

UMEÅ UNIVERSITY ODONTOLOGICAL DISSERTATIONS

New series No. 97 ISSN 0345-7532 ISBN 91-7264-223-8

# **Xylitol and its effect on oral ecology**

**– clinical studies in children and adolescents**

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2007

Cover picture: Xylitol has a cooling sensation when it dissolves in the mouth like the sense of snow.

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ISBN 91-7264-223-8  
Printed in Sweden by Print & Media  
Umeå 2007

**Don't underestimate the value of doing nothing, of just going along,  
listening to all the things you can't hear, and not bothering...**

*Pooh's Little Instruction Book*

*Till Mats, Oscar & Filip*

## Abstract

Xylitol, classified as a natural sugar substitute, has for about 35 years been known as an agent that may act against caries. The mechanism of action; how it inhibits mutans streptococci (MS) and the clinical dose-response relationship are not however fully investigated. The general aim of the investigations was to evaluate the effect of xylitol on oral ecology in children and adolescents. A series of experimental and controlled clinical trials were performed in which samples of saliva and plaque was collected and analysed with respect to xylitol content, pH, microbial composition and lactic acid production. In paper I, significantly reduced proportions of xylitol-sensitive MS in saliva were demonstrated after 18 weeks of regular use of two dose regimens of xylitol-containing tablets (1.7g and 3.4g xylitol/day) but the acidogenicity in dental plaque was not affected. In paper II, the effect on interdental plaque-pH of two different single dose intakes (2.0g and 6.0g) of xylitol was evaluated. The higher xylitol dose counteracted the pH-drop significantly ( $p < 0.05$ ) when the chewing was followed by a sucrose rinse while the lower dose did not differ from the control. In paper III, the xylitol concentrations in saliva after use of different common xylitol-containing products (0.1g-1.3g) were investigated. Statistically significant elevations of salivary xylitol levels were demonstrated for all products during the first 8-16 min when compared with baseline ( $p < 0.05$ ) but the individual variation was considerable. In samples of supragingival dental plaque, a high dose rinse (6.0g) increased the xylitol concentrations for a longer period ( $> 30$  min) than a low dose rinse (2.0g). In paper IV, it was demonstrated that 6.0g of xylitol in chewing gums, every day in 4 weeks, gave significantly less visible plaque and a significantly reduced sucrose-induced lactic acid formation ( $p < 0.05$ ) in saliva. Furthermore, the proportion of MS decreased significantly ( $p < 0.05$ ) compared to baseline. In paper V, the salivary uptake of [ $^{14}$ C]-xylitol was compared with a specific assay determining xylitol-sensitive MS and a fair positive correlation ( $p < 0.05$ ) between the two assays was found. In a controlled trial, the proportions of MS and the salivary xylitol uptake decreased significantly ( $p < 0.05$ ) in the xylitol gum test group after 4 weeks compared to baseline which was in contrast to the control gum group. No serious adverse effects were reported in any of the investigations.

The main conclusions from this thesis were: a) various xylitol-containing products increased the xylitol levels in saliva and plaque, b) 6.0g of xylitol could counteract the interdental pH-drop after sugar consumption and reduce lactic acid formation in saliva c) a daily dose of 6.0g xylitol reduced the amount of visible plaque and altered the salivary microbial composition, d) a transient shift of MS strains in saliva was demonstrated during periods of regular intake of xylitol products but no long-term impact was found after its termination. The relatively high amount of xylitol needed for a beneficial effect on the oral ecology calls for a further development of effective and safe routes for administration.

*Key words: chewing gum, dental plaque, dose-response relationship, interdental plaque-pH, oral microorganisms, saliva, xylitol*

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## List of papers

This thesis is based on the following five papers, which will be referred to by their Roman numerals:

- I. Stecksén-Blicks C, **Lif Holgerson P**, Olsson M, Bylund B, Sjöström I, Sköld-Larsson K, Kalfas S, Twetman S. Effect of xylitol on mutans streptococci and lactic acid formation in saliva and plaque from adolescents and young adults with fixed orthodontic appliances. *Eur J Oral Sci* 2004; 112: 244-248.
- II. **Lif Holgerson P**, Stecksén-Blicks C, Sjöström I, Twetman S. Effect of xylitol-containing chewing gums on interdental plaque-pH in habitual xylitol consumers. *Acta Odontol Scand* 2005; 63: 233-238.
- III. **Lif Holgerson P**, Stecksén-Blicks C, Sjöström I, Öberg M, Twetman S. Xylitol concentration in saliva and dental plaque after use of various xylitol-containing products. *Caries Res* 2006; 40: 393-397.
- IV. **Lif Holgerson P**, Sjöström I, Stecksén-Blicks C, Twetman S. Dental plaque formation and salivary mutans streptococci in schoolchildren after use of xylitol-containing chewing gums. *Int J Paediatr Dent* 2007; DOI:10.1111/j.1365-263X.2006.00808.x
- V. **Lif Holgerson P**, Sjöström I, Twetman S. Decreased salivary uptake of [<sup>14</sup>C]-xylitol after a 4-week period of xylitol chewing gum regimen. *Submitted*

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## Abbreviations

<b>AUC</b>	area under curve
<b>BAO</b>	blood agar oral plates
<b>CFU</b>	colony forming units
<b>CPM</b>	counts per minute
<b>[<sup>14</sup>C]-D-xylitol</b>	radio labelled xylitol
<b>MES</b>	3-[N-morpholino]ethanesulfonic acidhydrat
<b>MSB</b>	mitis salivarius bacitracin agar
<b>MS</b>	mutans streptococci
<b>NaCl</b>	sodium chloride
<b>PEP-PTS</b>	phosphoenolpyruvate-phosphotransferase system
<b>PTS</b>	phosphotransferase system
<b>TVC</b>	total viable count
<b>X<sup>R</sup>MS</b>	xylitol resistant mutans streptococci
<b>X<sup>S</sup>MS</b>	xylitol sensitive mutans streptococci





## Introduction

Dental caries is one of the most common infections in man. There was a substantial decline in prevalence during the later parts of the 20<sup>th</sup> century, mainly attributed to the increased use of fluorides (Marthaler, 2003). During recent years, there have been signs of increased caries prevalence in children (Haugejorden et al., 2002; Stecksén-Blicks et al., 2006). Fluorides, which are a symptomatic approach to caries, increase the resistance of the tooth surface. Another way to combat the development of caries is to use therapies that act on bacterial interaction with oral ecology (Tvetman, 2004).

## Oral ecology

### Oral bacteria

In the oral cavity, more than 700 different kinds of bacteria have been identified (Paster et al., 2001; Socransky and Haffajee, 2005). Some of these bacteria may cause dental caries. The caries bacteria are not foreign invaders of the oral cavity. They are members of the indigenous microflora that are present not only in the oral cavity but also in the gastrointestinal tract and other parts of the body. The indigenous microflora protects against infections caused by pathogenic bacteria. Shifts in the composition of the microflora may predispose individuals to diseases.

The oral soft tissues are the main habitat of salivary bacteria. The oral tissues continually shed oral mucosal cells and the bacteria are swallowed with the cells. Therefore, from a bacterial survival point of view, the non shedding surfaces of the teeth are very attractive.

Loesche introduced the specific plaque hypothesis (Loesche, 1976) and described dental caries as a disease where specific microorganisms play a key role. This concept originates from the finding of Fitzgerald and Keyes (1960) that dental caries develops in the interaction between the host, a sugar rich diet, and a certain streptococci. The hamsters in their study did not develop caries from sugar if they did not harbour the streptococci.

The bacteria can contribute to the development of caries in several ways. The most cariogenic group is mutans streptococci (MS), especially *S. mutans* and *S. sobrinus*. *S. mutans* colonize primarily fissures and grooves and *S. sobrinus* mainly the smooth surfaces (de Soet et al., 1992). Many clinical studies have shown that caries is

associated with an increase in the proportion of acidogenic bacteria such as MS and lactobacilli (Loesche, 1986; Marsh, 1999).

The MS are mainly associated with the initiation and may not contribute to the caries lesion into dentin (Ikeda et al., 1973; Edwardsson, 1974). Other bacteria are involved in the progression of the caries process; lactobacilli, actinomyces, and different proteolytic bacteria are found in human carious dentin and cementum (Liljemark and Bloomquist, 1996).

The cariogenic bacteria have similar characteristic pathogenic properties (Loesche 1986):

- They produce extracellular and intracellular polysaccharides. Extracellular polysaccharides include glucans and fructans that contribute to the plaque matrix. Intracellular polysaccharides are glycogen like storage compounds that can be used for producing energy and be converted to acids when there are no free sugars.
- They rapidly transport sugars in competition with other plaque bacteria. MS have several sugar transport systems; for example, they have a high-affinity for the phosphoenolpyruvate phosphotransferase system (PEP-PTS) and scavenge sugars in very low concentrations. Organic acids constitute the end products of bacterial metabolism.
- They can maintain sugar metabolism in extreme environmental conditions, such as low pH and even grow in these conditions.

When the bacteria are allowed to grow without mechanical disturbance, they can cause dental caries (Marsh, 1994).

After cleaning the tooth surface, the first bacterial layer (the pellicle) begins to adhere within a few minutes. There are different chemical and physical mechanisms that make the adherence of bacteria possible. For example, the bacterial cell walls can contain sticky components and cariogenic microorganisms have extracellular polysaccharides.

### Dental plaque

Dental plaque is the general term for the diverse microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin.

Plaque develops naturally on teeth and is an example of a biofilm. Under stable conditions, a homeostasis with the host occurs.

The development of dental plaque can be explained by different processes (Fejerskov and Kidd, 2003):

- a conditioning film formed by adsorption of salivary proteins, glycoprotein, and some bacterial molecules (pellicle);
- an attachment of single bacterial cells (primary colonisers);
- a growth of attached bacteria (micro colonies);
- a continued growth of micro colonies and secondary colonisers attached to primary colonisers (co aggregation); and
- a cell division of attached cells – confluent growth (mature plaque).



Fig.1 Dental plaque stained with colour tablets.

The pathogenic potential of the dental plaque depends on the number and type of the resident microflora. Cariogenic plaque normally contains high numbers of MS and lactobacilli. This kind of dental plaque has a great acidogenic potential.

The first minutes after consuming sugar is often dangerous to the teeth because organic acids start to form and the plaque-pH begins to decrease. Lactic acid is the principal component for the pH drop (Geddes, 1975). The plaque-pH may reach

values between 4 and 5 and will reach the so called “critical pH”. This value lies normally between 5.3 and 5.7. If these low pH levels are reached often, the risk for dental caries increases (Sheiham, 1983). Low pH generated from the acid production rather than the availability of sugar decreases homeostasis in the microbial community in the dental plaque (Bradshaw et al., 1989; Bradshaw and Marsh, 1998).

In addition to a “specific plaque hypothesis” (Loesche et al., 1976), there is a “non-specific plaque hypothesis”. The non-specific plaque hypothesis explores whether caries disease is caused by the overall activity of the total plaque microflora (Theilade, 1986). Combining the two earlier hypotheses, Marsh (2003) proposed the “ecological plaque hypothesis”. This hypothesis concludes that bacteria inducing caries development are selected out when the homeostasis is broken and a more acidic environment occurs. Any species of bacteria with relevant traits can contribute to the disease process (Fig. 2).

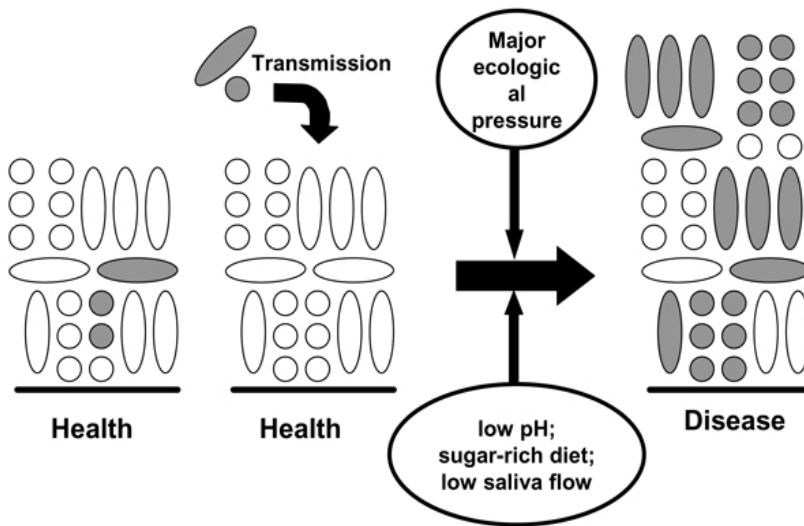


Fig. 2 The ecological plaque hypothesis. Figure adapted from Marsh (Marsh, 2006).

## Saliva

Salivary flow comes mainly from the three major salivary glands – the parotid, submandibular and sublingual glands – as well as from the minor glands in the oral mucosa. The saliva contains more than 99% water and less than 1% solids, such as proteins and electrolytes. The electrolytes include calcium and phosphate and proteins include mucins and mucoproteins, which contribute to pellicle formation. Saliva also contains sodium, potassium, and magnesium and small amounts of many other inorganic components. The pH-value of normal whole saliva varies between 6.8 and 7.2 (Fejerskov and Kidd, 2003).

The daily production of saliva varies between 0.5 and 1 litre. The flow rate of unstimulated saliva is normally 0.25-0.35 ml/min, and stimulation, such as using chewing gum, may increase the flow rate to more than 1.0 ml/min. In children, the amount of saliva secretion increases with age. The salivary flow rate is affected by many factors, such as nutrition, mood, season, and time of day etc. (Dawes, 1987).

The saliva contributes to digestion, mechanical cleansing, buffering, and acts antibacterial. Salivary clearance means that saliva eliminates substances that are introduced into the oral cavity. This is a physiological process and the focus has been on how the saliva takes care of sucrose, “Sugar clearance”. Inorganic ions in the saliva contribute to the remineralization process of the enamel.

In the oral cavity, there is always a small amount of saliva – a residual volume after swallowing that is spread out like a thin layer over the oral surfaces. The thickness of this layer varies at different sites. If sucrose (or other substances) is dissolved in this small amount of saliva, it will result in very high concentrations. The more saliva that is swallowed, the faster sucrose is eliminated and the clearance time becomes shorter. A high initial sucrose concentration will normally give rise to a high salivary flow rate, which results in a fast initial clearance rate. The salivary sugar clearance is individual and a function of parameters that vary for each subject, such as unstimulated salivary flow rate and the volume of saliva present in the mouth immediately before and after swallowing (Dawes, 1983; Lagerlöf et al., 1994).

Using the bicarbonate system (Fig. 3), the saliva buffers acids produced by bacteria. The buffering capacity of the saliva can restore a pH in dental plaque to the same level as before acid production. The amount of bicarbonate increases with the salivary flow rate. Bicarbonate has the ability to take up hydrogen ions to form carbonic acid.

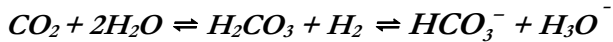


Fig. 3 The bicarbonate system is based on this chemical equilibrium. The reaction is catalyzed by the enzyme carbonic anhydrase.

If the concentrations of carbonic acid increase more carbon dioxide is formed in saliva and bicarbonate can bind hydrogen ions. At a pH above 5.5, the bicarbonate buffering system has a high buffering capacity (Dowd, 1999).

Saliva also contains other substances that increase the pH. Sialin is a small tetrapeptide formed in the parotid saliva. Urease, a dental plaque enzyme, transforms sialin to carbon dioxide and ammonia. Various diseases as well as medication can affect the function of the salivary glands and lead to hypofunctions of the glands (Sreebny and Valdini, 1987). Hypofunction can result in dental caries and injuries of the oral mucosa (Schubert and Izutsu, 1987). Sreebny (1989) described saliva as follows: “When present, saliva enables to enjoy some of life’s most serene pleasures: the delicate sensations of taste; the joy of eating; the exquisite the human voice; and the gratification of a kiss”.

## **Sugar and sugar substitutes**

### Sugar

Sugar is the generic name for the simpler forms of carbohydrates, including: monosaccharides (simple sugars) such as glucose, fructose and galactose; disaccharides (double sugars) such as sucrose, maltose and lactose; trisaccharides and oligosaccharides (but not including polysaccharides, such as starch, glycogen and cellulose). Monosaccharides and disaccharides are sometimes named according to the food in which they are found, for example “milk sugar” refers to lactose and “fruit sugar” refers to fructose (Moynihan, 1998).

Sweet fruits and honey were the first known sweet food. In India, sugar cane was processed before the birth of Christ, but it was the Arabs that invaded India about 640 AD that brought the sugar cane and the method to refine it to North Africa and Spain. Cane sugar was unknown in Europe until the middle ages and then for a long time it was a luxury available only to the upper class. These refined sugars became a symbol of status.

In the middle of the 19<sup>th</sup> century, the availability of refined sugars in Sweden increased. At this time, Europeans had learned to refine sugar from the sugar beet, which had been grown for a long time in Egypt and southern Europe without the knowledge about how to get sugar from it. The refined sugar became easier to produce and easier to buy and the diet in the countries changed with an increase of dental caries as a side effect (Sreebny, 1982). The Vipeholm study established that sugar consumed at meals did not have the same caries potential as if consumed between meals (Gustafsson et al., 1954; Zero, 2004). The annual per capita consumption of sucrose has been fairly stable in Sweden during the last 50 years, slightly above 40kg (Swedish Board of Agriculture, 2006).

### Sugar substitutes and sweeteners

Because bacteria that produce acids from sugars can cause caries, it has been a challenge to find sugar substitutes and artificial sweeteners. Sugar substitutes include lactitol, maltitol, mannitol, sorbitol, isomalt, and xylitol and are commonly used in foods to replace sugars (Moynihan, 1998). These are polyols (sugar alcohols) and are generally considered as more tooth friendly since they do not contribute to the formation of organic acids and the plaque matrix (Kalfas et al., 1990).

Acesulfame-K, Aspartame, Cyclamate and Saccharin are artificial sweeteners and not accepted as a source of nutrients by the cariogenic bacteria and do not decrease plaque-pH. They do not give any calories compared to sugar substitutes, sorbitol gives 4.0 kcal per gram and xylitol gives 2.4 kcal per gram.

### Sorbitol

Sorbitol is a six carbon sugar alcohol. Sorbitol is slowly adsorbed in the intestine and metabolized to fructose in the liver. It can only be used by approximately 5-10% of the bacteria in dental plaque and thus the acid production from sorbitol is normally low (Kalfas et al., 1990). The polyol is considered to be low cariogenic. If the consumption of sorbitol is frequent, the plaque bacteria may undergo adaptation and ferment the polyol and the cariogenic potential may increase (Kalfas et al., 1990). Too much sorbitol can cause diarrhoea (>20-30g). Maltitol and mannitol are used in combination with sorbitol and act on plaque as sorbitol does.

## Xylitol

Xylitol is a five carbon polyalcohol, which is widely distributed in nature (Fig. 4). Most fruits, berries, and plants contain xylitol. Washüttl et al. (1973) listed the richest nature sources of xylitol to be plums, strawberries, raspberries, cauliflower, and endives. In human metabolism, 5-15g of xylitol is formed daily (Hollman et al., 1964). Xylitol, metabolised in the liver, is converted to D-xylulose and glucose by polyol dehydrogenase. Activity from this enzyme may induce and select intestinal microflora (Krishnan et al., 1980; Bässler 1969). The absorption rate of ingested xylitol is quite slow and high oral doses may induce osmotic diarrhoea. Unadapted adults can consume 30-60g oral xylitol per day without side effects, while after adaptation the dose can be increased up to 400g daily (Mäkinen and Scheinin, 1975).

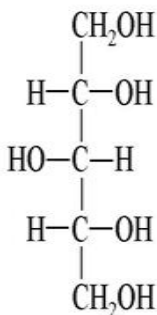


Fig. 4 The chemical structure of the xylitol molecule.

Xylitol can be used in the diet of diabetics. As it is slowly absorbed, the initial metabolic steps are independent of insulin and it does not cause rapid changes in blood glucose concentration (Förster, 1974). Xylitol can also be used as a source of energy in intravenous nutrition because tissues can use xylitol under postoperative and posttraumatic conditions (Georgieff et al., 1985).

Xylitol is industrially produced from xylose (wood sugar), which is available in the forms of xylans (hemi cellulose polysaccharides that consist of xylose). Xylose is present especially in hardwood material, such as birch, beech, nutshells, straw, corn, and bagasse. Birch sap does not normally contain free xylitol in measurable amounts. The manufacturing of xylitol is made by a few simple chemical steps



using xylan-rich plant materials (Fig. 5). Crystallized xylitol is used in for example chewing gums and tablets; grinded xylitol is a component of tooth paste.

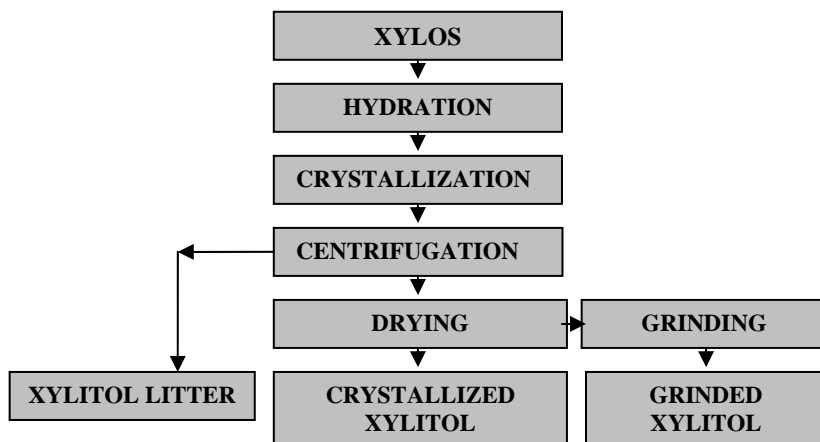


Fig. 5 Schematic view of industrial xylitol manufacturing.

Xylitol is a natural carbohydrate like substance. The industrially produced xylitol molecules have the exact same structure as the molecules that occur naturally and in humans. Xylitol is hydrophilic and may compete with water molecules for the hydration layers that surround protein molecules in biological environments. Furthermore, xylitol may form complexes with inorganic ions such as  $\text{Ca}^{2+}$ , stabilizing the calcium phosphates in saliva (Mäkinen and Söderling, 1984).

Growth inhibition of bacteria by xylitol *in vitro* has been observed in many different bacterial species: *Escherichia coli*, some strains of *Lactobacillus casei*, *Streptococcus pneumoniae*, several *Actinomyces* species, food-spoilage organisms, and several strains of *Streptococcus mutans* (Knuutila and Mäkinen, 1975; Vadeboncour et al., 1983; Beckers, 1988). The hampered growth is based on metabolic reactions. Most microorganisms do not incorporate xylitol because they do not have any suitable transport mechanism. Oral bacteria use the PTS system (phosphotransferase system) for sugar transport (Birkhed et al., 1985; Assev et al., 1996; Touger-Decker and van Loveren, 2003). Xylitol is incorporated with help from the fructose specific PTS and phosphorylated to xylitol-5-phosphate (Wåler et al., 1984; Wåler, 1992; Trahan 1995; Trahan et al., 1996; Roberts et al., 2002; Tanzer et al., 2006). This substance inhibits further intracellular metabolism of the bacterial cell and the

process consumes energy. The phosphate can also be dephosphorylated and transported back to saliva or plaque (xylitol futile cycle) (Söderling and Pihlanto-Leppälä, 1989; Pihlanto-Leppälä et al., 1990; Kakuta et al., 2003). An electron microscopic study showed that growth inhibition of MS by xylitol resulted in degraded cells, autolysis, and the formation of vacuoles (Tuompo et al., 1983). These changes reduce the possibility for the cell to adhere to a surface. After exposure for xylitol, increased bacterial tolerance to xylitol cannot be ruled out (Roberts et al., 2002). A shift towards xylitol resistant strains of MS has been shown in saliva. It has been suggested that these strains have a reduced ability to adhere to tooth surfaces (Trahan et al., 1992; Trahan et al., 1996; Söderling et al., 1997).

The Turku sugar studies were initiated in the beginning of 1970s. At first, there were two clinical studies, one feeding study and one chewing gum study (Scheinin and Mäkinen, 1975). In the feeding study, almost complete substitutions of dietary sucrose by fructose or xylitol were accomplished. The study included 115 subjects divided into three groups. They received sucrose, fructose, or xylitol as dietary sweeteners. Normal consumption of xylitol was 50-67g per day. The study showed an 85% reduction of dental caries in the xylitol group compared to the sucrose group, whereas a 30% caries reduction was shown in the fructose group compared to the sucrose group. The chewing gum study included 100 dental students that were divided into a sucrose group and a xylitol group. The daily dose of each sugar was 6.7g. After one year, the reduction of caries increment in the xylitol group was 82% compared to the sucrose group. It was concluded that a complete substitution of sucrose not was needed to reduce caries increment (Scheinin and Mäkinen, 1975).

After the Turku sugar studies, many clinical trials have been initiated. Studies with caries increment as outcome measure include the “Belize study”, which was performed during 1989-1993 in Belize in Central America. Over 1200 children participated. They were divided into nine treatment groups: one control group (no supervised gum use); four xylitol groups (range of supervised xylitol consumption: 4.3 to 9.0g/day); two xylitol-sorbitol groups (range of supervised consumption of total polyols: 8.0 to 9.7g/day); one sorbitol group and one sucrose group (supervised consumption: 9.0g/day respectively). The results suggested that systematic use of polyol-based chewing gums reduces caries rates in young subjects; xylitol gums were more effective than sorbitol gums (Mäkinen et al., 1995).

In the “Michigan xylitol programme”, the participants were from 6 years old to geriatric ages. They were provided with saliva stimulants, mostly chewing gums, for 2 weeks to 56 months. The clinical and microbiological results suggested that xylitol was more effective than sorbitol in preventing caries (Mäkinen et al., 1996). Alanen

et al. (2000) demonstrated in a field study from Estonia that a supervised use of both xylitol-containing candy and chewing gums reduced caries incidence in schoolchildren compared with corresponding control groups. In contrast, Machiulskiene et al. (2001) performed a study in Lithuania with more than 600 participating schoolchildren. They were divided into different groups, and after three years there were no differences between the xylitol, sorbitol, and control group. The study concluded that the chewing process reduced caries increment.

Other areas where xylitol seems to be useful are in preventing the transmission of *Streptococcus mutans* from mother to child (Söderling et al., 2000; Peldyak and Mäkinen, 2002; Thorild et al., 2005) and in preventing acute otitis media (Uhari et al., 2000).

Studies have shown that the intake of xylitol has to be at least 5-10g/day in fractionated doses to have an anticaries effect; studies with lower doses show generally a less favourable outcome (Isokangas et al., 1988; Petersen and Razanamihaja, 1999; Alanen et al., 2000; Mäkinen et al., 2000; Machiulskiene et al., 2001). There is, however, also a question whether or not xylitol can cause side effects when being used in larger quantities (Scheie et al., 1998; Storey et al., 2006; Vernacchio et al., 2006). A debate is also ongoing whether it is the xylitol itself that gives the oral health effects or whether it is the effect from the saliva stimulation (Scheie and Fejerskov, 1998; Hayes 2001; van Loveren, 2004).

## **Chewing gums**

Sugared chewing gum may contribute to the cariogenicity of the diet. Sucrose chewing gum decreases plaque-pH (Edgar et al., 1975) and clinical studies have demonstrated an increase in caries incidence with the use of sugared chewing gum (Glass, 1981).

The development of sugar free chewing gum provided the possibility of a non cariogenic alternative to sugared chewing gum. Sugar free chewing gum seems to be more effective, but both sugared and sugar free chewing gums can significantly reduce the acid response (Manning and Edgar, 1993). Caries incidence is less in chewers of sugar free chewing gum compared with sugared gum (Mäkinen et al., 1995) and this agrees with the plaque-pH results (Park et al., 1995). As the chewing starts, the saliva secretion rate increases and the stimulation of the saliva are highest during the first minutes. After 20 minutes, the flow is still increased (Dawes and Macpherson, 1992). When chewing stimulates saliva production, the composition

of the saliva changes and the concentration of bicarbonate, phosphate, and calcium increase. These changes in the composition of stimulated saliva lead to a greater ability to prevent a fall in pH and a greater tendency to favour hydroxyapatite crystal growth. In addition, the increased volume of stimulated saliva increases the ability to clear sugars and acids from the teeth. Sugar free chewing gum is a very practical and acceptable saliva stimulus after intake of sugar containing foods. Many studies around the world have confirmed the effect from chewing sugar free chewing gums (Jensen 1986; Park et al., 1990; Söderling et al., 1991; Manning and Edgar, 1993). The plaque reducing effect of sugar free chewing gum seems to be more pronounced when the chewing gum contains xylitol (Mäkinen et al., 1995a; Mäkinen et al., 1995b). Other substances in chewing gums that may contribute to a decrease in caries development are urea, dicalcium phosphate, and sodium trimeta phosphate (Imfeld et al., 1995; Lingström et al., 2003). Two reviews of the chewing gum suggest that there is convincing evidence for the oral health benefits of sugar free chewing gum in the control of caries (Itthagarun and Wei, 1997; Edgar, 1998).

## Aims

In spite of all the research that has been done, there are a number of unanswered questions regarding xylitol. This thesis aimed to seek answers for some of the issues. The general aim was to further investigate the effect of xylitol on oral ecology in children and adolescents.

The more specific aims were to:

- \* Paper I: to investigate differences in the microbial ecology in dental plaque and saliva adjacent to fixed orthodontic appliances after using two dose regimens of xylitol-containing tablets.
- \* Paper II: to study the effect of high and low amounts of xylitol on the interdental plaque-pH, with and without a subsequent sucrose challenge, in children with habitual consumption.
- \* Paper III: to determine the xylitol concentrations in saliva after use of different xylitol-containing products and to investigate whether or not a dose-response relationship would be obtained in dental plaque.
- \* Paper IV: to investigate the effect of xylitol on oral bacteria in saliva and the amount of visible dental plaque after a 4 week period of chewing a high amount of xylitol. A secondary aim was to study whether there were any differences in the outcome measures between children with and without caries experience.
- \* Paper V: to evaluate a simple method to disclose a microbial shift in saliva and investigate the short- and long-term effects of daily use of xylitol-containing chewing gums on MS and [<sup>14</sup>C]-xylitol uptake in saliva.

# Material

## Subjects

The study designs were approved by the regional ethical review board in Umeå, the former local ethical committee at Umeå University. All children volunteered to participate after verbal and written information and informed consent was collected from the children as well as from their custodians. All participants were provided with regular dental care within the Public Dental Health Service.

### Paper I

The material consisted of 56 randomly selected adolescents and young adults of both sexes that underwent orthodontic treatment with fixed appliances at the Public Specialist Clinics in Umeå and Hyltebruk, Sweden. The majority were girls (n=31) and the mean age was 15.8 years, ranging between 13-20 years.

### Paper II

The participants of this study were 11 healthy children and adolescents with a mean age of 12.4 years, range 10-15 years (4 boys and 7 girls).

### Paper III

The study group consisted of 12 healthy children, 6–13 years of age (6 boys and 6 girls) with a mean age of 11.5 years.

### Paper IV

The study group consisted of 128 children (7-12 years old), and they were pupils at a comprehensive school (grade 1 to 6) in a small municipality in northern Sweden.

### Paper V

In the pilot study, 15 adults participated; in the main study, 109 children participated, partly the same as in study IV. The adults were healthy volunteers and the children were pupils at a comprehensive school, see subjects for paper IV.

## **Test products**

### Xylitol tablets (Paper I & III)

Each tablet contained 421.5mg xylitol, but no other sugars. The tablet had the same composition as Xerodent®, Dumex Alpha, Sweden, but with no fluoride.

### Chewing gums (Paper II & III)

Each chewing gum contained 66 weight % xylitol and no other sugars (Xylimax, Fennobon Oy, Finland).

### Chewing gums (Paper IV & V)

The control gums were produced by Fennobon Oy, Karkkila, Finland for the study only. They contained sorbitol (63.5 weight %) and maltitol (4.5 weight %). 1 piece of chewing gum = 0.69g of sorbitol and 0.05g of maltitol. The test chewing gum contained xylitol (77 weight %). 1 piece of chewing gum = 1.03g of xylitol (Fennobon Oy, Karkkila, Finland).

### Xylitol candy (Paper III)

Candy containing 0.56g xylitol/piece (Läkerol, Leaf, Finland) was used.

### Toothpaste (Paper III)

Toothpaste containing 10% xylitol (Dentosal Junior, Denmark) was used.

### Xylitol solution (Paper III)

Solutions were prepared from crystallized xylitol at the laboratory with a concentration equivalent to: 1.0g, 2.0g, and 6.0g xylitol in 10 ml solution.

### Paraffin (Paper I-V)

The same kind of paraffin pieces (solidification point 46-48°C, Merck, Germany) was used for saliva sampling in Paper I to V and for chewing gum control in Paper II and III.

# Methods

## Study designs

### Paper I

The study had a prospective design with three parallel arms. The participants were randomly assigned into one of the following groups: group A (n=23) consumed 2 xylitol tablets 2 times a day; group B (n=23) consumed 2 tablets 4 times a day; and group C (n=10) served as control with no intake of xylitol tablets. Stimulated whole saliva and samples of dental plaque were collected at baseline and after 6, 12, and 18 weeks. After laboratory processing, the outcome measures were MS levels in saliva and plaque, proportion of X<sup>S</sup>/X<sup>R</sup> MS in saliva, and lactic acid formation rate in the dental plaque samples.

### Paper II

The study had a randomised single-blind crossover (Latin square) design (Fig. 6). At the initial visit, the participants were supplied with xylitol chewing gums and instructed to chew 6 pieces (≈4.0g) per day in a fractioned way (2 pieces in the morning, 2 pieces after lunch, and 2 pieces in the evening) throughout the entire 4 week study period. They were recalled for three test sessions (A, B and C). The subjects were randomly assigned to three groups testing single intakes of (1) paraffin (CTR; no xylitol), (2) low dose xylitol (LX; 2.0g xylitol), or (3) high dose xylitol (HX; 6.0g xylitol) in a randomised order with a washout period of one week.

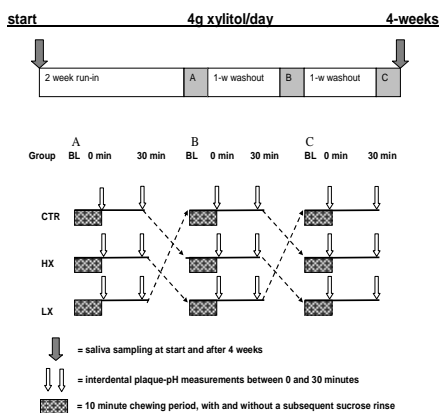


Fig.6 Study design Paper II.



### Paper III

The study consisted of two sets of experiments, A and B. The same study group was used for both experiments. Experiment A was performed with a single blind design and experiment B was performed with single blind crossover design.

*Experiment A:* The experimental products (a-f) were administered by one of the authors in single doses as follows:

- a) two pieces of xylitol chewing gum were chewed for 5 min;
- b) two pieces of a sucking tablet containing xylitol were melted during 5 min;
- c) two pieces of candy with xylitol were consumed during 5 min;
- d) tooth brushing with a xylitol-containing toothpaste for 2 min;
- e) rinsing with 10 ml of a neutral aqueous solution containing 1g xylitol for 2 min;
- f) two pieces of unsweetened paraffin that were chewed for 5 min (control).

*Experiment B:* The participants were told to avoid tooth brushing for 2 days in order to accumulate supragingival plaque. A baseline pooled plaque sample was gently collected and immediately thereafter the subjects were randomly assigned to rinse with aqua-solution containing xylitol or water only. Follow-up plaque samples were collected after 5, 15, and 30 minute. The experimental procedure was repeated twice according to the crossover design, separated by washout periods of two weeks.

Outcome measures for both set A and B were concentration of xylitol in saliva (A) and plaque (B) after intake of the different products.

### Paper IV

This study had a randomized, double blind design, performed on individual basis. Before the group allocation, all children were clinically examined and stratified as having or not having caries experience. The criteria for caries experience (CE) were one filled and/or decayed (in dentine) primary or permanent tooth surface or more. Those without caries experience were marked as “CF” and this procedure was done in order to secure an equal number of children with caries experience in the test and the control groups. After baseline clinical registrations and collection of salivary samples, the participants were instructed to chew on either test gums containing xylitol or control gums sweetened by sorbitol and maltitol three times a day for 4 weeks. The outcome measures were (1) salivary MS counts, (2) visible plaque score, and (3) lactic acid production in saliva.

## Paper V

The pilot study had a cross sectional design, and paraffin stimulated whole saliva samples were collected after a thorough mouth rinse with water approximately 3 hours after breakfast. The samples were immediately handled for determination of X<sup>S</sup> MS and uptake of radio labelled xylitol.

The main study had a randomized double blind prospective design with two parallel arms. The participants were instructed to chew on gums sweetened by either sorbitol/maltitol or xylitol three times a day for 4 weeks. Paraffin stimulated saliva samples were collected at baseline and after 4 weeks and 6 months. The outcome measures were MS and TVC counts in saliva, proportion of MS, and salivary uptake of radio labelled xylitol.

## **Clinical methods**

### Saliva sampling (Paper I-V)

#### Stimulated saliva (Paper I-V)

Paper I: The subjects were asked to collect 1ml of saliva in a test tube during paraffin chewing.

Paper II-V: After a thorough mouth rinse with tap water, the subjects were asked to collect 1-3 ml of saliva in a test tube during paraffin chewing. The time used for saliva collection was noted and the salivary secretion rate was calculated.

#### Unstimulated saliva (Paper III)

Initially, a professional tooth cleaning with pumice paste was carried out followed by a thorough rinse with distilled water. A baseline sample of unstimulated whole saliva was collected from the floor of the mouth with aid of a plastic pipette. The different experimental products were administrated by one of the authors in single doses, and the participants were told to consume the products for a designated time. After the designated time, any left over of the experimental products were spat out or removed. Follow-up samples of unstimulated saliva were collected at 1, 3, 8, 16, and 30 minutes after the use of each experimental product. The samples were collected by one of the authors without knowledge of which product that had been administrated before the sampling.

### Dental plaque sampling (Paper I & III)

In Paper I, samples of supragingival plaque were gently collected from the buccal surfaces of the upper front teeth adjacent to the bracket bases with the aid of a sterile explorer.

In Paper III, the participants were told to avoid tooth brushing for 2 days in order to accumulate supragingival plaque. Before the experimental session, they were not allowed to eat or to chew any gum for two hours. A baseline pooled plaque sample was collected from the buccal and interdental surfaces of anterior and posterior teeth with aid of a sterile blunt explorer. Immediately thereafter, the subjects were randomly assigned to rinse for 1 min with a xylitol solution (2g/10ml, 6g/10ml) or distilled water. Follow-up plaque samples were collected 5, 15, and 30 minutes after rinsing. This experimental procedure was repeated twice according to the crossover design, separated by washout periods of two weeks.

### Dental plaque registration (Paper IV)

Erythrosine rondells (Rondell Red, Nordenta, Sweden) were used to stain the teeth red. The patients were then asked to carefully rinse the mouth with tap water and the teeth were then examined to identify red-stained areas. The amount of plaque was determined with the Simplified oral debris index (DI-S) (Table 1) by Greene and Vermillion (1964). DI-S is one part of the Simplified oral hygiene index (OHI-S). After the dental plaque determination, the children were provided with a toothbrush and toothpaste and instructed to remove the stained plaque.

Table 1

The simplified oral debris index (DI-S), by Greene and Vermillion, 1964.

Score	Amount of plaque
0	no dental plaque
1	plaque <1/3 of the crown
2	plaque >1/3, but <2/3 of the crown
3	plaque >2/3 of the crown

### Plaque-pH measurements (Paper II)

These tests were identically performed at each session. After a thorough mouth rinse with tap water, the subjects were asked to chew on the assigned chewing gums for 10 minutes. The interdental plaque-pH was measured before chewing (baseline), directly after chewing (0 min), and then at 2, 5, 10, 15, 20, 25, and 30 minutes after chewing. Thereafter, the children rinsed their mouth with water and a new baseline pH was established. Then a new chewing period was performed and followed by a rinse with sucrose solution after which the pH measurements were carried out as above. The investigator who made the recordings was unaware of the chewing gum regimen. The pH in supragingival interdental plaque was measured at pre-selected proximal sites with aid of the Micro-touch method (Fig. 7) as described by Scheie et al. (1992) using a Beetrode pH microelectrode (NMPH5) and a reference electrode (DRIREF 5SH) from World Precision Instruments, Inc., Sarasota, USA. The pH meter (Model 340) was from Mettler Toledo AG Schwerzenbach, Switzerland.



Fig. 7 Measuring the interdental plaque-pH with the Micro-touch method (Scheie et al., 1992).

## **Laboratory methods**

### Bacterial cultivation and enumeration (Paper I-V)

The salivary samples were serially diluted in 10-fold steps with a potassium phosphate buffer with NaCl. Aliquots were placed in duplicate on BAO for detection of TVC and on MSB agar for enumeration of MS (Gold et al., 1973). The agar plates were incubated at 37°C under aerobic conditions for 48 hours, except in

Paper I where the plates were incubated for 72 hours for comparison with plates in the autoradiography method. The MS strains were identified by morphological characteristics and the number of colonies were counted with the aid of a stereomicroscope (10-30x magnification) and expressed as CFU per ml.

The plaque samples were weighed on a microbalance, dissolved in distilled water, and sonicated a few seconds for homogenisation before being processed as the saliva samples described above.

#### Quantification of xylitol resistant MS (Paper I)

The autoradiography method described by Dréan and Trahan (1990) formed the base for this analysis. A sterile cellulose nitrate filter was placed on a modified MSB agar containing 30% sucrose. The plate was then inoculated with aliquots of the plaque or saliva suspensions and cultivated in 72 hours in 37°C under aerobic conditions. The identified CFUs was distinctly indicated on the membrane filter with aid of a sharp needle in order to facilitate later identification. The membrane was transferred to the lid of the Petri dish and washed thoroughly in phosphate buffer and finally in a neutral Ringer solution without calcium. After gentle removal of excess fluid, the membrane filter was replaced in the dish and covered with a 00H-filter paper. 1.0ml of a radio labelled Ringer-phosphate solution was then added, containing 0.1 mCi/ml of [<sup>14</sup>C]-D-xylitol, followed by incubation in 37°C for 15 minutes. The filter paper was then carefully removed and the cellulose membrane with the bacterial growth was washed in an equal mix of formalin and phosphate buffer. The dried membrane was placed on an x-ray film and exposed for 24, 48, and 72 hours in -80°C. The films were processed and the number of CFUs with clear xylitol uptake (X<sup>S</sup>MS) was related to the previously indicated colonies.

#### Quantification of xylitol uptake in saliva (Paper V)

In order to quantify xylitol uptake, 1.0ml of saliva and 10µl of a radio labelled isotope (ARC 1744 Xylitol, D(1-<sup>14</sup>C) conc. 0.1 mCi/ml, Larodan Fine Chemicals AB, Malmö, Sweden) were incubated together for 60 min at 37°C. After centrifugation (13,200 rpm for 10 min), the supernatant was carefully separated from the pellet and both were kept frozen until further processing. For the analyses, the thawed pellet was suspended in 100µl MQ water and placed into a scintillation tube while 100µl of the supernatant was placed directly into the tubes. 5ml of a liquid scintillation cocktail (Ready Safe, Beckman, Sweden) was added and

the disintegration was measured with an LKB 1214 rackbeta liquid scintillation counter (LKB-Wallac, Turku, Finland) for one minute and expressed as CPM. The xylitol uptake in saliva was calculated as the ratio between the CPM in the pellet and the combined supernatant/pellet counts and expressed as percent.

#### Xylitol concentration (Paper III)

The xylitol determinations were carried out enzymatically using the polyol dehydrogenase based kits from Boehringer Mannheim (R-Biopharm AG, Germany) and a spectrophotometer (Ultrospec 100 PRO, Amersham Biosciences, Uppsala, Sweden). The xylitol concentration was expressed as mg/ml of the saliva and plaque suspensions. The precision of the analytical method was tested against standard solutions and the variation was less than  $\pm 3\%$  in the baseline range ( $\approx 0.1$  mg/ml) and  $\pm 10\%$  in the range of 30-40 mg/ml.

#### Lactic acid assay (Paper I & IV)

The method for determination of lactic acid concentration differed in the papers. In Paper I, plaque samples were used and in Paper IV the concentration was assessed in whole saliva samples.

In Paper I, acid production was initiated by mixing equal volumes of the plaque suspension with a MES reactive solution (3-[N-morpholino]ethanesulfonic acidhydrat, pH 5.5), containing 2% sucrose that was incubated for 20 minutes in 37°C and then stored on ice for additional 5 minutes. The fermentation was stopped by centrifugation and the supernatant was withdrawn and stored frozen until further analyses. After thawing, L- and D-lactic acid concentration was determined enzymatically in a spectrophotometer and expressed as formation rate,  $\mu\text{mol/g/min}$ .

In Paper IV, the determination of lactic acid was made using 0.5ml saliva pipetted into two tubes, and acid production was initiated when sucrose was added to the tubes. The tubes were incubated for 30 and 60 minutes respectively in 37°C. The fermentation was stopped by centrifugation and the supernatant was withdrawn and stored frozen until further analyses. After thawing, L- and D-lactic acid concentration was determined enzymatically in a spectrophotometer and expressed as formation rate,  $\mu\text{mol/g/min}$ .

## **Statistical analysis**

### Paper I

The data were subjected to analysis of variance for repeated measures. Bacterial counts were transformed into log 10 units before statistical analysis.

### Paper II

The area under the pH-curve (AUC) above pH 6.0 was calculated and compared between the groups with ANOVA. Data on bacterial counts were subjected to ANOVA in order to test differences between baseline and the 4-week follow-up.

### Paper III

Follow-up data were compared with baseline using ANOVA for repeated and pairwise measures. Tukey's post-hoc test was applied.

### Paper IV

The index for dental plaque were scored and categorised before evaluation with chi-square tests. Bacterial and biochemical data were subjected to analysis of variance (ANOVA) or Student's t-test.

### Paper V

In the pilot study, the scores of the two assays were compared with a chi-square test. In the field study, bacterial and biochemical data were subjected to analysis of variance (ANOVA) or Student's t-test.

The level of statistical significance was in all studies set at 5% ( $p < 0.05$ ). All statistical analysis was processed with aid of SPSS software (version 11.5 and 12.0 Chicago, Ill, USA).

## Results

### MS counts, X<sup>S</sup> MS and lactic acid formation in dental plaque (Paper I)

After 6 weeks, a statistically significant ( $p < 0.05$ ) short-term drop of the MS counts was found in the saliva samples of group B (3.4g xylitol). In group A (1.7g xylitol) and C (control), the salivary MS counts were not significantly altered compared to baseline ( $p > 0.05$ ), and the plaque levels remained statistically unchanged in all groups during the study period. Compared with baseline, a statistically significant ( $p < 0.05$ ) reduction of the number of X<sup>S</sup> MS in group A and B was disclosed after 6, 12, and 18 weeks of habitual consumption of xylitol-containing lozenges, whereas no such reduction was shown in the control group. There was, however, no difference between the two xylitol groups (Fig. 8). A slight decrease of the lactic acid formation rates was seen in the two xylitol groups compared with baseline, but the differences were not statistically significant ( $p > 0.05$ ).

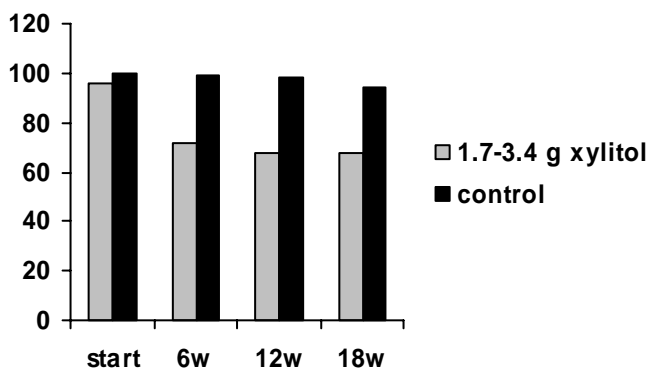


Fig. 8 A statistically significant ( $p < 0.05$ ) reduction of X<sup>S</sup> MS was shown in the xylitol groups but not in the control group.

### Interdental plaque-pH (paper II)

Without subsequent sucrose rinse, the 10 minute chewing period increased the interdental plaque-pH in all groups, but the elevation was highest in the high xylitol (HX) group. The difference between the HX and the control group was statistically significant ( $p < 0.05$ ) during the first 5 minutes after chewing with respect to the



AUC. Conversely, when the chewing was followed by a sucrose rinse, the plaque-pH dropped in all groups although less pronounced in the HX group. A statistically significant difference between the HX and LX groups during the initial 5 minutes after the sucrose challenge was observed ( $p < 0.05$ ) (Fig. 9).

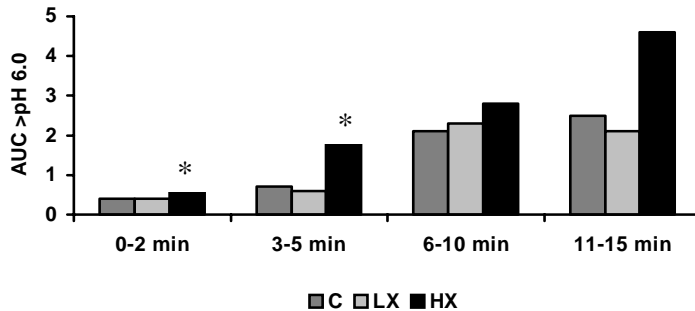


Fig. 9 AUC (over pH 6.0) in 15 minutes after 10-minute chewing of gum with contrasting amounts of xylitol followed by 1-minute rinse with sucrose solution. Group C = paraffin, Group LX = 2g xylitol and Group HX = 6g xylitol, ( $n=11$ ). Stars denote statistically significant differences ( $p < 0.05$ ).

### Xylitol concentration in saliva and plaque (Paper III)

Statistically significant elevations of salivary xylitol levels were demonstrated for all products during the first 8-16 minutes when compared with baseline ( $p < 0.05$ ). The highest 1 minute post ingestion values in saliva were found after chewing gums and the lowest levels were obtained after toothpaste. No significant differences were demonstrated between chewing gums, sucking tablets, candy, and rinses at any sampling time (Fig. 10). The baseline mean values of the xylitol concentration in dental plaque ranged between  $0.1 \pm 0.1$  mg/ml and  $0.5 \pm 0.8$  mg/ml. A dose-response relationship was demonstrated as a statistically significant ( $p < 0.05$ ) increase after 5, 15, and 30 minutes with the higher amount (6.0g) and after 5 and 15 minutes with the lower amount (2.0g). At the 5 minute follow-up, the mean xylitol concentration was  $8.6 \pm 5.4$  mg/ml following the high xylitol rinse and  $5.1 \pm 4.0$  mg/ml after the low xylitol rinse ( $p=0.08$ ). The corresponding values after 15 minutes were  $5.4 \pm 4.1$  and  $1.4 \pm 0.9$  mg/ml respectively ( $p < 0.01$ ) (Fig. 11).

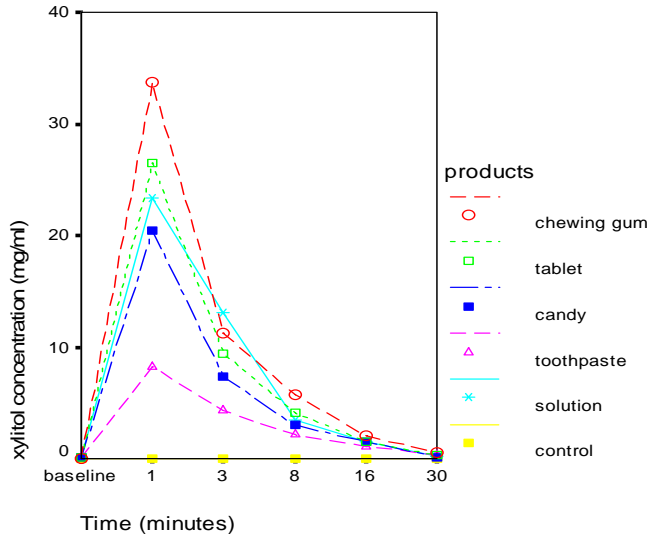


Fig. 10 Xylitol concentration in saliva after intake of different xylitol products.

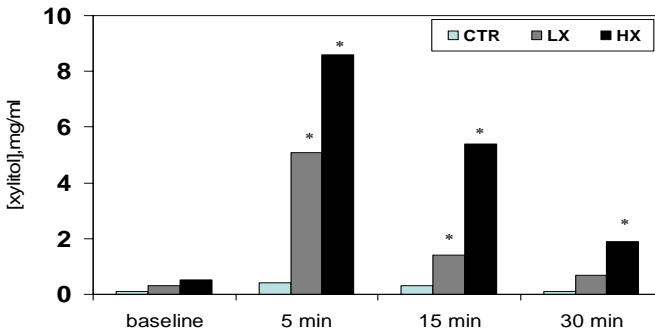


Fig. 11 The dose-response relationship in dental plaque demonstrated after rinsing with a high (6.0g) and a low (2.0g) xylitol-containing solution. \* denotes a statistically significant difference ( $p < 0.05$ ).

### MS, lactic acid formation and amount of plaque (Paper IV)

The mean caries prevalence (DMFS+dmfs) for the total study group was  $1.2 \pm 2.3$ , and the corresponding value for those with caries experience (CE;  $n=40$ ) was  $3.8 \pm 2.7$  (DMFS  $1.4 \pm 1.0$  and dmfs  $2.4 \pm 1.7$ ). The salivary secretion rate varied from 0.5 to 2.3 ml/min. The mean percentage of the proportion of MS in relation to TVC dropped in group B from 1.2% at baseline to 0.45% after 4 weeks; this difference was statistically significant ( $p < 0.01$ ) (Fig. 12). The MS/TVC-ratio differed also between groups A and B after 4 weeks and there was a statistically significant difference ( $p < 0.05$ ). There was a significant reduction in salivary MS counts after 4 weeks among the caries-free children in the xylitol group B ( $p < 0.05$ ), but this was not the case in any of the other subgroups. A statistically significant reduction ( $p < 0.05$ ) in the percentage distribution of the baseline and immediate post-treatment plaque scores was disclosed in both the xylitol (B) and the control (A) group after 4 weeks when compared to baseline. The number of sites with no visible plaque (score 0) increased in both groups. There was no difference between the groups or between children with and without caries experience after the 4 week study period. The baseline levels of lactic acid in the sucrose-challenged whole saliva samples were significantly reduced by approximately 25% ( $p < 0.05$ ) after 4 weeks in both the xylitol and the sorbitol/maltitol groups.

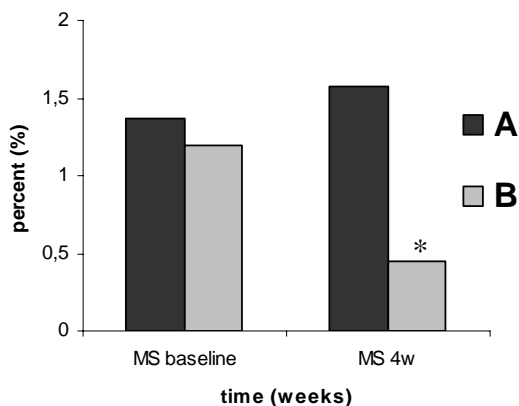


Fig 12 The mean percentage of the proportion of MS in relation of TVC. Group B showed a statistically significant difference ( $p < 0.01$ ) from baseline to 4 weeks.

### Xylitol uptake in saliva (Paper V)

There were no significant differences in mean values from baseline to 6 months of salivary MS and TVC in either of the groups. The proportion of MS in relation to TVC is shown in Figure 13. In group B, the mean percentage dropped from 1.2 at baseline to 0.4 after 4 weeks ( $p=0.01$ ). After 6 months, the mean percentage had increased to 0.7 ( $p>0.05$ ). The MS/TVC-ratio differed also between group A and B after 4 weeks with statistically significant difference ( $p<0.05$ ).

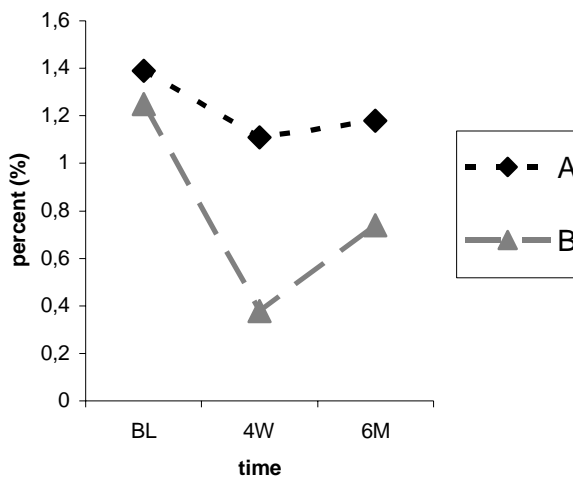


Fig. 13 Proportion (%) of MS in relation to TVC in group A (sorbitol/maltitol) and group B (xylitol) ( $p<0.05$ ).

The baseline mean values of bacteria with xylitol uptake varied between 27.4% and 31.4%, and there was no difference between the test and control group. After 4 weeks, this proportion showed a statistically significant decrease ( $p<0.05$ ) in the xylitol group (B), but not in the control group (A). Six months after the intervention ended the proportion was higher than at baseline.

## Discussion

The various investigations in the present thesis were undertaken in order to seek some answers to clinical questions regarding xylitol, and the findings will be discussed accordingly and in the light of what is known about its mechanisms of action.

### Does xylitol reduce the prevalence of MS?

This question was addressed in Papers I, IV and V. The thinking behind a selective effect on MS is mainly based on the findings by Trahan et al. (1985), who established that xylitol was transported and phosphorylated through the cell wall by a constitutive fructose-PTS (phosphotransferase) system. Thus Wåler et al. (1992) showed that dental plaque suspensions could take up xylitol and accumulate xylitol-5-phosphate and this and subsequent intracellular accumulation may be toxic for selected strains of *S. mutans* (Trahan et al., 1985). In a study from 1986, Assev and Röllä reported that a specific *S. mutans* strain can expel xylitol after an intracellular dephosphorylation of xylitol-5-phosphate, a “futile cycle”; however, this may only work as long as there is energy available for the metabolic process. Söderling and Pihlanto-Leppälä (1989) observed an expulsion of intracellular xylitol-5-phosphate, such as xylitol in *S. mutans* 25175. They also established that a two-step energy-dependent process takes place. Xylitol-5-phosphate is first hydrolysed to xylitol and inorganic phosphate. Next xylitol is expelled from the cells (Pihlanto-Leppälä et al., 1990).

In the present series of investigations, a short-term drop in the salivary levels of MS was disclosed after a daily intake of tablets with a comparatively low dose of xylitol (Paper I) as well as temporarily reduced proportions of MS in saliva after a 4 week chewing gum period (Papers IV and V). In Paper I, no effect on the MS growth in plaque was found and the clinical significance of this transient reduction in saliva may be questioned. The findings agree with previous reports by Scheie et al. (1998) and Giertsen et al. (1999), but they are somewhat in conflict with other studies in which more dramatic effects on MS growth in plaque and saliva has been disclosed in children (Mäkinen, 1998), orthodontic patients (Isotupa et al., 1995) and in adults (Milgrom et al., 2006). However, another study similar to the design of Paper I has reported a significant decrease in amount of dental plaque but no reduction in MS (Söderling et al., 1997). Based on the collective findings, it may be speculated whether the anticipated effect of xylitol on MS is based on quality and function rather than on the quantity as measured by routine microbial cultivation. In any

case, no long-term suppression of oral MS could be demonstrated in the present thesis.

### Does the use of xylitol select for “less cariogenic” X<sup>R</sup> MS strains?

This is perhaps the most controversial and, at the same time, the most interesting question concerning the anti caries effects of xylitol. The two main phenotypes of MS can be recovered from the oral cavity: (1) the predominant strains with capacity of taking up xylitol (X<sup>S</sup>), and (2) less prevalent mutant types (X<sup>R</sup>) that cannot transport xylitol into the cell due to lack of or low cell wall PTS-activity. Because the mutants are not inhibited by xylitol, a long-term exposure may give an ecological advantage to the X<sup>R</sup> MS strains (Trahan, 1995). This may then lead to an increased proportion of X<sup>R</sup> MS in dental plaque. This “overgrowth” may be beneficial since these strains are considered to be less adhesive than the sensitive strains. Consequently, it has been postulated that X<sup>R</sup> strains are less cariogenic than X<sup>S</sup> strains (Trahan, 1995), but this has not been convincingly verified (Assev et al., 2002).

The relationship between X<sup>S</sup> MS and X<sup>R</sup> MS was investigated in Paper I and to some extent in Paper V. In Paper I, a significant shift in the proportion of X<sup>S</sup> and X<sup>R</sup> strains of MS in saliva was disclosed with an autoradiography method described by Dréan and Trahan (1990). In Paper V, a simplified way of estimating the proportion of salivary bacteria with ability to take up and harbour intracellular xylitol was tested. The main objective for this was to find a simple and less time consuming method suitable for use in field trials, although it should be emphasized that the two assays by no means should be directly compared with each other. In Paper I, a statistically significant decrease in the proportion of MS with [<sup>14</sup>C]-xylitol uptake was revealed, but most interestingly this shift did not seem to be dependent on xylitol concentration. The xylitol-induced effect obtained with both the “low” and “mid” concentrations were quite similar in magnitude. The design of Paper V did not allow any evaluation of the dose-response event. Moreover, whether or not the duration of the microbial shift is affected by concentration is not known. Based on Paper V, the X<sup>R</sup>/X<sup>S</sup> ratio was back to baseline after 6 months with the high-dose regimen and there are no reasons to think that it would exceed that period with the lower doses.

The observation that oral ecological shifts may take place in spite of comparatively low levels of xylitol was highly interesting in the light of the “mother-child” studies by Söderling et al. (2000) and Thorild et al. (2003; 2004; 2006). In both investigations, the effect of maternal chewing of xylitol gums on the vertical

transmission of MS was evaluated as well as the effect on caries development during the pre-school years. Both trials delayed MS colonisation and the caries incidence was significantly lower in children whose mothers chewed xylitol gums during the period of primary tooth eruption. This effect was achieved with only 1-3g of xylitol per day and was partly explained by a reduced adhesiveness of the maternal MS that compromised the transmission. The findings of the present thesis may lend some support to that speculation. On the other hand, a recent project that gave preschool children a low daily xylitol dose (0.5-1.0g) found no effect on MS colonisation (Oscarson et al., 2006). Interestingly, the commonly displayed positive relationship between MS prevalence in mothers and their offspring (Thorild et al., 2002) could not be demonstrated in the xylitol group, a finding that was in clear contrast to the controls. Furthermore, Meurman et al. (2005) have recently shown a positive relationship between the frequency of xylitol intake and the percentage of X<sup>R</sup> MS, suggesting that the actual dose might be less important than the frequency.

Although a xylitol microbial shift was demonstrated in the present thesis, the question whether or not the increased proportion of X<sup>R</sup> bacteria in saliva was less cariogenic or of any clinical benefit remains to be elucidated.

#### How much xylitol is present in the oral cavity after intake and use of oral products?

This question was addressed in Paper III in which the levels of xylitol in saliva and dental plaque were determined after the use of some commonly advocated dental products. The findings were clear-cut. The mean xylitol concentration in whole saliva increased immediately after intake to levels that theoretically can execute an antibacterial effect (Tapiainen et al., 2002). The duration of the elevated values seemed mainly to depend on the amount of xylitol in the different products and lasted for a maximum of 8-16 minutes. From an antibacterial point of view, however, it is not clear whether a high peak concentration of xylitol is preferred over an extended time of slightly elevated levels. Furthermore, the influence of the various administration forms needs to be further investigated. In the present study, the highest concentrations were achieved after the chewing gums, a finding that was somewhat remarkable as chewing stimulates the saliva secretion and oral clearance the most. Another notable finding was the “low” peak concentration after the toothpaste, but due to the slow clearance the 8-minute follow-up was fully comparable with the candy that initially contained ten times more xylitol. Therefore, a study that focused on a fixed amount of xylitol in gums, rinses, candies, and tablets would be helpful to elucidate this issue.

The concentrations of xylitol in dental plaque after rinses with contrasting concentrations of xylitol were also measured in Paper III. The main findings were that the xylitol levels were of the same magnitude as in whole saliva after 5 minutes and they remained elevated for a much longer period than in saliva. This is likely of clinical importance since it indicates that the diffusion of xylitol into the supragingival plaque is rapid and that the plaque bacteria may be influenced by xylitol for extended periods. In Paper III, this period exceeded 30 minutes, but unpublished data from our laboratory has recently demonstrated significantly increased xylitol concentrations in young supragingival plaque up to 1 hour after 5 minutes of chewing on chewing gums.

In both the saliva and the dental plaque samples, a considerable individual variation was obtained concerning the xylitol concentrations. The variation was expected for the plaque samples as these were pooled from several labial and interdental sites and the follow-up samples could not be sampled from exactly the same sites. Moreover, in spite of the 2-day restriction on tooth brushing, some of the participants exhibited limited amounts of visible plaque. The great individual variation in the saliva was less expected and may be explained by factors such as secretion rate, consistency of saliva, and the “intensity” in which the subjects handled the xylitol products in their mouth. The analytical method was quite stable, and the reliability was checked with double determinations of selected samples.

### Does xylitol reduce the amount of dental plaque?

The simple answer obtained in Paper IV was “yes”, but the fact that the children participated in a clinical trial could of course have influenced the improvement in oral hygiene. Even before the “Turku sugar studies”, information suggested that xylitol could reduce the amount of dental plaque. The mechanism behind this is probably due to the extracellular polysaccharides that are produced by oral and cariogenic bacteria. In the presence of xylitol, the polysaccharides become more soluble, leading to a reduced plaque mass and diminished adhesiveness, resulting in a reduced plaque volume on the tooth surfaces. An early study (Scheinin and Mäkinen, 1971) showed that a partial substitution of the sucrose in the diet by xylitol for 4 or 5 days reduced the amount of dental plaque. Söderling et al. (1989) found, in a comparative study with three groups of seven adults on a regimen of xylitol, sorbitol, or xylitol/sorbitol chewing gum, that the xylitol-containing gums decreased the amount of plaque, whereas the sorbitol gum resulted in a significant increase in the amount of dental plaque. In the present series of investigations, the amount of erythrosine stained plaque was evaluated in paper IV. A statistically significant reduction of the amount of visible plaque was disclosed in the xylitol



group after a 4 week period of chewing when compared to baseline, but this was also the case in the control group that used gums sweetened with sorbitol and maltitol. The participating children had on average relatively high scores at baseline; it should be emphasised that plaque reduction was not only statistically significant but also a clear improvement from a clinical point of view. Because no non sweetened control gum was used, it was not possible to separate the pure “mechanical” effects of tooth brushing, chewing and saliva stimulation from the sugar alcohols. Any conclusions on an exclusive xylitol effect on the plaque amount based on the present thesis would be premature. In the literature, however, reports on the beneficial effects of xylitol on dental plaque volume are frequent and predominant (Loesche et al., 1984; Söderling et al., 1989; Söderling et al., 1997; Mäkinen, 2005; Shyama et al., 2006), although opposite findings also are available (Scheie et al., 1998; Giertsen et al., 1999).

### Does xylitol reduce the acidogenicity of oral bacteria?

This question was addressed in a direct way in Paper II by interdental pH measurements and indirectly in Papers I and IV by sugar induced fermentations of collected samples of plaque and saliva. The mechanism of action is commonly explained by the futile xylitol cycle and previous studies have generally suggested that the reduced acid production is the key anti-caries mechanism of xylitol. Intracellular xylitol-5-phosphate cannot be metabolized and used for acid production (lactic, acetic, and propionic acid) by a variety of oral bacteria (Trahan, 1995; Assev et al., 1996). This finding is confirmed in studies with both laboratory and clinical strains of mutans streptococci (Trahan and Mouton, 1987; Trahan et al., 1991).

Previous studies have investigated the effect of xylitol in mouth rinses, chewing gums, and lozenges on the pH in sucrose-challenged dental plaque with conflicting findings (Söderling et al., 1989; Aguirre-Zero et al., 1993; Lingström et al., 1997; Koparal et al., 2000). The majority of the studies suggested a certain advantage for the polyol, but others found minor or no effects in the pH response to sucrose (Lingström et al., 1997; Giertsen et al., 1999). The anti-acid effect is especially true when xylitol is the only energy source available, but one catch is the fact that many other carbohydrates are available in real life situations. Consequently, a number of studies have failed to unveil a xylitol-hampered acidogenicity in vivo (Wennerholm et al., 1994; Scheie et al., 1998; Giertsen et al., 1999). The reasons for the contrasting findings are not fully clear, but some factors (e.g., administrative vehicle and regimen, carbohydrate availability, the individual plaque composition, and laboratory processing) may be influenced by xylitol dosage.

In Paper II, the above complexity was clearly illustrated. A 6.0g single dose of xylitol had a short but limited effect on the interdental plaque-pH, whereas a more “normal” dose (2.0g) did not differ from paraffin controls in a group of habitual xylitol consumers. The outcome in paper II was almost in perfect harmony with the results presented in Papers I and IV where the acid production rate was measured “ex vivo” in freshly collected samples of plaque and saliva after addition of sucrose. A slight but statistically non significant effect was seen in suspensions of dental plaque subjected to low and moderate amounts of xylitol ( $\leq 3.4\text{g}$ ); however, when the daily dose was increased to 6.2g per day, the decrease reached statistical significance. The present findings are strongly supportive of Milgrom et al. (2006) who suggested a dose-response to xylitol chewing up to around 6g followed by a plateau effect up to 10g. The question in this paragraph could simply be answered with “yes”, but the clinical implication from this series of studies is that if one wants to gain from the reduced acidogenicity over the long-term perspective, the intake of xylitol must be quite substantial irrespective of whether it is used in a single dose regimen (Paper II) or in a fractioned way (Paper IV).

Is there a dose-response relationship?

This issue has already been discussed above and as indicated the answer may vary by the type of action. When this thesis finding and other findings were compiled in the table below, an interesting pattern emerged.

Table 2.

outcome measure	beneficial ... for health	dose-related effect demonstrated	
		in this thesis	by others
<b>“pathological side”</b>			
decreased acidogenicity	X	yes	yes
reduced plaque volume	X	NA	yes
caries lesion development	X	NA	yes
<b>“protective side”</b>			
reduced MS	X	no	yes (partly)
increased X <sup>R</sup> MS proportion	?	no	NA
mother-child MS transmission	X	NA	no

NA = not addressed

To achieve a threshold level needed to obtain significant clinical effects, 5-6g per day in fractionated doses is required (Ly et al., 2006). This is probably true when looking on xylitol as a substitute for fermentable carbohydrates on the pathological side of the caries balance, while the antibacterial capacity on the protective side of the balance seems to be less dose dependant. Thus a speculation from a clinical point of view is that less xylitol is needed to affect bacterial composition and adhesiveness compared to the amounts needed to diminish fermentation.

### Tablets or chewing gums?

The present series of studies were not designed to answer this specific question although common sense probably would advocate products that encourage chewing and saliva stimulation. Based on experiences from these and other ongoing investigations, the compliance was better with the gums and the complaints were fewer. Moreover, chewing gum is more or less an accepted habit for most ages all around the world. A common thinking is that the products should have xylitol as single sweetener since it has been claimed as most effective in preventing caries and to promote remineralisation (Mäkinen et al., 1995). With this in mind, one should remember that the availability of pure xylitol products for the average customer is limited on the Swedish market. In Paper IV, no dramatic differences were disclosed between the xylitol test and the control gums containing sorbitol/maltitol, yet a small advantage was given to the test gums. Candy containing xylitol can also be an effective caries preventive measure as confirmed in a study from Estonia where school children were given xylitol candy or chewing gums at school for 3 years. The caries increment was decreased with 61% and 57% in the candy and chewing gum group respectively (Alanen et al., 2000). Thus the various types of gums, tablets, candies, and lozenges available on the market provide the therapists a wide range of choices to find the best suitable and acceptable solution for each patient. The possibility to change products and, most important, to alter the flavour are important for the individual during a long-term use of xylitol. Noteworthy, no serious side effects or adverse advents were experienced with any of the commercial xylitol products used in the present thesis.

One major obstacle with the use of both gums and candies for xylitol administration is high frequency and the rather large number of pellets that are required to deliver the therapeutic amounts. In addition, the costs for a long-term use could be a barrier. Therefore, novel low-cost delivery systems for xylitol should be developed and especially targeted to various ages (Featherstone, 2006).

### Can xylitol be regarded as a public health measure?

According to a recent review of randomized field trials and observational studies, it was concluded that the evidence is strong enough to support the regular use of xylitol sweetened gum as a way to prevent caries and it can be promoted as a public health measure (Burt, 2006). One problem with that statement is that it is partly based on clinical trials that do not meet the inclusion criteria of most Health Technology Assessment agencies such as the Cochrane Collaboration and the Swedish Council on Technology Assessment in Health Care (SBU; Lingström et al., 2003). As a comparison, SBU stated in a systematic review published in 2002 that the evidence for xylitol in caries prevention was insufficient and inconclusive. Efficacy, however, may not be equal to effectiveness and this is highly dependant on the prevalence of caries in the population. A drawback is that few xylitol studies have been conducted in truly selected risk patients except from the trials performed in the high caries community of Belize (Mäkinen et al., 1995). The most critical question for any public health intervention is the cost effectiveness – the number needed to treat or the amount or costs of a therapy to avoid one “event”. A brief calculation based on the successful Estonian chewing gum trial by Alanen et al. (2000) revealed that approximately 1,500-2,000 pieces of chewing gum would be needed to avoid one decayed surface. Thus the direct cost only for the gums would likely exceed 200 Euro in this “best scenario” and it should be remembered that several other recent trials have reported a less favourable outcome. The lesson learned from the use of fluoride in public health may be helpful also for xylitol. For example, the concept of using “xylitol-based” rinses in outreach school programs in immigrant or low-income communities would be interesting approaches. A slow-release device suitable for individuals with caries risk may also be useful. Xylitol may very well be the intervention of choice in early childhood caries or in low-motivated adolescents, but clinical studies with high level of evidence on that topic are difficult and expensive to implement and complete. In our research group, there are two ongoing projects that may increase the knowledge on the high risk issue. In the meantime, it is preferred to consider xylitol as a targeted measure for individuals rather than a public health measure in Sweden.

### Clinical recommendations

In concert with previous research, the present thesis has demonstrated that xylitol has antibacterial properties that may alter the ecology in the oral cavity. Although no trial with clinical caries as outcome measure was presented here, the findings could partly be used for the following clinical recommendations:

- patients at caries risk could be recommended to use xylitol products as a complement to the daily exposure to fluoride;
- at least 6g of xylitol/day is needed to counteract acidogenicity and benefit from the anti-caries properties;
- xylitol products that actively stimulates saliva secretion, such as chewing gums, are the first choice;
- the products should contain as much xylitol per unit as possible and preferably as the single sweetener;
- the daily intake should be fractioned at least 3 times over the day (for example, 2 pieces of chewing gum directly after breakfast, lunch, and dinner);
- the chewing period should not be shorter than 10 minutes.


## Conclusions

The main conclusions from this thesis are as follows:

- a transient shift of MS strains in saliva was demonstrated following a regular daily intake of xylitol.
- a 6g daily dose of xylitol could alter salivary microbial composition during the intervention period, but no long-term impact was observed 6 months after xylitol intervention.
- a 6g single dose of xylitol had a short and limited beneficial effect on interdental plaque-pH.
- xylitol-containing products gave elevated concentrations of xylitol in saliva and dental plaque for at least 8 minutes after intake.
- a 6g daily dose of xylitol reduced dental plaque and lactic acid production in saliva in schoolchildren after chewing for 4 weeks.

## Acknowledgements

This thesis is something strange, nothing that I ever thought I would do. Without the help and support from everybody around me, especially the one as follows, it would not have been possible.

First, my supervisor **Svante Twetman**, I haven't got words for your never ending enthusiasm in research, always having new projects on your mind. You have taught me that there are some things that are more important than research – first it is one's family and then it's skiing, preferably down hill...  You are so cool... and I promise I will “tagga ner” (some day!).

My co-supervisor **Christina Stecksén-Blicks**, thanks for reading my manuscripts and in a kind way pointing out what had to be changed. You have taught me to think in a critical way and I'm very grateful for all help and advice you have given me!

My supervisor at the laboratory, **Inger Sjöström**, what would I do without you? You have introduced me to a world which I never thought I would be part of, it has been a pleasure working with you and I envy the one that will replace me! You have a great experience and is very clear-sighted! Thanks for honey and apples – I know where your house is – only one minute away from home!

**Hjördis Olsson**, thanks for data support and support with different paperwork. Your help can not be too highly praised!

**Hans Stenlund**, thank you for statistical help and advice.

**Sotos Kalfas**, co-author, thank you for good collaboration and for answering my questions regarding microbiology in an easy way.

**Karin Sunnegårdh-Grönberg**, for being a very nice colleague, former (and coming) room-mate and caring friend, with lots of advice and experience of the world as PhD-student (and the rest of the world too...).

**Malin**, my dear friend and colleague. Thank you for listening to my presentations, being nervous, sometimes more than me? Giving me courage to go on when I wanted to quit. I'll listen to your presentations, I promise, but first tea and sugar cake!

Without the every day different weird topics, silly talks and great laughs from the “fikarum” at the research floor, I wouldn’t have survived. **Elisabeth, Mari(a), Kjell, Mari, Hrishi, Nina, Liza, Karina, Mirva, Anna, Anette, Ulla, Rolf, Emma, Inger, Eva, Hjördis, Maggan, Christer.** Thank you!

**Monika Öberg**, co-author, thank you for good collaboration and for providing me with suitable participants to study II and III.

**Britt Bylund, Martin Olsson and Kerstin Sköld-Larsson**, co-authors, thank you for good collaboration!

**Anitha Persson**, thanks for nice talks about everything and nothing! Our Stephan curves can only increase nowadays!

**Ingegerd Johansson**, thank you for the pushes in the right direction when I wanted to start my PhD-studies.

**Carin, Ingrid, Birgitta, Eva, Catharina and Pamela**, dentists at Paediatric Dentistry. Thanks for your interest in what I’m doing!

**Britt-Sofie and all dental nurses at the clinic of Paediatric Dentistry**, thanks for help regarding clinical things as mirrors, probes, tweezers, toothbrushes, rooms and chairs!

**Everybody at the clinic of Cariology and Dental hygienist education**, 7<sup>th</sup> floor, THU. When I think of you, it feels like home!

All **children** that participated in the studies, their **parents** and their **teachers** – here comes “tuggummi tanten” - thank you for your participation and keep on chewing!

**The Swedish Dental Society, the Patent Revenue Fund and the County Council of Västerbotten**, thank you for the financial support.

**Paula Matala and Fennobon Oy**, thank you for providing me with chewing gums to the studies.

My family, **Mats, Oscar and Filip**, my **father Erik** and **mother Vivi-Ann**, my parents-in-law, **Ove and Birgitta** thanks for all support and patience with a sometimes really tiresome wife, mother, daughter and daughter-in-law, only thinking about xylitol and chewing gum... I love you!



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