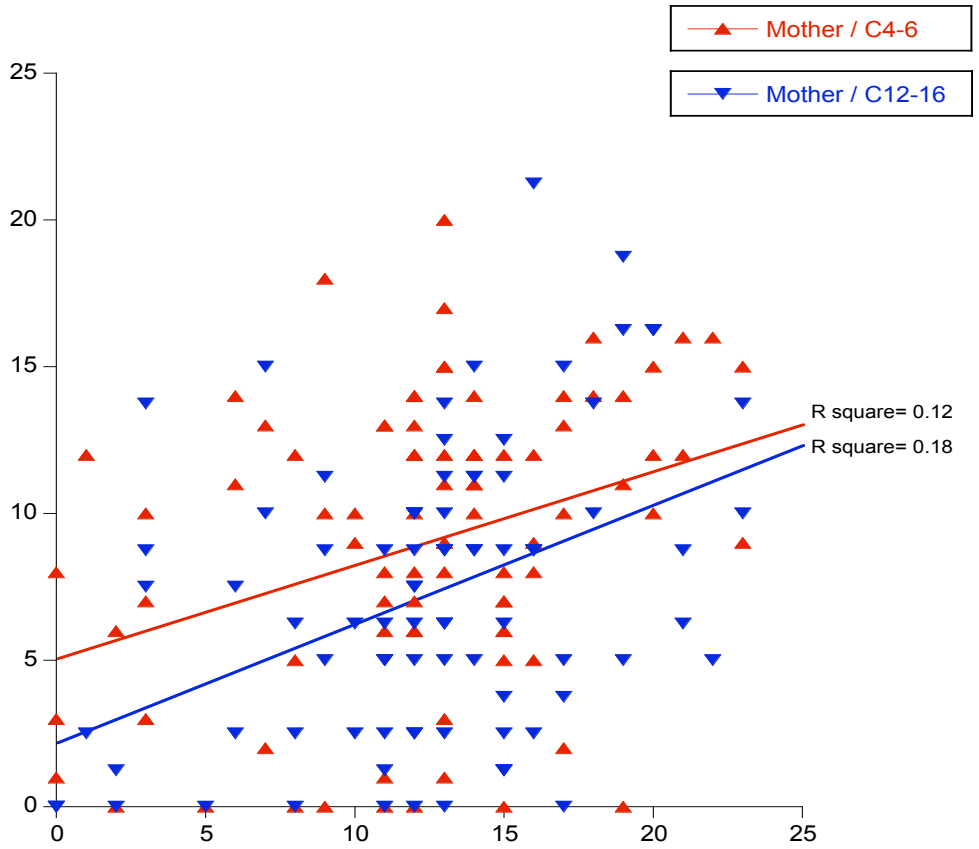


Figure 3. Correlation between the caries experience of the mothers and each of their younger (C_{4-6}) and older children (C_{12-16}).

Study II: supragingival plaque microbial associations

Microbial correlations between the mothers and their children

A significant association was found in relation to 17 of the total of 18 bacterial strains in the mother/C_{4-6} pair and 13 of the total of 18 in both the mother/C_{12-16} and C_{4-6}/C_{12-16} pairs (Table 8). The Lactobacillus strains had the highest score agreement while *P. micra* had the lowest.

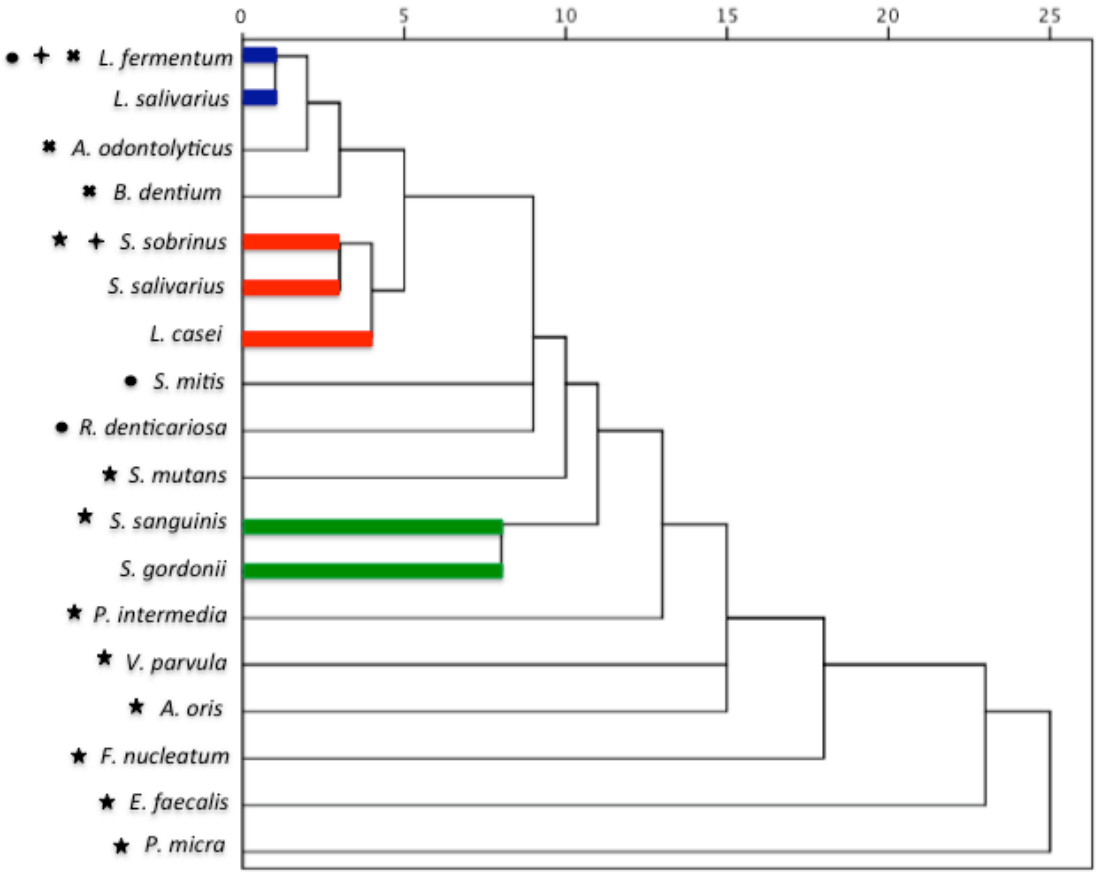
Table 8. Results of the statistical analysis for microbial correlation in the mother/C_{4-6}, mother/C_{12-16} and C_{4-6}/C_{12-16} pairs.

Bacterial strain	Mother/C_{4-6}	Mother/C_{12-16}	C_{4-6}/C_{12-16}
S. mutans	*	*	ns
S. sobrinus	*	*	ns
S. sanguinis	*	*	**
S. salivarius	*	ns	ns
S. gordonii	*	*	**
S. mitis	*	*	**
L. casei	*	*	**
L. fermentum	*	*	**
L. salivarius	*	ns	ns
A. odontolyticus	*	ns	**
A. oris	*	*	**
P. intermedia	*	ns	ns
F. nucleatum	*	*	**
V. parvula	*	*	**
R. dentocariosa	*	*	**
B. dentium	*	ns	*
P. micra	ns	*	**
E. faecalis	*	*	**

*: $p < 0.05$ **: $p < 0.01$ ns: non-significant

Cluster and principal components analyses

The dendrogram obtained by cluster analysis of the 18 bacterial strains for all the study participants together is shown in Figure 4. Three clusters were identified; 1) *L. fermentum* and *L. salivarius, 2) S. sobrinus*, *S. salivarius* and *L. casei*, and 3) *S. sanguinis* and *S. gordonii*. The two-dimensional plots presenting the results of the principal components analysis revealed a similar pattern of microbial associations in the mothers and children, as shown in Figure 5.



Three main clusters are highlighted:
Blue: *L. fermentum* and *L. salivarius*
Red: *S. sobrinus*, *S. salivarius* and *L. casei*
Green: *S. sanguinis* and *S. gordonii*

The different symbols refer to associations between the main clusters and other bacterial strains (whether part of a cluster or not)

Figure 4. Dendrogram of the cluster analysis of the 18 taxa, using an average linkage sort. The data employed were the counts of the 18 taxa in supragingival plaque samples taken in 258 subjects (mothers and their children).

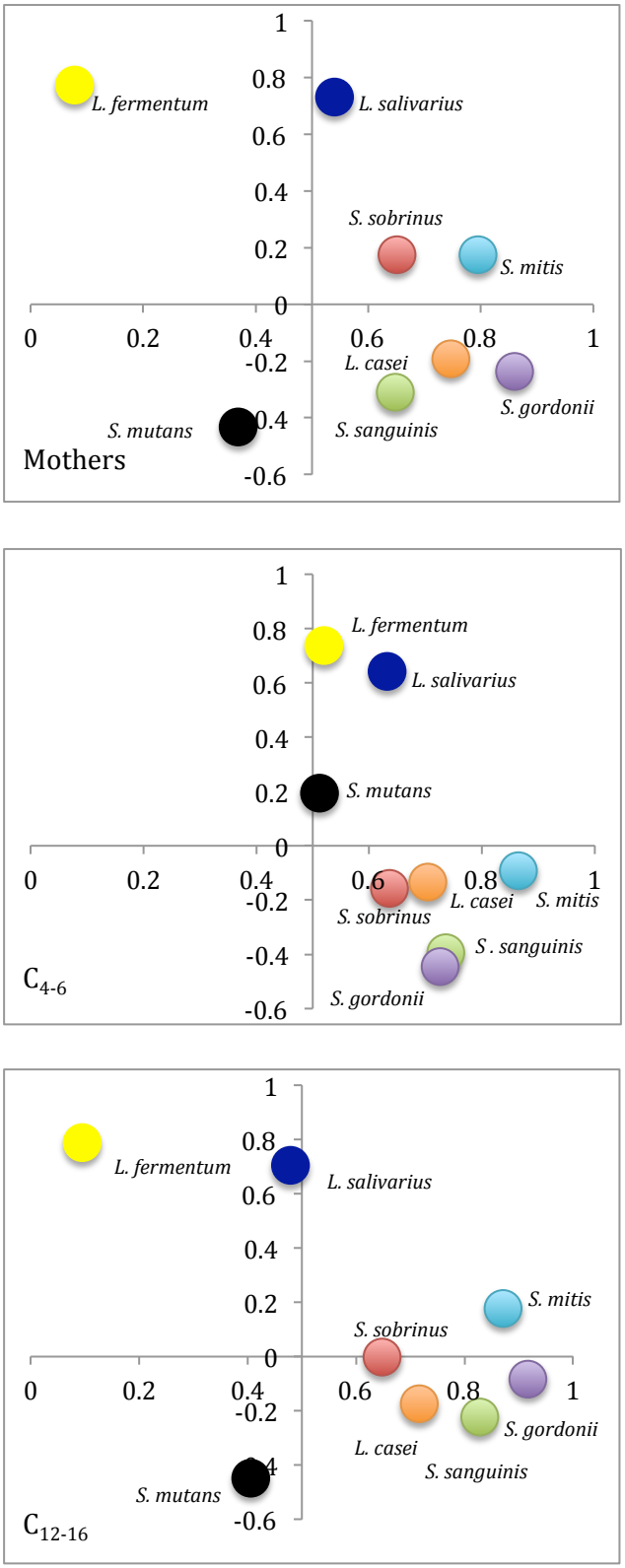


Figure 5. Factor analysis by community ordination using principal component analysis for each of the mothers, 4- to 6-year-old children (C_{4-6}) and 12- to 16-year-old children (C_{12-16}).

Study III: supragingival plaque microbial counts in relation to caries experience

No relationship was observed between the bacterial scores and the caries experience for any of the caries-related bacteria in the mothers or their children. No significant associations were detected between the bacterial scores and the D/d categories in the mothers or in their younger or older children except for a tendency observed in relation to *Streptococcus mutans*.

Study IV: caries-preventive effects of a high-fluoride regimen

Out of the total 17 families (34 subjects) enrolled, three families attended only the baseline visit and one family failed to show up for the final visit. As a result, 13 families (26 subjects) completed the clinical trial. 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 28 at baseline, two weeks and four weeks and 26 at six weeks respectively. All the participants reported that they had followed the given toothbrushing instructions.

Fluoride concentration

As shown in Figure 6, the concentration of fluoride in the approximal fluid increased gradually and significantly at the two-, four- and six-week follow-up visits, as compared to baseline ($p < 0.05$).

Plaque-pH measurements

The use of the 5,000 ppm fluoride toothpaste was accompanied by changes in interproximal plaque acidogenicity, including significant reductions in $AUC_{5.7}$, $AUC_{6.2}$ and maximum pH fall, and an increase in minimum pH, as seen in Figures 7 and 8 ($p<0.05$). The overall changes (pH curves) from baseline to the end of the trial are illustrated in Figure 9.

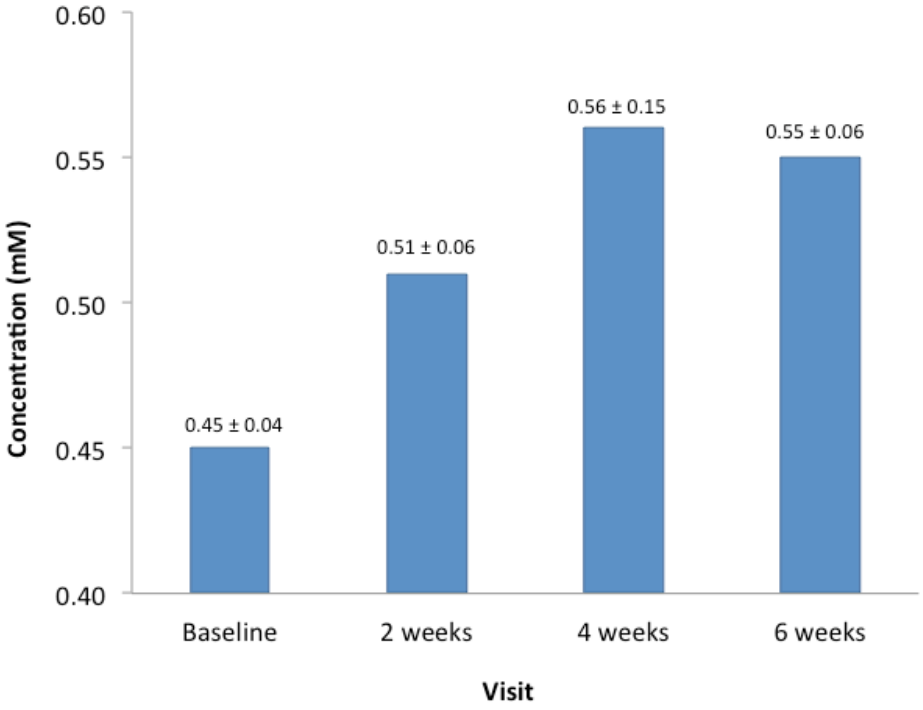
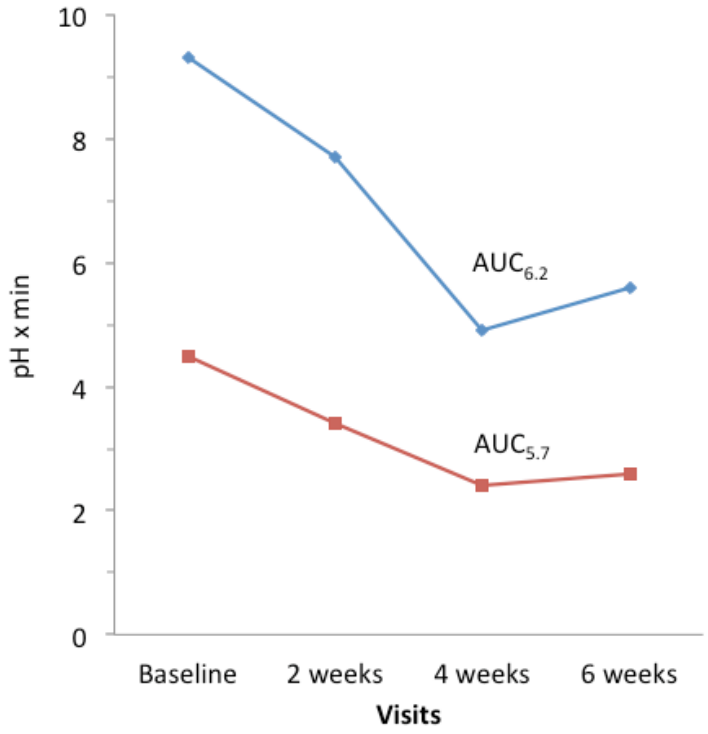
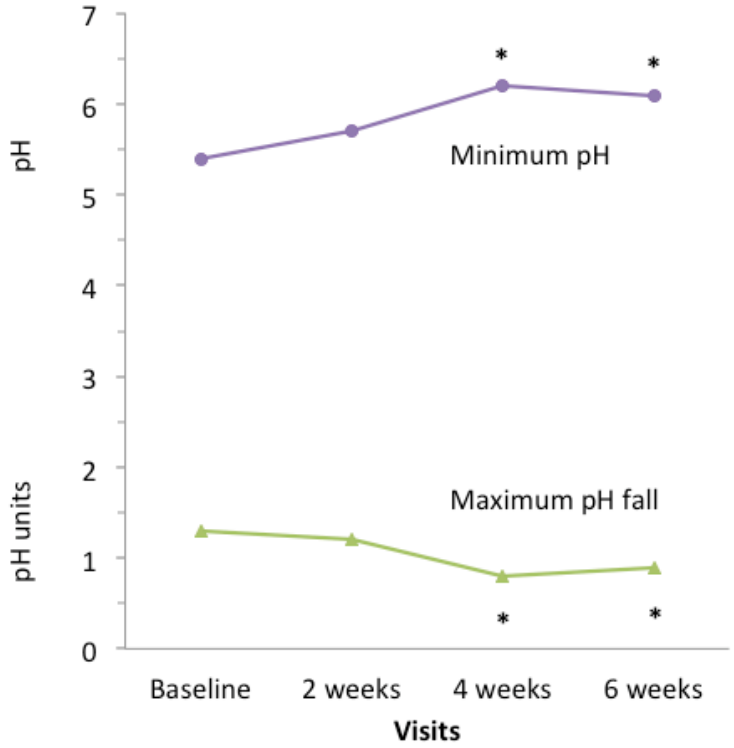


Figure 6. Changes in approximal fluid fluoride concentrations (mM; mean ± SD) throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks).



* Statistically significant compared with baseline

Figure 7. Approximal plaque acidogenicity calculated as area under the curve ($AUC_{6.2}$, $AUC_{5.7}$; mean) assessed using the pH "strip method" up to 20 minutes after a 10% sucrose rinse throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks).



* Statistically significant compared with baseline

Figure 8. Approximal plaque acidogenicity (maximum pH fall, minimum pH; mean) assessed using the pH "strip method" up to 20 minutes after a 10% sucrose rinse throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks).

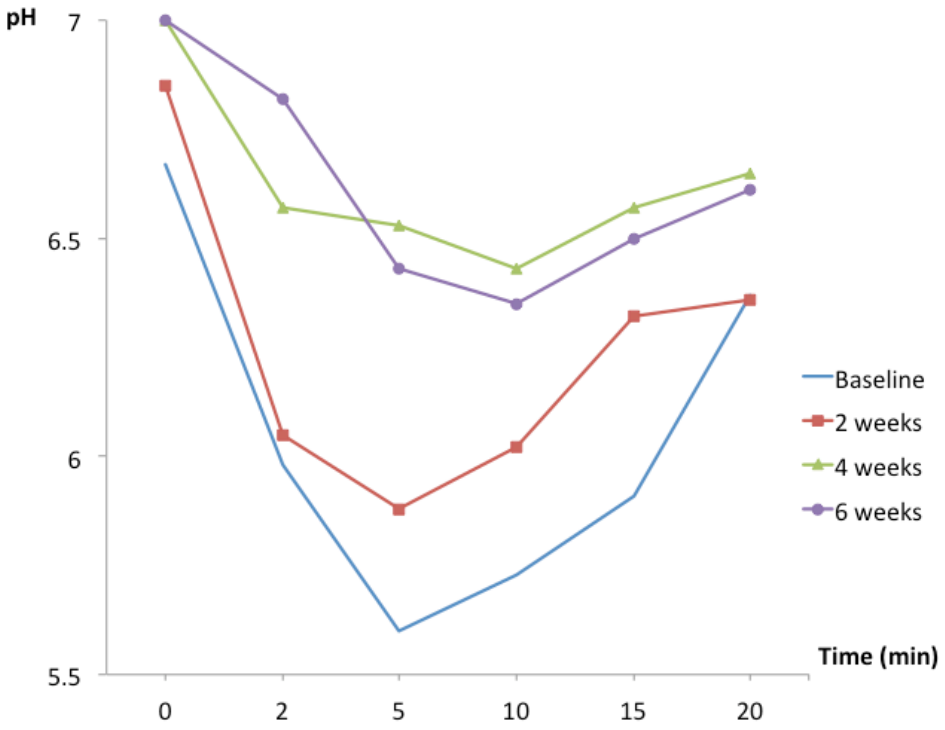


Figure 9. pH curves (mean values) throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks) as assessed by the pH "strip method" after a rinse with 10% sucrose.

Organic acids analysis

The average concentrations of the major (acetate and lactate), minor (butyrate, formate, propionate, succinate) and total acid anions in resting and fermenting plaque remained more or less the same throughout the four visits (Figure 10).

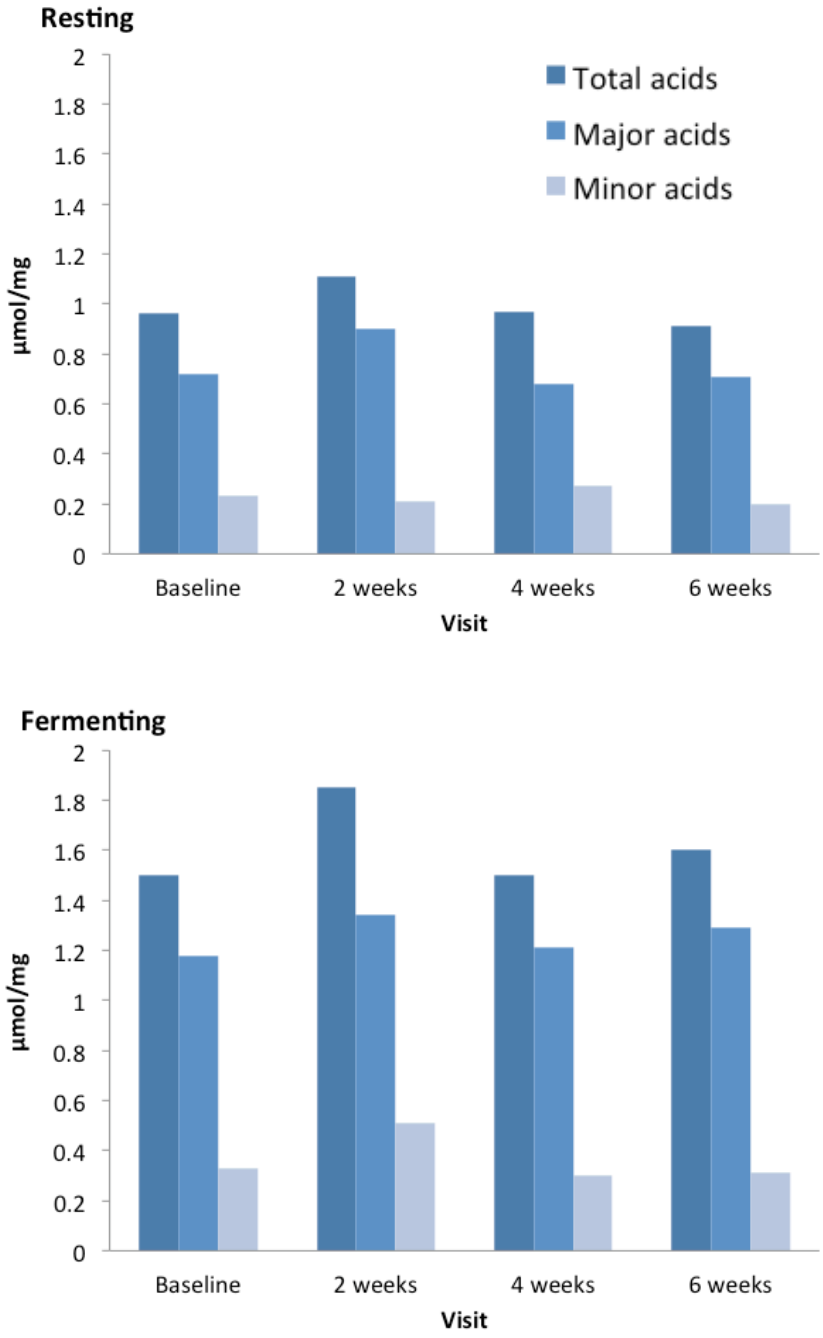


Figure 10. Organic acid concentrations (major, minor and total acids) in resting and fermenting plaque samples (µmol/mg) throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks).

Chair-side lactic acid concentration

A statistically significant decrease in the percentage of high scores for lactic acid production on the tongue was observed at the six-week follow-up visit, as compared to baseline, as shown in Table 9 ($p<0.05$). These changes were mainly seen within the same risk category (high risk: scores 7-9).

Table 9. Distribution of the Clinpro™ Cario-L-Pop™ (CCLP) test scores (n [%]) used to assess the tongue lactic acid production rate (baseline, 2 weeks, 4 weeks and 6 weeks).

	Visits/scores	9	8	7	6	5	Total n
n (%)	**Baseline**	10 (35.7)	6 (21.4)	5 (17.9)	4 (14.3)	3 (10.7)	28
	2 weeks	6 (21.4)	15 (53.6)	3 (10.7)	4 (14.3)	0 (0)	28
	4 weeks	1 (3.6)	6 (21.4)	13 (46.4)	7 (25.0)	1 (3.6)	28
	6 weeks	1 (3.8)	3 (11.5)	11 (42.4)	10 (38.5)	1 (3.8)	26

Most frequent values highlighted in grey

Salivary parameters

The salivary buffer capacity was significantly changed subsequent to the high-fluoride regimen at four weeks and six weeks compared with baseline, as seen in Table 10 ($p<0.05$). Reductions in MS counts were also observed and were statistically significant at six weeks compared with baseline, as seen in Table 5 ($p<0.05$). A similar tendency was noted for the LB counts, as seen in Table 10 (ns).

Table 10. Score distribution (%) of salivary buffer capacity (low/medium/high), mutans streptococci (MS) counts (from low to high: 1/2/3/4) and lactobacilli (LB) counts (from low to high: 1/2/3/4) throughout the study (baseline, 2 weeks, 4 weeks and 6 weeks).

Salivary parameter	n (%)			
	Baseline	2 weeks	4 weeks	6 weeks
Buffer capacity				
Low	3 (11.5)	4 (15.4)	2 (7.7)	2 (7.7)
Medium	22 (84.7)	18 (69.2)	16 (61.5)	14 (53.9)
High	1 (3.8)	4 (15.4)	8 (30.8)	10 (38.4)
MS count				
1 (lowest)	4 (15.4)	4 (15.4)	7 (26.9)	10 (38.5)
2	5 (19.3)	5 (19.3)	8 (30.7)	7 (27)
3	2 (7.7)	3 (11.5)	6 (23.1)	7 (27)
4 (highest)	15 (57.6)	14 (53.8)	5 (19.3)	2 (7.5)
LB count				
1 (lowest)	4 (15.4)	4 (15.4)	4 (15.4)	3 (11.6)
2	5 (19.3)	8 (30.8)	5 (19.3)	6 (23.1)
3	11 (42.2)	7 (26.9)	9 (34.5)	16 (61.5)
4 (highest)	6 (23.1)	7 (26.9)	8 (30.8)	1 (3.8)

Study V: variations in the caries-risk profile after a high-fluoride regimen

The use of 5,000 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 near future at each visit following baseline (Figure 11). The chance of avoiding caries increased significantly ($p<0.01$) during the trial for all individuals and for the two groups separately; from 28% at baseline to 54% at six weeks for the mothers and from 31% to 56% for the teenage children (Table 11). A statistically significant difference in the chance of avoiding caries between the mothers and their children was found at 2 and 4 weeks, as shown in Table 11.

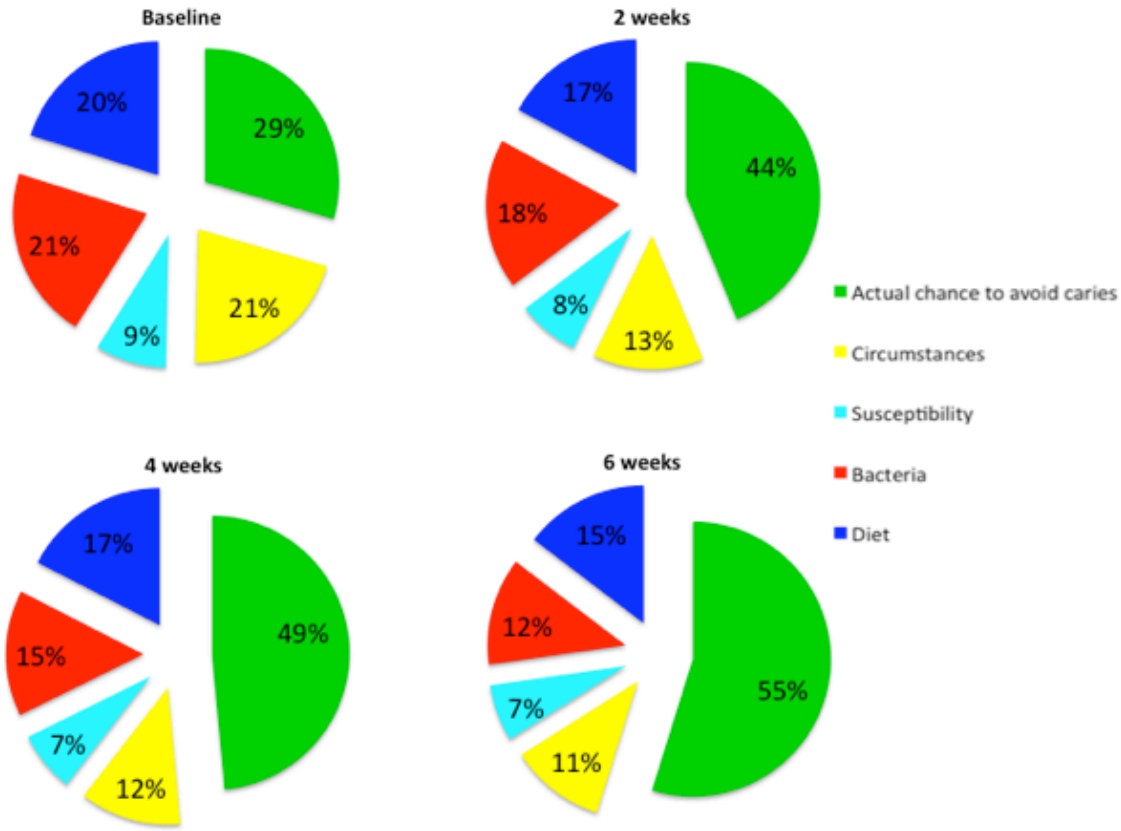


Figure 11. Mean changes in the caries risk profile illustrated by the Cariogram pie chart (actual chance to avoid caries, circumstances, susceptibility, bacteria and diet) at each examination (baseline, 2 weeks, 4 weeks and 6 weeks) for all study participants.

A linear trend for all individuals was observed for the MS count ($p<0.01$) with an increase in the number of participants with a salivary concentration of MS $<10^3$, as shown in Figure 12. The same trend was also observed for the LB (Figure 12, $p=0.04$) and buffer capacity scores (Figure 13, $p=0.03$).

Table 11. Results from the Cariogram analyses (chance to avoid caries, circumstances, susceptibility, bacteria and diet) at each examination (baseline, 2 weeks, 4 weeks and 6 weeks) for the mothers and their teenage children separately.

	Mean ± SE				
Cariogram Variables	**Baseline**	**2 weeks**	**4 weeks**	**6 weeks**	***p-value*[1]**
Chance to avoid caries					
Mothers	28.0 ± 4.8	39.4 ± 5.3	44.4 ± 5.9	53.7 ± 3.8	*<0.01*
Children	31.0 ± 5.5	48.1 ± 5.4	52.9 ± 6.2	55.8 ± 3.6	*<0.01*
p-value[2]	*0.80*	*<0.01*	*0.02*	*0.09*	
Circumstances					
Mothers	20.8 ± 1.1	14.6 ± 1.1	12.2 ± 1.4	11.7 ± 1.1	*<0.01*
Children	20.8 ± 1.2	12.2 ± 1.1	11.7 ± 1.1	10.7 ± 1.0	*<0.01*
p-value	*0.16*	*0.08*	*0.13*	*0.27*	
Susceptibility					
Mothers	9.3 ± 0.7	8.6 ± 0.8	8.1 ± 0.8	7.3 ± 0.6	*0.29*
Children	7.9 ± 1.0	6.5 ± 0.9	6.4 ± 0.8	6.2 ± 0.8	*0.51*
p-value	*0.39*	*0.90*	*0.48*	*0.62*	
Bacteria					
Mothers	20.0 ± 2.3	18.7 ± 2.1	16.4 ± 2.2	11.8 ± 1.3	*<0.01*
Children	21.8 ± 2.3	17.6 ± 2.2	13.4 ± 2.2	13.3 ± 1.7	*<0.01*
p-value	*0.69*	*0.70*	*0.70*	*0.70*	
Diet					
Mothers	21.7 ± 1.5	18.9 ± 1.9	18.7 ± 2.1	15.3 ± 1.4	*0.09*
Children	18.8 ± 2.0	15.4 ± 1.9	16.4 ± 2.5	14.0 ± 1.2	*0.10*
p-value	*0.44*	*0.84*	*0.30*	*0. 85*	

[1] Comparison between visits

[2] Comparison between mothers and their children

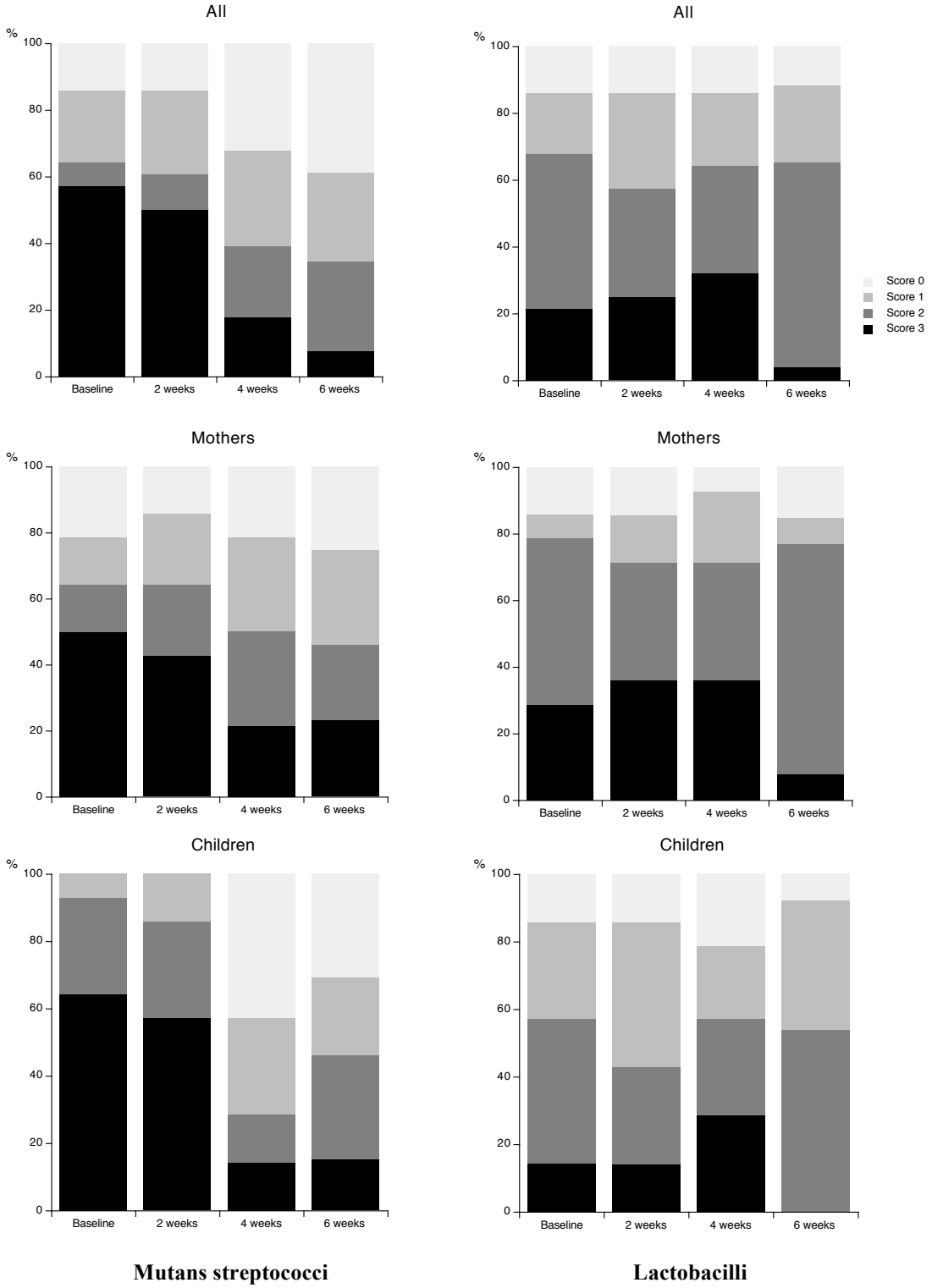


Figure 12. Sample distribution across the different recall examinations (baseline, 2, 4 and 6 weeks) for salivary mutans streptococci (MS, left) and lactobacilli counts (LB, right) for all individuals and for mothers and children separately. The four scores correspond to the following values; for MS: 0 = 0-< 10^3, 1 = 10^3-10^4, 2 = 10^5-10^6, 3 = > 10^6 CFU/ml and for LB: 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).

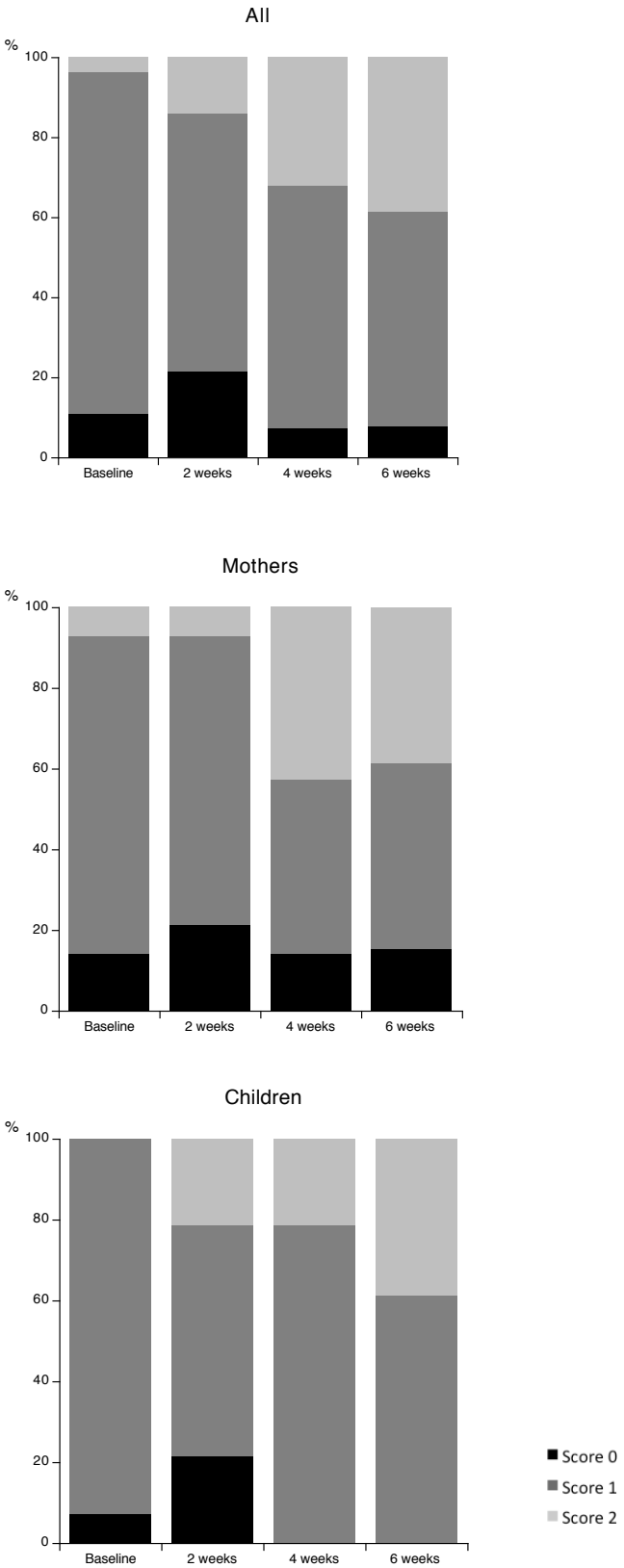


Figure 13. Sample distribution across the different recall examinations (baseline, 2, 4 and 6 weeks) for salivary buffer capacity levels assessed as low, medium or high for all individuals and for mothers and children separately. The three scores correspond to the following values: 0 = low, 1 = medium, 2 = high. (n = 28 at baseline, 2 and 4 weeks and 26 subjects at 6 weeks).

A statistically significant difference was also found when comparing the mothers and their children for MS and LB counts ($p<0.05$). On the other hand, a numerical yet non-significant trend was found for the buffer capacity ($p>0.05$). The mixed-design analysis showed that the chance of avoiding caries was not statistically associated with the mothers or children ($p>0.05$), while all the other Cariogram sectors (variables) were statistically associated ($p<0.01$) with the chance of avoiding caries (Table 12).

Table 12. Mixed-design analysis of variance of the different sectors of the "Cariogram" (the chance to avoid caries as the dependent variable) and the difference between mothers and children.

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

Circumstances, susceptibility, bacteria and diet (fixed effects)
Participant (random effect)
Number of observations = 110 (28 at baseline, 2, and 4 weeks, 26 at 6 weeks)
Number of groups = 4 (corresponding to the total number of examinations: baseline, 2, 4 and 6 weeks)

Discussion

The aim of the present thesis was to conduct a series of cariological studies on families with the aim of describing the caries experience in mothers and their children and caries-related associations between them, exploring supragingival microbiology and its relationship to the caries experience, implementing a high-fluoride regimen with consecutive assessment of its anticariogenic effects through variations in certain plaque and salivary characteristics, as well as changes in the caries risk.

Caries experience and caries-related factors in Saudi mothers and their children

Study I revealed a high caries experience in all three family members (mothers, 4- to 6- and 12- to 16-year-old children). The mean DMFT reported for the mothers is in accordance with that of a previous study (Farsi, 2008). However, the caries experiences for both the younger and older children was slightly higher compared with earlier studies (Akpata et al., 1992; Al-Tamimi and Petersen, 1998; Al-Shammery, 1999; Al-Malik and Rehbini, 2006; Wyne, 2008).

Caries has previously been related to different socio-economic factors, with higher caries prevalence in the low socio-economic classes (Marthaler, 2004). An earlier study found an association between low caries prevalence and high socio-economic standards in six-year-old Saudi children (Al-Mohammadi et al., 1997). A similar trend towards lower caries experience with higher family income was found in the present study, which was statistically significant in the older children. In addition, caries in Saudi pre-school and school children has been found to be more prevalent among those whose mothers are less well educated (Al-Tamimi and Petersen, 1998; Al-Malik et al., 2001). The mothers' DMFT was lower in relation to higher education in the current study. When it came to their children, a lower but non-significant caries experience was found in relation to higher maternal education. A tendency towards higher caries experience was seen in our study in relation to larger families, which, for the younger children, matches the consideration of family size as one of the main risk factors for caries in Saudi primary school children (Amin and Al-Abad, 2008).

The observed decline in dental caries in most industrialised countries can be primarily attributed to the daily use of fluoridated toothpaste (Bratthall et al., 1996; Marinho et al., 2003; Twetman et al., 2003). Despite the availability of different types of fluoridated toothpaste in the Kingdom of Saudi Arabia, more than half the mothers in the

present study were unaware of whether or not their toothpaste was fluoridated. In addition, the observed late introduction of oral hygiene routines in children is also consistent with other reports on a Saudi population (Al-Otaibi and Angmar-Månsson, 2004; Amin and Al-Abad, 2008).

In many Arab countries, the caries prevalence is increasing in parallel with the recent economic growth, with a subsequent increase in the consumption of refined sugars (Amin and Al-Abad, 2008). The intake frequency of sugary snacks in the present study was high among the children. A trend towards higher caries experiences was noted in relation to higher snacking frequency, but this was not statistically significant. One possible explanation could be that only children with a high caries experience and snacking frequency were included.

As shown in a previous study, there was a significant difference in the reported caries experience in relation to the salivary buffer capacity in the mothers (Farsi, 2008). In addition, a tendency towards higher caries experience in the mothers and both their younger and older children was observed, with higher mutans streptococci and lactobacilli counts.

Regular toothbrushing, snacking frequency and gingival health were related between the mothers and both their younger and older children. The plaque index, salivary buffer capacity and mutans streptococci counts were related between the mothers and the older children. This could be due to differences in salivary secretion rates between adolescents and younger children, as salivary glands are fully developed by the age of 15 (Crossner, 1984). Variations in age-related oral microflora could be another reason.

The previously mentioned findings highlight the necessity to direct national dental services in the Kingdom of Saudi Arabia towards adopting caries-prevention strategies, with families as the main target, and emphasising the "early oral health care concept" (Meyer et al., 2010). In order to provide more insight into the relationships of caries and associated factors between the mothers and their pre-school and school children, future research should include information regarding plaque acidogenicity and microbiological profiles in the mothers and their children.

Supragingival plaque microbial associations

Study II has shown that children exhibit plaque microbial levels similar to those of their mothers and supports the findings of a previous study (Tanner et al., 2002a). A similarity of this kind was evident in relation to almost all the bacterial strains in the mother-younger child pairs and to a

slightly lesser extent in the mother-older child pairs. One possible reason for this difference could be that older children make their own choices regarding dental health habits and practices (Alm et al., 2008). In addition, the influence of parental dental health behaviours tends to be more evident in early rather than late childhood (Mattila et al., 2000; Mattila et al., 2005b). Differences in dental health behaviour between mothers and their older children might therefore contribute to a difference in their oral bacterial ecology.

The clusters described by the dendrogram in Study II go hand in hand with the fact that dental caries is induced by cariogenic plaque comprising numerous species, including *S. mutans*, low-pH streptococci, *Rothia*, *Actinomyces*, *Lactobacillus* and *Bifidobacterium* spp (Filoche et al., 2010). The dendrogram points to a close relationship between streptococci and lactobacilli and a distant association with other bacterial species such as *F. nucleatum*, *E. faecalis* and *P. micra*. Streptococci are among the early colonisers dominating dental plaque (Nyvad and Kilian, 1987; ten Cate, 2006). Lactobacilli and streptococci (mainly mutans streptococci) are among the key cariogenic bacteria, which act synergistically due to their acidogenic and aciduric properties (van Houte, 1994; Kleinberg, 2002; Jakubovics, 2010; Marsh et al., 2011). On the other hand, *F. nucleatum* is recognised as an important "bridge" between early and late colonisers (ten Cate, 2006; Jakubovics, 2010; Marsh et al., 2011).

Principal components analysis (PCA) revealed a similar pattern of microbial associations in the mothers and children mainly for different *Streptococci* (*S. mutans*, *S. sobrinus*, *S. mitis*, *S. gordonii* and *S. sanguinis)* and *Lactobacillus* species (*L. casei*, *L. salivarius* and *L. fermentum*). One possible explanation for the presence of a significant agreement for *S. salivarius* counts observed in the mother/younger child pair is the fact that it is one of the first species to colonise the oral cavity from birth and is therefore more likely to be present in the younger children than in the older children (Socransky and Manganiello, 1971).

To summarise, Study II's correlation analyses demonstrated more bacterial score agreements in the mother-younger child pair than in the mother-older child pair. On the other hand, PCA revealed almost identical microbial associations in comparisons between the mothers and older children, which differed slightly from the associations that were observed when comparing the mothers with their younger children. Several factors could contribute to these differences. One is that the supragingival plaque microbial composition most probably differs between deciduous and permanent dentitions (Aas et al., 2008). In addition, mothers and older children have more developed immune systems than younger children. Older children especially those of pre-

pubertal age, are subject to hormonal changes, which also influence the bacterial composition of plaque. Two age groups of children were included in Study II in order to obtain a clear age cut-off that would differentiate between the younger and the older children with regards to the previously addressed factors.

In addition to the mentioned observations, the presented data support the assumption that age and the individual *per se* may play a role in determining the heterogeneity and diversity of supragingival plaque microbiota.

Supragingival plaque microbial counts in relation to caries experience

The majority of studies using the checkerboard DNA-DNA hybridisation technique have focused on studying plaque bacterial ecology in relation to periodontal disease (Ximénez-Fyvie et al., 2000; Socransky et al., 2004; Haffajee et al., 2008; Dahlén et al., 2010; López et al., 2011). These studies revealed a directly proportional relationship between the bacterial count and periodontal pocket depth. A relationship between the counts of supragingival bacteria and the caries experience (DMFT/dmft), could not be demonstrated in Study III. However, a relationship between the caries experience and caries-related salivary bacterial counts (mutans streptococci and lactobacilli) assessed using CRT Bacteria® was found in the same study sample in Study I. This corresponds well with an earlier study in which a stronger association was found between dental caries and cariogenic organisms analysed by culture technique in comparison to the checkerboard method (Hintao et al., 2007).

A number of factors related to the characteristics of dental plaque, the caries disease process and the choice of sampling method may explain Study III's finding. The bacterial interaction in dental plaque could make a greater contribution to caries development than the specific count of certain cariogenic species. In addition, the plaque microbial community differs at different stages of plaque maturation (early plaque *versus* mature plaque). Furthermore, the location of the sampling sites and number of sites selected may influence the relationship. The count of plaque bacteria could also be affected by a number of caries-related factors other than the actual caries experience such as the location/depth (enamel *versus* dentine), stage (incipient *versus* cavitated), level (acute, chronic or arrested) and activity of the carious lesion (active *versus* inactive). Furthermore, the checkerboard DNA-DNA hybridisation technique has a high cut-off limit for bacterial quantification making the

detection of bacteria in low counts problematic, which may be further complicated by cross-reactions (Socransky et al., 1998). Finally, the unequal distribution of subjects within the checkerboard scores with a majority of low bacterial counts in the present study, could account for the lack of significant results.

Contrary to the findings in Study III, a previous study showed that higher counts of specific bacterial species were associated with health, caries initiation and caries progression by using a reverse-capture essay (Aas et al., 2008). Moreover, pooled plaque samples were analysed in Study III as opposed to samples from different caries-susceptible sites in the oral cavity. As caries is a localized disease, it is essential that biofilm samples are taken from specifically determined tooth sites; pooling samples does not appear to be appropriate (Nyvad et al., 2013). In addition, the DMFT/dmft index could be regarded as a crude method to describe the individual caries experience and conveys no information about caries activity.

Caries-preventive effects of a high-fluoride regimen

Study IV is the first intervention study to assess the effects of using 5,000 ppm fluoride toothpaste on caries-related salivary and plaque parameters over a time period exceeding two weeks. It demonstrates the ability of 5,000 ppm fluoride toothpastes to reduce the cariogenicity of saliva and plaque and supports their benefit in high caries risk patients.

The efficacy of any fluoride-preventive strategy depends on its oral bioavailability. As the approximal fluid is a mixture of plaque fluid and saliva, any change in its fluoride content represents changes in the fluoride concentration of saliva and plaque. The increase in approximal fluid fluoride concentration in Study IV goes hand in hand with the results of a recent investigation (Nordström and Birkhed; 2009). The significant increase can be attributed to the use of the 5,000 ppm fluoridated toothpaste and the avoidance of post-brushing rinsing with water. A positive correlation between the F concentration in dentifrices and the F concentration in plaque has been previously reported (Duckworth et al., 1989). Subsequent studies further show that post-brushing water rinsing reduces the retention of fluoride in interdental plaque (Sjögren et al., 1996; Zero et al., 2010).

In addition, the pH “strip method” was used for the first time in Study IV to assess the effects of 5,000 ppm fluoride toothpaste on plaque acidogenicity (plaque pH). All pH-related variables differed considerably following the use of the 5,000 ppm fluoride regimen. These findings reflect the important role fluoride plays in inhibiting acid production by

plaque bacteria, which eventually increases plaque pH and disrupts the acid tolerance of cariogenic bacteria (Marquis et al., 2003; Koo, 2008). This significant reduction in plaque pH can be interpreted as a net result of changes in various biofilm properties induced by the use of the 5,000 ppm fluoride toothpaste. One important finding in Study IV is the observed shift in the time of minimum pH from five minutes at baseline and two weeks, to 10 minutes at four and six weeks. This suggests that fluoride slows down acid production.

Study IV is also the first study in which Clinpro™ Cario L-Pop™, a chair-side test, has been used to assess the effects of a high-fluoride regimen on the lactic acid production rate on the tongue. This production was reduced after using the 5,000 ppm fluoride dentifrice, but it remained for all the participants within the same risk category. This could indicate that a longer use of the 5,000 ppm fluoride toothpaste is required to reduce the lactic acid production to a lower risk level, but also that the tongue is not the most optimal site to reflect changes produced by a high fluoride regimen. Nevertheless, the findings in Study IV suggest that even the relatively short-term use of 5,000 ppm fluoride toothpaste could reduce the caries risk.

No reduction in the major, minor and total acid anion concentrations in resting and fermenting plaque was noted in Study IV. There could be different reasons for this finding. It may be that longer-term-use of the high-fluoride regimen is needed to detect significant reductions in organic acid concentrations. In addition, no sugar intake restrictions or specific dietary instructions were given in Study IV, which could have sustained an ideal environment for bacteria to thrive and produce acids, even though an elevated concentration of fluoride was present. Moreover, some of the participants might not have complied with the instructions to be followed one hour prior to every scheduled appointment. This could in turn have led to the higher lactate observed in resting plaque instead of fermenting plaque seen in some of the participants. In addition, it has previously been shown that approximal resting plaque has a higher acid concentration than buccal resting plaque, while the response to a sucrose rinse is less pronounced in approximal plaque as compared to buccal plaque (Damen et al., 2002; Gerardu et al., 2007). The shorter exposure time to sucrose in Study IV (one minute sucrose rinse instead of two minutes) may have resulted in the partial clearance of sucrose and major acids from plaque before the actual sampling of fermenting plaque at 10 minutes, diminishing the difference between resting and fermenting acid concentration in approximal plaque (Geradu et al., 2007). In addition, difficulties associated with sample reproducibility could lie behind the high variability of the data.

Regarding the salivary-related parameters, statistically significant changes were observed in the buffer capacity and mutans streptococci counts following the use of the 5,000 ppm fluoride regimen. These positive findings prove that in vivo, only high concentrations of fluoride can reduce the number of cariogenic bacteria in dental plaque (van Loveren, 2001). These findings underline the opportunity to increase fluoride oral bioavailability in saliva by increasing its concentration in toothpastes. Since oral fluoride reservoirs are mediated by calcium, their formation is limited by the low concentration of calcium in oral fluids (Vogel, 2011). By using products that overcome this limitation, the formation of these fluoride reservoirs can be greatly increased and eventually lead to substantive increases in its bioavailability (Vogel, 2011). The fluoridated toothpaste used in Study IV had a neutral pH base with tri-calcium phosphate, which in turn provides a novel procedure for effective fluoride delivery to saliva and plaque and thereby the enhancement of remineralisation. Toothpastes could be regarded as a suitable vehicle for increasing fluoride concentration, but there are concerns about the toxicological effects of high fluoride concentrations. In adults, this is limited by the fact that toothpastes are expectorated and an insignificant amount is swallowed. However, in younger children, this remains an important consideration (Ekstrand, 2006). The younger siblings (4-6 years old), who were included in Study I, were therefore excluded from Study IV because of these toxicological concerns.

One interesting observation in Study IV was the plateau-like pattern seen particularly in the measurements related to fluoride concentration, tongue lactic acid production rate and salivary MS count, towards the end of the study. This may reflect one of two things: either a form of adaptation in the oral microflora with higher fluoride availability in the oral environment or a kind of plaque fluoride saturation. The fluoride concentration measured in Study IV is that of free fluoride. This plateau could therefore actually reflect the fact that more fluoride has been deposited in the form of reservoirs and only a limited amount is in free from. This observation could indicate that the use of 5,000 ppm fluoridated toothpaste may induce only a transient, rapid increase in salivary and plaque characteristics. However, further long-term studies are needed to confirm this finding. Although all the participants reported compliance with the study protocol, this does not rule out the possibility of insufficient compliance by some individuals.

Variations in caries-risk profile after a high-fluoride regimen

Study V was an experimental study designed to validate the hypothesis that the use of 5,000 ppm F toothpaste modifies caries risk. A significant change in the caries risk as assessed by the "Cariogram" software was observed after the six weeks' use of the high-fluoride regimen, both for all participants and when evaluating mothers and their teenage children separately.

A reduced "Cariogram" model was used to evaluate the change in caries-risk profile in relation to the use of high-fluoride toothpaste (5,000 ppm). As a result not all the standard variables to be included in the "Cariogram" were inserted into the program at the four different time points. They included the salivary secretion rate, plaque amount, and diet frequency. Even though all the subjects had a normal secretion rate at baseline, it was decided to exclude this information, as the secretion rate was not measured at all time points. Since 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. Petersson et al. (2010b) used a reduced "Cariogram" model in school children and found that the exclusion of the salivary variables significantly impaired the accuracy of caries prediction. Among such variables, the mutans streptococci counts had the greatest impact on the predictive ability of the model, while the salivary secretion rate and buffer capacity displayed only a small effect.

The main finding in Study V was the gradual and 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 six weeks for all participants. This immediate response following the short-term use of the high-fluoride toothpaste could be attributed to an antimicrobial effect, evident from the significant reduction in the mutans streptoccoci count found in the study (Buzalaf et al., 2011; Lussi et al., 2012).

The teenagers showed a faster change in the actual chance of avoiding caries occurring between baseline and two weeks, while the mothers presented a later change at four to six weeks. This could be due to a less mature biofilm in the teenagers, which can benefit more easily from the high-fluoride regimen.

In the "Cariogram", the fluoride programme was set at "2" (fluoride toothpaste, no supplements) at baseline and "1" (additional fluoride measures, infrequently) at two, four and six weeks. This change in itself changes the weight of 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 (data not shown). This indicates that the variables influenced by the high-fluoride regimen (mutans streptococci and

lactobacilli counts, and buffer capacity) accounted for this change in caries risk. This corresponds well with the increased fluoride retention in the biofilm and reduced plaque acidogenicity found after a sugar challenge for the same group presented previously in Study IV. Moreover, the data also correspond well with a previous study of 5,000 ppm fluoride dentifrice (Nordström and Birkhed, 2009). A recently published study, evaluated the effect of two years daily use of high-fluoride toothpaste in comparison with a dentifrice containing 1,450 ppm fluoride on caries incidence and caries progression in adolescents, in which a lower progression rate was found for those using the higher fluoride regimen (Nordström and Birkhed, 2010). The same study also reported that the effect was strongest with poor compliance.

Although not evaluated in Study V, it may be anticipated that the plaque amount might have changed throughout the trial as previously shown by the use of a high-fluoride regimen (Nordström et al., 2009). So, if this variable had been included in the analysis, an even greater increase in the “actual chance to avoid caries” might have been observed.

Strengths and limitations

Study I

The design of Study I offered several advantages. It permitted a description of the caries experience in three different age groups. It also created an avenue to explore different biological and non-biological determinants of dental caries. By targeting families, various mother-child relationships relevant to dental caries became feasible for investigation. Unlike the focus on one gender (mainly males) in most of the publications describing the caries experience in KSA, Study I included both genders. Even though the study was performed on a Saudi sample, the findings can be regarded as valid for families from other countries with similar oral health problems.

On the other hands, the study was performed on a convenience sample from the city of Jeddah in the western province of KSA. As a result, the external validity of the study in terms of generalising the findings to the entire Saudi population is restricted. Further multi-regional studies from rural and urban provinces of the country are needed to provide a more representative picture of the national caries experience. Another limitation of Study I is the non-involvement of the fathers, which was primarily due to practical limitations, as it was very difficult to have all family members present on the same visit. The inclusion of the fathers would have provided a more comprehensive picture of the influence of parent-related factors on the caries experience in their children. Even if the fathers may not be as directly involved as the mothers in the oral health education of their children in societies like that in KSA, they may function as role models, especially for their sons.

Study II

This microbiological study is one of the few studies that have implemented the checkerboard DNA-DNA hybridisation technique using a caries-specific panel (Tanner et al., 2002a; 2002b; Corby et al., 2005; Aas et al., 2008; Kanasi et al., 2010). In addition, it is the first study in which this whole genomic DNA-based analysis has been used to compare supragingival plaque microbiology between mothers and their children.

As some sites in the mouth are favoured by certain bacterial species, one possible shortcoming of Study II is that a pooled plaque sample was analysed instead of samples from multiple or specific sites in the oral cavity. Pooling supragingival plaque into a single tube may

present technical problems such as difficulty in lysing all the bacterial cells present in a very dense bacterial suspension (Papapanou et al., 1997). However, heavy plaque sampling was avoided in Study II. Another limitation is that relatively few strains were analysed in the study despite the fact that the checkerboard DNA-DNA hybridisation technique allows the simultaneous analysis of numerous bacterial strains (Socransky et al., 1994). A panel of 18 bacterial strains was chosen since the main focus of the study was cariogenic bacteria. A similar panel to the one used in Study II was implemented in a recent study (Nelun Barfod et al., 2011). In comparison to other microbiological techniques, the checkerboard DNA-DNA hybridisation method has several advantages, but on the other hand one of its main limitations is its high cut-off point for bacterial quantification making the detection of bacteria in low counts problematic (Socransky et al., 1998). In addition, it quantifies both viable and dead bacteria, the later which is of limited interest. Another concern is the cross-reactions, which infrequently occur with the checkerboard DNA-DNA hybridisation technique (Socransky et al., 1998; Socransky and Haffajee, 2005). These reactions are limited and generally occur more between genera than species. Methodologically, checking for the extent of such cross-reactions is a standard procedure.

Although significant microbial associations were observed in Study II, any representation of these microbial complexes, whether by cluster analysis or by community ordination techniques, suffers from the limitation that an attempt is made to represent multi-dimensional relationships in two or three dimensions (Socransky et al., 1998). Different presentations of relationships are therefore bound to suggest microbial associations quite different from those obtained by basic correlation analyses. There is also a potential risk of human error in the interpretation of multi-dimensional data and further studies evaluating supragingival microbial relationships are required (Socransky et al., 1998).

Study III

The checkerboard DNA-DNA hybridisation technique has been used extensively to explore supra- and subgingival plaque microbiology in relation to periodontal disease (Haffajee et al., 1998, Socransky et al., 1998; Ximénez-fyvie et al., 2000; Socransky et al., 2004; Dahlén and Leonhardt, 2006; Haffajee et al., 2008; López et al., 2011). As with Study II, Study III is one of the few studies that have used this molecular technique in relation to dental caries.

There are two limitations to the sampling method used in Study III. Unlike saliva, it is difficult to standardise the amount of bacteria in a

plaque sample. Standardisation can be easily achieved with saliva by expressing the amount of bacteria in a 1-mL sample. Moreover, pooling supragingival plaque into one test tube may create difficulties as mentioned previously. Heavy plaque sampling was minimised in Study III by taking only a sufficiently visible amount of supragingival plaque. On the other hand, the question of whether too little plaque was collected cannot be ruled out.

A relationship between plaque bacterial counts and caries experience might therefore have been obtained if the investigation had been performed at site-specific level and if caries-related parameters, such as depth, stage and activity, had been taken into account, as mentioned in the discussion section of this thesis.

Study IV

Recently, attempts have been made to evaluate the preventive properties of high-fluoride regimens. Study IV specifically examined how 5,000 ppm fluoride toothpaste can alter certain salivary and plaque characteristics, which have not been included in previous studies performed on this kind of regimen. The findings in this study clearly demonstrate the short-term caries-preventive effects of 5,000 ppm fluoride dentifrice.

On the other hand, not all potential salivary and plaque variables were evaluated in Study IV. The possibility that other salivary and biofilm properties apart from those studied were also affected cannot be excluded. Further research should aim at evaluating the long-term effects of 5,000 ppm fluoride toothpastes on biofilm properties, such as microbial composition and activity.

Despite the fact that a power calculation was performed justifying the inclusion of 34 participants, the total number of study participants may be regarded as limited and the drop out rate in Study IV is therefore considered high (23.5%).

Study V

There is a scarcity in the available literature on the beneficial roles of high-fluoride programmes. Study V was the first to implement the "Cariogram" software in evaluating the caries-risk modifications generated by the short-term use of 5,000 ppm fluoride dentifrice. The results of the study have clearly shown the ability of such a high-fluoride regimen to rapidly modify the caries risk.

Although statistically significant differences were obtained in Study V, the small number of subjects enrolled in this study might be seen as a

shortcoming. Larger scale studies are needed to confirm such findings and to assess the long-term caries-risk reduction ability of 5,000 ppm fluoride toothpaste.

Moreover, evidence relating to the validity of existing caries-risk assessment systems is limited (Tellez et al., 2013). It is unknown whether the identification of high-risk individuals can lead to more effective patient management, caries prevention, arrest or reversal (Tellez et al., 2013). There is therefore a need to develop valid reliable methods for caries-risk assessment, which are based on the best evidence for prediction and disease management rather than expert opinion.

Conclusions

The main conclusions from this thesis are as follows:

1. **T**he caries experience in Saudi mothers and their children was high, with similar contributory caries-related factors.

2. **S**upragingival plaque microbiota were correlated between the mothers and their children. In addition, similar supragingival plaque microbial associations were present in the three family members.

3. **U**nlike the saliva samples and the chair-side method, interproximal pooled plaque samples analysed using the "checkerboard DNA-DNA hybridisation technique" did not appear to reveal any significant relationships between the bacterial counts and the caries experience.

4. **T**he short-term use of 5,000 ppm F toothpaste had the ability to reduce the cariogenic potential of dental plaque and saliva.

5. **S**ix weeks' use of 5,000 ppm F toothpaste had the ability to reduce the caries risk, which was clearly demonstrated using the "Cariogram" software.

Clinical relevance and implications

The series of studies included in the present thesis highlight the necessity to focus on caries-preventive strategies and implement a minimally invasive concept in future dental treatment strategies. It is important for the public health authorities to be cognisant of any deterioration in the level of oral health and dental caries, so that efforts can be increased to prevent these problems. There is no room for laxity and believing that dental caries is not a problem today and that it will continue to decline. Nor should this be limited to the developing countries. Even in the developed countries, oral health concern still exist, as the average life expectancy of the general population is increasing meaning that people are more prone to retain their dentition for a longer period of time.

The efforts should begin as early as possible by implementing the "Early Oral Health Care Concept", permitting the education of expectant parents in terms of oral health care and diseases. This should be coupled with other strategies including public health motivation strategies and school-based oral health educational and prevention programmes. These strategies should emphasise proper toothbrushing and interdental cleaning, encourage the consumption of a proper and balanced diet and establish regular dental office screenings and visits.

Rather than blaming patients for their lack of oral hygiene and poor dietary habits, as clinicians, we must therefore ask ourselves, "Have we performed our duties appropriately?". We may have succeeded in delivering state-of-the-art restorative treatments, but we might have underestimated the importance of motivating and educating our patients. As caries is a multifactorial disease, preventive strategies must be designed to target all the caries-related factors contributing to the patient's problem. Moreover, we should explain, educate and motivate our patients in relation to their oral health problems and what is advocated using pedagogic models such as the "Cariogram". The dental consultations and dentist-patient interviews should be interactive not dictatorial in order to promote better comprehension by the patient and greater compliance. Another important recommendation is to target families at risk not just individuals, in order to include those at risk of caries to a wider extent.

Regarding high-fluoride products for caries control, the majority of studies performed on these products have reported results favouring their use, especially in high caries-risk patients. However, this should not blind us and prevent us from considering the possibility that cariogenic bacteria will develop resistance to high-fluoride concentrations (Marsh and Martin,

2009a). Another aspect to consider is the high cost of 5,000 ppm fluoride toothpastes in relation to the regular 1,450-1,500 ppm fluoride toothpastes. Some may argue that recommending additional means of protection, such as fluoride mouthrinses and interdental cleaning aids along with regular fluoridated toothpastes would be more affordable to the patient than prescribing high-fluoridated dentifrices. Cost is therefore another point to consider when designing preventive protocols, especially in private practices.

To summarise, an evidence-based holistic preventive and treatment approach customised to match each patient's needs should be followed in order to provide our patients with the best oral health care. In comparison with other methods for oral fluoride administration, the high-fluoride toothpaste regimen can be regarded as an easy efficient way to increase individual fluoride exposure.

Future perspectives

To obtain a greater insight into the caries status in KSA, public and private oral health authorities should call for participation in national surveys covering the different provinces of the country and including both rural and urban areas. This will provide the basis to understand the factors contributing to the determined prevalence of the disease. From that point and onwards, properly designed preventive strategies should be initiated. Investigations should include different age groups and both genders. Parent-child relationships should be considered and thoroughly investigated. The aim must be to achieve the WHO's oral health goal for different age groups.

Since the field of microbiological analyses is witnessing advances at a rapid pace, there is great potential to use novel microbiological quantitative techniques to correlate bacterial counts with the caries profile.

Future plaque acidogenicity studies should consider the use of the pH "strip" method, as it offers an easy, reliable and fairly accurate technique for recording plaque pH. The Clinpro™ Cario L-Pop™ chair-side test has been recently introduced and additional investigations are required to determine its sensitivity and precision in recording changes in the lactic acid production rate and the categorisation of caries risk.

Caries-risk assessment models such as the "Cariogram" have proven to be rather accurate risk estimators, but they also represent an interesting tool to evaluate indirectly the ability of anticariogenic products to modulate the caries risk. Their use should therefore not focus solely on determining caries risk at a certain time point or using it as a pedagogic model.

High-fluoride regimens appear to be one of the latest approaches in caries prevention. More research is therefore required in order to thoroughly understand the anticariogenic properties of these products. Length of efficacy, potential side-effects and biological concerns such as fluoride resistance should also be evaluated.

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