

**MICROBIOLOGICAL EFFECTS
AND CLINICAL USE OF
XYLITOL IN PREVENTING
ACUTE OTITIS MEDIA**

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CLINICAL USE OF XYLITOL IN
PREVENTING ACUTE OTITIS MEDIA**

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium of the Department of Paediatrics, on August 23rd, 2002, at 12 noon.

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Abstract

The purpose was to evaluate the microbiological mechanism of action of xylitol and to assess its use in clinical practice for preventing acute otitis media (AOM).

To test whether the effect of xylitol on *S. pneumoniae* is inhibited by fructose, a total of 20 strains of *S. pneumoniae* were exposed to xylitol in the presence of fructose and other carbon sources. Addition of 5% xylitol to the media resulted in marked growth inhibition, an effect which was totally eliminated in the presence of 1%, 2.5% or 5% fructose but not in the presence of 1% or 5% glucose, 1% galactose or 1% sucrose. The inhibition of pneumococcal growth is probably mediated via a fructose phosphotransferase system in a similar manner to that seen in mutans streptococci. Sorbitol alone did not affect the growth of pneumococci, and thus sorbitol is unlikely to provide any clinical benefit in the prevention of AOM.

To evaluate the effect of xylitol on the ultrastructure of *S. pneumoniae* and *Haemophilus influenzae* (*H. influenzae*) and on the pneumococcal phenotype, five strains of *S. pneumoniae* and one strain of *H. influenzae* were examined by electron microscopy after xylitol exposure. Xylitol damaged the ultrastructure of the pneumococci. Some of the bacteria were lysed and the cell wall of the remaining ones became more diffuse and the polysaccharide capsule was ragged. The resulting morphology was identical to that of the transparent pneumococcal phenotypic variant. The properties of the transparent variants of pneumococci could explain the clinical efficacy of xylitol in preventing AOM despite the lack of effect on the nasopharyngeal carriage of pneumococci. The cell wall of *H. influenzae* became slightly thicker, but the morphology remained otherwise unchanged.

To evaluate the pharmacokinetics of xylitol locally in the nasopharynx, xylitol concentrations were measured in the saliva of 65 children by enzymatic assay after giving them xylitol chewing gum or syrup at doses equal to those used in clinical trials. Concentrations high enough to have an antimicrobial effect were attained, but the xylitol disappeared from the saliva within 15 minutes, which indicates that high peak concentrations may be more important for efficacy than the time for which the concentration exceeds the level needed for an antimicrobial effect.

To find a more convenient dosing regime for xylitol prophylaxis, xylitol was administered to 1277 children only during an acute respiratory infection (ARI) in a randomised placebo-controlled trial. The occurrence of AOM during ARI was 34/166 (20.5%) in the xylitol mixture group as compared with 32/157 (20.4%) among the children receiving the control mixture. Among older children receiving control chewing gum, xylitol chewing gum or xylitol lozenges, AOM was experienced by 24/218 (11.0%), 31/220 (14.1%) and 34/219 (15.5%) respectively. None of the differences between the groups was statistically significant. Xylitol should be used continuously in AOM prophylaxis, as it proved ineffective when used only during URI.

Keywords: Otitis media, Xylitol, Streptococcus pneumoniae, Child

To the memory of Leevi Luotonen

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Oulu, June 2002

Terhi Tapiainen

Abbreviations

AOM	acute otitis media
ARI	acute respiratory tract infection
CI	95 % confidence interval
ET	Eustachian tube
<i>H. influenzae</i>	<i>Haemophilus influenzae</i>
<i>M. catarrhalis</i>	<i>Moraxella catarrhalis</i>
MEE	middle ear effusion
MEF	middle ear fluid
NNT	number needed to treat
OME	otitis media with effusion
PYR	person years at risk
RSV	respiratory syncytial virus
RT	absolute rate difference
<i>S. mutans</i>	<i>Streptococcus mutans</i>
<i>S. pneumoniae</i>	<i>Streptococcus pneumoniae</i>
URI	upper respiratory tract infection

List of original papers

This thesis is based on the following articles, which are referred to in the text by Roman numerals (I-IV).

- I Tapiainen T, Kontiokari T, Sammalkivi L, Ikäheimo I, Koskela M, Uhari M (2001) Effect of xylitol on growth of *Streptococcus pneumoniae* in the presence of fructose and sorbitol. *Antimicrob Agents Chemother* 45: 166-169.
- II Tapiainen T, Sormunen R, Kontiokari T, Ikäheimo I, Uhari M Morphology of *Streptococcus pneumoniae* and *Haemophilus influenzae* after xylitol exposure. Submitted for publication.
- III Tapiainen T, Renko M, Kontiokari T, Uhari M (2002) Xylitol concentrations in the saliva of children after chewing xylitol gum or consuming a xylitol mixture. *Eur J Clin Microbiol Infect Dis* 21: 53-55.
- IV Tapiainen T, Luotonen L, Kontiokari T, Renko M, Uhari M (2002) Xylitol administered only during respiratory infections failed to prevent acute otitis media. *Pediatrics* 109(2). URL: <http://www.pediatrics.org/cgi/content/full/109/2/e19>

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1 Introduction

Acute otitis media (AOM) is one of the most frequent diseases of childhood. Almost every child suffers at least from one episode before school age and a third of all children have recurrent AOM (1,2). It causes discomfort to the children and families, but it can also lead to additional problems. AOM is the most common reason for antibiotic treatment in childhood (3), and increasing antibiotic consumption has been shown to be related to the emerging phenomenon of antimicrobial resistance (4). AOM episodes seldom lead to acute complications such as mastoiditis and meningitis, but they can impair the hearing, since the middle ear is filled with effusion. Fluctuating or prolonged hearing impairment in early childhood may result in long-term consequences for speech and language development (5,6). The economic burden of the disease is illustrated by the estimated annual cost of 130 million euros arising from AOM in Finland (7).

Prevention of AOM would be the best way of solving these problems. Many attempts have been made to reduce its occurrence, but continuous antimicrobial prophylaxis is problematic due to the threat of antimicrobial resistance (4). Surgical procedures are performed on a wide scale, to the extent that every third child in Finland undergoes adenoidectomy and 10% receive tympanostomy tubes (7).

Xylitol, a natural sugar alcohol with a five-carbon structure, has been introduced as a novel way of preventing AOM in children, as it impairs the growth of the main otopathogen *Streptococcus pneumoniae* (*S. pneumoniae*) and reduces the adherence of *S. pneumoniae* and *Haemophilus influenzae* (*H. influenzae*) to the nasopharyngeal cells (8,9). The regular use of xylitol has been shown to reduce the occurrence of AOM in children by 30-40% in two clinical trials (10,11). The aim of this study is to investigate further the mechanism of action of xylitol in preventing AOM and to find a more convenient but still effective way of using it in clinical practice.

2 Review of the literature

2.1 Acute otitis media

2.1.1 Definitions, epidemiology and diagnosis

Otitis media is an inflammation of the middle ear, which includes a variety of medical conditions with different signs and symptoms. Acute otitis media (AOM) is defined as the presence of middle ear effusion (MEE) with acute onset of symptoms of inflammation of the middle ear or other respiratory symptoms. Otorrhoea occurring through tympanic perforation or tympanostomy tubes with acute symptoms is also classified as AOM. Middle ear effusion without acute symptoms is called otitis media with effusion (OME) (12,13), or alternatively secretory otitis or glue ear (14), and is classified as chronic when it has persisted for 3 months. Recurrent AOM is defined as the appearance of three new AOM episodes within 6 months or four during one year (13). The early stages of otitis media with myringitis, or inflammation of the mucous membrane of the middle ear without MEE, is referred as otitis media without effusion (12). The definitions of AOM vary in clinical practice and in the literature, the middle ear effusion component being included only in half of the published articles concerning AOM (15). The obscure definition of AOM makes it difficult to compare and interpret the results of separate studies.

AOM is the most frequent diagnosis in children under 15 years of age in physicians' practices in the United States (16), and is the outcome of approximately every fifth upper respiratory infection (17). Its peak incidence occurs at the age of 6 to 12 months, so that 40-60% of children have experienced at least one episode by the age of one year and its cumulative incidence can be as high as 80% by 3 years (1,2). Recurrent AOM is common, as 9-18% of children experience at least three episodes of AOM during the first year of life (1). The occurrence of AOM and recurrent AOM has been reported to be increasing (18).

The diagnosis of AOM is based on the detection of MEE or otorrhoea in the presence of acute symptoms (12), but entails considerable uncertainty in clinical practice (19), as the examination of ear status is demanding due to poor cooperation and the presence of aural cerumen, which needs to be mechanically removed in over half of the infants examined in order to visualize the tympanic membrane properly (20). An evaluation of physician accuracy in diagnosing otitis media conducted among 514 pediatricians and 188 otolaryngologists (21), who watched nine video-presentations of ideal pneumatic otoscopic examinations, including cases of AOM, OME and retracted tympanic membrane without effusion, showed the average rate of misdiagnosis to be 50% among the pediatricians and 27% among the otolaryngologists, the most common misdiagnosis being that of OME as AOM. Symptoms cannot be a basis for AOM diagnosis, as it has no specific symptoms (22). Rhinitis and other respiratory symptoms are common, since AOM frequently occurs as a consequence of an acute viral upper respiratory tract infection (URI) (23,24). Earache and other ear-related symptoms are quite specific for AOM but insensitive, especially since the presence of earache is difficult to evaluate among the youngest children (25). The tool most frequently used to diagnose AOM is pneumatic otoscopy, in which cloudiness and impaired mobility of the tympanic membrane are good indicators, whereas redness of the tympanic membrane and the appearance of the tympanic membrane in the absence of any test of mobility are of poor diagnostic value (26,27). In trial settings the sensitivity of pneumatic otoscopy for detecting MEE has been found to reach 84-90% and the specificity 58-90% (28-30). A more objective way of detecting MEE is to use tympanometry, which relies on acoustic admittance, i.e. a measure of the ease with which acoustic energy flows into the middle ear (31). The sensitivity of this method for diagnosing AOM is reported to be 83-91% and its specificity 63-86% (27,28,30,32). In most evaluations of the accuracy of tympanometry, myringotomy has been performed only after a clinical suspicion of AOM. Koivunen et al. (33) reported the specificity of hand-held minitympanometry to be 93% and the sensitivity 79% when myringotomy was performed on all children undergoing an operation for recurrent AOM. The weight of MEF correlated negatively with static admittance, and the few false negative cases were ones with only traces of MEF (33).

2.1.2 Treatment

AOM is usually treated with antimicrobial agents that are effective against *S. pneumoniae*, *H. influenzae* and *Moraxella catarrhalis* (*M. catarrhalis*), which are the three most common causal bacteria (3). AOM is the leading reason for the use of antimicrobials in most developed countries (3). An attempt has been made in the Netherlands to reduce the use of antimicrobials by instructing physicians to wait and observe patients with AOM closely before prescribing antimicrobials, but the efficacy of this strategy in reducing antimicrobial use at the national level has not yet been evaluated (34). The justification for antimicrobial treatment is the idea that the eradication of pathogens will lead to clinical success and resolution of MEE (35). It has been estimated that one third of all patients could benefit from antimicrobial treatment, as indicated by

repeated middle ear fluid (MEF) cultures, while 60% of cases recover clinically despite bacteriological failure and 10% persist despite bacteriological success (36,37).

Randomised clinical trials comparing initial antimicrobial treatment with placebo therapy for the management of AOM have employed various endpoints and achieved diverse results. These results have been used in meta-analyses to estimate the overall effect on specified endpoints (12,38-40), indicating that antimicrobials have a significant but modest effect on the short-term clinical resolution of AOM. Although the relative decrease in clinical symptoms 2-7 days after diagnosis in antimicrobial groups has been estimated to be up to 40%, the absolute rate decrease (RD) varies only between 4 and 14%, as approximately 80% of children recover without antimicrobial treatment. Thus the number of AOM episodes needed to treat (NNT) in order to gain benefit for one child at 2-7 days varies between 7 and 25. The results cannot be applied to children without any antimicrobial treatment, as all the trials have allowed antimicrobial therapy to be started in the placebo group if the clinical course has not been successful, or else they have given placebo therapy only to those with non-severe AOM (41-43). Children younger than 1 to 2 years of age have not been enrolled in most studies included in these meta-analyses (42-45), and data on the effect in the case of infants is limited, although there is one trial that suggests that the extent of the effect is similar in children aged under 2 years, i.e. seven to eight episodes need to be treated to gain benefit for one child at day four after diagnosis (46). Although the effect of antimicrobials on the resolution of MEE 4 weeks after treatment is not statistically significant (12,38,39), there is no evidence that antimicrobials fail to affect the resolution of MEF. Data on daily middle ear status after treatment would be required for a thorough analysis, and such data are difficult to obtain.

The most common complication of AOM is mastoiditis, the incidence of which is suspected to rise if antimicrobial treatment is not used (47). In a study by Rudberg in the 1950's the occurrence of mastoiditis was 17% (44/254) in the control group compared with 1.5% (4/267) among those receiving sulphonamides for AOM and 0% (0/844) among those receiving penicillin (48). The mastoiditis incidence rate in the Netherlands, with an antimicrobial prescription rate for AOM of 30%, has recently been reported to be 3.8/100 000 person years, compared with 1.2-2/100 000 person years in countries with prescription rates of up to 100% (49).

Shorter courses of antimicrobials could be beneficial for reducing the selection of resistant bacteria. Most studies show no difference in effectiveness between a short course of antimicrobial therapy (usually 5 days) and a long course (50). The emergence of antimicrobial resistance, and especially of penicillin-resistant *S. pneumoniae*, may lead to changes in the recommended regimens for treating AOM in many countries. The treatment of AOM caused by penicillin-resistant *S. pneumoniae* with intramuscular ceftriaxone or high-dose amoxicillin clavunate has been reported to be effective (51,52).

Myringotomy combined with antimicrobials does not improve the outcome relative to antimicrobials alone (41,53,54). Tympanocentesis is still performed in order to obtain a sample for microbiological culture in the case of patients who are seriously ill, immunologically deficient, newborns or suspected of having complications (12).

2.1.3 Consequences

2.1.3.1 Antimicrobial resistance

More than 90% of all antimicrobial treatment provided in the United States during the first two years of life is for AOM (55), as a child receives an average of 41.9 days of antimicrobial therapy for AOM in the first year of life and 48.6 in the second (56). There is substantial evidence that the increased consumption of antimicrobials results in enhanced antimicrobial resistance (57). The regional occurrence of antimicrobial-resistant strains correlates with the regional use of antimicrobials (58). Antimicrobial resistance will decrease if the consumption of the antimicrobial agent can be reduced (59). The selection of resistant strains after spontaneous mutations is infrequent without any contacts with antimicrobial agents, as wild mammals do not have antimicrobial-resistant faecal bacteria (60).

Although the antimicrobial resistance of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in Finland has been reported to be increasing (61), in total of 96% of *S. pneumoniae* strains are still fully susceptible to penicillin and only 14% of *H. influenzae* strains produce beta-lactamase among middle ear isolates from children (62). The countries with the most liberal policy in the use of antimicrobials tend to have the greatest proportion of resistant bacteria, the most difficult situations in Europe being in Spain and France, where 50-90% of *S. pneumoniae* strains are not susceptible to penicillin (63,64) and up to 70% of *H. influenzae* strains produce -lactamase (64). In the United States the prevalence of multi-drug resistant *S. pneumoniae* is increasing (65) and the prevalence of non-penicillin-susceptible *S. pneumoniae* isolates from middle ear fluid (MEF) exceeds 75% in the worst areas (66).

The amounts of antimicrobials prescribed for AOM should be reduced in order to restrict the emergence of antimicrobial resistance. Since spontaneous bacterial recovery from AOM is most frequent in episodes caused by *H. influenzae* and *M. catarrhalis* and less frequent in those caused by *S. pneumoniae* (36), it could be speculated that a knowledge of the aetiology of AOM could help in targeting the therapy. At the same time, in view of the fact that the outcome for an individual patient cannot yet be predicted in clinical practice, the most reasonable approach for reducing antimicrobial consumption and antimicrobial resistance would be to improve diagnostic accuracy in primary care (19) or to prevent AOM.

2.1.3.2 Cost

Total cost of AOM in Finland in 1997 was estimated to be 138 million USD (27.6 USD per capita) (7), which is quite close to the estimated cost of 611 million USD (20.9 USD per capita) in Canada in 1994 (67). The estimated cost of a single episode in the same survey was 228 USD (7), while in California in 1997 it was 268 USD (68). Visits to physicians and surgical procedures account for the greatest proportion of the direct costs, whereas the costs of antimicrobial medication are less significant (7,67,68). Indirect costs

account for between 23% (67) and over half of the total (7,68), the main cause being parental absence from work. The annual costs are highest among children under 3 years of age, who have the greatest incidence of AOM (2,7). As the costs of AOM mostly arise from visits to physicians, who are needed for a proper diagnosis, and parental work loss, efforts to change the treatment protocols are unlikely to affect the situation markedly, whereas the prevention of AOM could reduce all the direct and indirect costs. Therefore even an expensive method for preventing AOM could be cost-effective.

2.1.3.3 *Hearing loss and late sequelae*

AOM can result in conductive hearing impairment, which may be prolonged if the acute episode is followed by persistent OME (69). In a study of 222 infants and 540 older children with OME, the average speech awareness threshold in the infants was 24.6 dB and the average air conduction threshold in older children 27 dB (70). Furthermore the infants also needed an approximately 28 dB more intense stimulus in the speech-sound discrimination test than adults to achieve the same level of performance (71). The peak incidence of AOM and OME occurs among children aged 6 to 12 months (2), which is the critical period for language development (72,73). Language development requires normal hearing, as children automatically map the repeating patterns of the speech that they hear and statistically recognize the crucial elements leading to language development (72,73). Since otitis media results in mild or moderate hearing loss, it could result in periodic degraded language input, leading to poorer language and speech skills immediately and poorer verbal skills later (12). This effect could be modified by factors such as caregiver sensitivity, child IQ and ambient noise (74). It is possible that basic language skills may recover as soon as hearing is normalized, but the poorer attention paid to oral language remains and leads to poorer narrative skills and more solitary play (75).

The first evidence of an association between otitis media and language problems came from retrospective case-control studies (76-79). Luotonen *et al.* observed that more than 4 AOM episodes before the age of three years was associated with impaired reading comprehension scores as late as the age of nine years (6), whereas in a recent Swedish birth cohort of 2156 children those with numerous AOM episodes had higher grades when leaving the ninth year of school, although the grades of those with treated bilateral SOM tended to be weaker (80). Most prospective studies, which estimate the duration of MEE more accurately, indicate that there is a weak to moderate association of early otitis media with language skills and later cognitive skills (5,81-85). Such associations are difficult to evaluate, however, since children with OME have different stages of hearing impairment, ranging from minimal to moderate. A study which included prospective examinations of hearing levels of children with OME at the age of 12 to 18 months showed that the prevalence of speech delay was 2% among children with hearing levels of less than 20 dB at the age of 3 years but 33% among those with levels greater than 20 dB (86), which emphasizes the connection between hearing impairment and language development.

The language development of children with chronic OME treated with early insertion of ventilatory tubes has been compared with that of children with delayed intervention in randomised trials, which can avoid problems of confounding variables (87-89). In a study by Maw et al. with 186 participants, the children with delayed insertion of ventilatory tubes had poorer language skills 9 months after randomisation, but the difference disappeared at 18 months, when almost all the children had undergone surgery (87). Two other studies did not detect any difference in language development between the groups after 1-2 years of follow-up (88,89), although Rovers et al. did find that an improvement in hearing correlated with enhanced language development even when the intervention was ineffective (88). As it is difficult to measure hearing and language skills precisely in children and usually only clear deficits can be found, the results do show a correlation between untreated chronic OME and impaired language development (87).

2.2 Pathogenesis of acute otitis media

The development of acute otitis media is a complex process that starts in the nasopharynx, which is connected to the middle ear by the Eustachian tube. Viral upper respiratory tract infection (URI) causes inflammation, impairs the functioning of the Eustachian tube and enhances the bacterial colonization of the nasopharynx (90-95). The URI-induced inflammation, a decrease in pressure in the middle ear and the entry of both bacteria and viruses into the middle ear via the Eustachian tube result in inflammation and effusion in the middle ear.

2.2.1 Anatomical and physiological factors

The role of the Eustachian tube is essential for the development of AOM. The physiological functions of this tube include the regulation of middle ear pressure and protection of the middle ear by virtue of its functional anatomy and immunological and mucociliary defence mechanisms, and also the drainage of secretions from the middle ear into the nasopharynx by means of muscular and mucociliary clearance functions (12,96). The nasopharyngeal end of the Eustachian tube opens during swallowing, sneezing or yawning, but nasopharyngeal secretions do not enter the middle ear in healthy children (97). If the Eustachian tube is experimentally blocked, middle ear pressure decreases, causing the development of serous effusion (98). The anatomy of the Eustachian tube in children predisposes them to middle ear diseases. It gradually alters before adulthood as the length of the tube increases, its angle relative to the horizontal plane increases from 10 degrees to 45 degrees and its compliance decreases (92,99,100). Otitis-prone children have poorer tubal function than children without recurrent AOM (101), and the bony nasopharynx has also been reported to be smaller (102).

2.2.2 Pathogens

2.2.2.1 Viruses

Respiratory viruses both predispose individuals to AOM and have also been found as the sole pathogenic agent in the middle ear (23,24,103-109). Almost all children with AOM have symptoms of upper respiratory tract infection (URI) at the time of diagnosis (107). There is a close epidemiological correlation between viral epidemics and AOM incidence, as has been shown most convincingly between respiratory syncytial virus (RSV) and AOM (23,24,105). Concurrent viral infection in children with AOM has been verified by the detection of respiratory viruses in samples from the throat, nasopharynx or middle ear in 22% to 75% of AOM cases (23,24,105-108). Viruses alone, without a positive bacterial culture, are detected in the MEF in 2% to 22% of AOM cases (104-106,108,109), the most frequent being RSV, although rhino, influenza, parainfluenza, adeno and enteroviruses can also invade the middle ear (24,104-106,108,109). RSV is especially likely to precede AOM, as was evident in a controlled study that showed that the risk of AOM is greater in a child with URI during RSV infection than in children with URI of other viral aetiology (110). On the other hand, the significance of rhinoviruses for the pathogenesis of AOM probably exceeds that of RSV (111), since rhinoviruses circulate in the community throughout the year while RSV is the main viral pathogen in AOM only during the RSV season.

The respiratory viruses affect the development of AOM in many ways. The functioning of Eustachian tube is impaired during URI, which often leads to negative pressure in the middle ear, predisposing it to the development of MEE (93,112,113). Respiratory viruses cause inflammation and epithelial damage in the Eustachian tube, which leads to its obstruction due to the accumulation of mucus and inflammatory cells in the tubal lumen (114). Viral infections also impair mucociliary action in the epithelium of the respiratory tract, which disturbs the clearance function of the Eustachian tube (115). The relationship of viral infection to AOM has also been demonstrated in a study where the influenza A virus was inoculated intranasally into adults (91), with the consequence that 59% (16/27) developed negative middle ear pressure, 15% (4/27) MEE and 4% (1/27) purulent MEE. The results of a similar experiment using rhinovirus were analogous with the exception that only the adults with prior negative middle ear pressure developed MEE (90).

Viral infections enhance bacterial adherence to pharyngeal cells *in vitro* and in patients with URI (94,116,117). The colonization of nasopharynx by *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* increases during AOM episodes (95). Influenza A infection also impairs the functioning of the polymorphonuclear leukocytes that are needed for the clearance of *S. pneumoniae* (118).

The clinical course of AOM is modified by the presence of respiratory viruses and interaction between viruses and bacteria (107,119-121), and the persistence of symptoms or bacteria in MEF may be due to viral infection or combined viral-bacterial infection (119,120). The presence of both viruses and bacteria is also more likely to lead to bacteriological failure of antimicrobial treatment (119).

2.2.2.2 *Streptococcus pneumoniae*

S. pneumoniae is the most frequent pathogen isolated from MEF in patients with AOM (122), being detected in 26-48% of cases by bacterial culture methods (62,122-125). When a more sensitive technique such as antigen detection or the polymerase chain reaction (PCR) is used, positivity for *S. pneumoniae* increases by 50% compared to the results from conventional bacterial culture (123,126). Bacterial cultures often remain negative when effusion has persisted for 1 to 3 months, but the presence of bacterial DNA can frequently be detected in chronic MEE (127).

The nasopharyngeal carriage of *S. pneumoniae* is important in the pathogenesis of AOM. The bacteria are transmitted by respiratory droplets, after which they attach to the mucosal epithelium and colonize the nasopharyngeal epithelium (128). Attachment is mediated through a bridge between bacterial surface components and epithelial cell receptors composed of glycolipids (128). The carriage rate of *S. pneumoniae* varies from 9% to 52% between populations in healthy children (95,129), but increases during a respiratory infection without AOM and is at its highest, 45% to 91%, during AOM episodes (95,129). During pneumococcal AOM the nasopharyngeal carriage rate of *S. pneumoniae* reaches 97 to 100% (129). Although a known middle ear pathogen can almost always be found in the nasopharynx, the recognition of AOM of unknown aetiology through nasopharyngeal culture is impossible due to the variety of bacteria present in the nasopharynx. The mean duration of nasopharyngeal colonization in children varies between 19 days and 4.5 months in different studies (130,131). The acquisition of a new strain increases the risk of AOM, which develops within a month of acquisition in 15% of cases (130). The factors that increase nasopharyngeal colonization in children include young age, day-care attendance, parental smoking, the winter season and antibiotics (128). Colonization can be interrupted by host immunity and antagonized by other oropharyngeal flora such as viridans streptococci (128).

A knowledge of the structure, function and virulence of *S. pneumoniae* can help us in developing new preventive measures for AOM. Being a Gram-positive pathogen, *S. pneumoniae* is surrounded by a polysaccharide capsule which protects it from host immunity and is essential for its virulence. A total of 90 serotypes can be distinguished in terms of the structure of the polysaccharide capsule and differences in antigenic properties (132). In a recent Finnish study the most frequent serotypes in MEF isolates from children with AOM were 19F, 23F, 6A, 6B and 14, which accounted for 73% of all cases (62). Many other virulence factors of *S. pneumoniae* have been identified, such as an intracellular toxin named pneumolysin, surface proteins such as pneumococcal surface protein and pneumococcal surface adhesin A and pneumococcal enzymes such as neuraminidases and autolysin (133,134). The whole genome of a virulent isolate of *S. pneumoniae* has recently been sequenced, which may help in determining new virulence factors (135).

In addition to the virulence factors listed above, *S. pneumoniae* is able to undergo intra-strain phenotypic variation which alters the properties of the strain and its ability to cause diseases. This phenotypic variation simultaneously affects multiple cell-surface structures needed for the interaction between *S. pneumoniae* and its host (136), leading to phase variation in the appearance of the colonies when viewed with oblique, transmitted

light in transparent media. The transparent variants are more likely to colonize the nasopharynx, whereas the opaque variants are more virulent and more likely to cause invasive diseases (136,137). After intranasal inoculation of influenza A virus into chinchillas, the opaque variants of *S. pneumoniae* cause significantly more negative pressure in the middle ear and to persist there longer than the transparent variants (138).

2.2.2.3 Other bacteria

H. influenzae and *M. catarrhalis* are the most common bacteria involved in the development of AOM in addition to *S. pneumoniae*. These three bacteria are found in 70% of MEF samples taken during AOM (62,122). *Staphylococcus aureus*, *Streptococcus pyogenes* and aerobic Gram-negative rods are found infrequently in MEF (62,122). In neonates, however, *Staphylococcus aureus*, coagulase-negative staphylococci and Gram-negative rods are found more often than in older children (139). *H. influenzae* is isolated from the MEF in 12% to 27% of AOM events (62,122-125), the proportion being higher in children with recurrent AOM (62). *M. catarrhalis* was not established as a middle ear pathogen until the 1980's (140), and its percentage in MEF isolates varies over a wide range of 1% to 23% between populations (62,122-125). In approximately 15% of AOM events the MEF culture remains negative for all bacteria (62,122,124,125).

H. influenzae and *M. catarrhalis* colonize the nasopharynx in a similar manner to *S. pneumoniae* and their carriage also increases during viral respiratory infections and AOM episodes (95). In a longitudinal study of 306 infants during the first year of life, *M. catarrhalis* proved to be the most prevalent pathogen in nasopharyngeal cultures (141). Even so, *M. catarrhalis* and *H. influenzae* can be regarded as less virulent pathogens than *S. pneumoniae*, as the symptoms of AOM episodes caused by them are less severe than those caused by *S. pneumoniae* (142). In addition, the rate of spontaneous disappearance of *M. catarrhalis* from the MEF during AOM is 80%, which is markedly higher than that of *H. influenzae* or *S. pneumoniae*, 50% and 20%, respectively (143).

2.2.3 Immunology

Local mucosal defence in the nasopharynx and Eustachian tube plays a critical role in the prevention of AOM, since viruses and bacteria invade the middle ear through these passages. This mucosal defence consists of the mucociliary system, the antimicrobial molecules of innate immunity, and the adaptive immune system. The mucociliary system forms a barrier against invading pathogens, as normal ciliary function, the mucus produced by secretory cells in the Eustachian tube and adequate hydration together serve to protect the surface of the epithelium (144). Antimicrobial molecules of innate immunity, such as lysozyme, lactoferrin and collectins, have been identified in the Eustachian tube in animal models (145,146). The local immune defence against otopathogens matures earlier in children than does systemic immunity (147,148). Mucosal IgA antibodies limit the duration and frequency of otopathogen colonization in

the nasopharynx (149). Adenoids participate in local defence supplying phagocytic cells and secretory IgA that can control the nasopharyngeal flora (150).

The nature of the immune system predisposes infants to AOM. Protective maternal IgG antibodies decrease gradually during the first six months of life, while endogenous IgG, IgA and IgM reach their maximum concentrations in later childhood. Infants usually have low levels of the serum antibody to *S. pneumoniae* (151), and the antibody response to those serotypes that most commonly cause AOM is poor (152). *H. influenzae* often induces strain-specific immunity in children, which does not protect them from other strains of the bacterium (153). Otitis-prone children may have a poorer immune response to otopathogens than others, and the proportion of nasopharyngeal bacteria coated by secretory IgA has been reported to be lower in them (149). Otitis-prone children also fail to produce antibodies against the highly conserved proteins of *H. influenzae*, which would be more broadly protective than strain-specific antibodies (154). IgG subclass deficiencies have also been associated with recurrent respiratory tract infections (155).

2.3 Prevention of acute otitis media

2.3.1 Intervention in risk factors

The occurrence of AOM is affected by both host factors and environmental factors. Predisposing host factors include young age, male gender, racial factors, allergic rhinitis, immune deficiency, cleft palate, craniofacial abnormalities and genetic predisposition (12), none of which can be modified to prevent AOM. A retrospective study carried out in Norway and a prospective twin study in the United States estimate the heritability of AOM to be 45-64% in males and 74-79% in females (156,157). The environmental risk factors for AOM include contacts with other children, especially in day-care outside the home, parental smoking, use of a pacifier and lack of breastfeeding (158-162). The significance of each environmental risk factor has been evaluated in a meta-analysis of 18 relevant studies (163), showing the most important factor to be day-care outside the home, which increased the risk ratio up to 2.5 compared with home care. The numerous child contacts occurring in day-care increase both viral infections and pneumococcal nasopharyngeal colonization, and thus predispose children to AOM (159,160,164). Changes in risk factors in a community can have significant consequences in terms of the occurrence of AOM. Local authorities in Finland have been obliged by law since 1990 to arrange day-care for all children under the age of 3 years, which has resulted in a three-fold rise in the number of children younger than 3 years attending day-care in the cities of Oulu and Tampere, for example. Simultaneously, preventive surgery for recurrent AOM among children of that age increased significantly in these areas, adenoidectomies by 17% and tympanostomies by 30% (165).

There are only a few randomised trials which estimate the effect of intervention in risk factors on the prevention of AOM. As attending day-care is a significant risk factor for URI and AOM due to frequent transmission of respiratory pathogens, it is likely that improved infection control could result in reduced morbidity. There have been three

randomised field trials (166-168) in which day-care personnel have been advised to improve practical hygiene, e.g. hand washing and toy cleaning, to reduce the infection rate, whereupon the occurrence of URI in these centres has been compared with that in control centres. In two of the trials the occurrence of URI episodes was reduced significantly in all age groups, and in one this applied only to children younger than 2 years. In the two trials showing a significant effect in all age groups the reduction in the occurrence of URI was 9% (confidence interval (CI) 4-16%) in a series of 20 day-care centres in Finland and 20% (CI 7-32%) in 47 day-care centres in Canada (166,167), while the occurrence of AOM was reduced by 27% (CI 17-36%) in the former study (166).

The promotion of breastfeeding, discouragement of parental smoking and restriction of the use of pacifiers are all potential ways of preventing AOM. Parental counselling with regard to the risk factors can be an effective approach. A randomised intervention to provide parents attending 14 infant health clinics with information about the known harmful effects of prolonged, constant pacifier use (169) resulted in a 21% decrease in pacifier use and a 29% decrease in the occurrence of AOM relative to a control group. In clinical practice, however, awareness of the modifiable risk factors for AOM can be surprisingly low among physicians, so that only approximately 60% of paediatric residents and 71% of paediatricians in the United States were aware that breastfeeding can confer protection from AOM, for instance (170). If general practitioners were to be instructed about modifiable AOM risk factors, the efficacy of screening and parental counselling could be increased significantly (171).

2.3.2 *Chemoprophylaxis*

Antimicrobials that are effective for treating AOM have also been widely used for its prevention. Daily administration of antimicrobials at one-half of the usual dosage for treating AOM has been the most common form of prophylaxis provided for children with recurrent AOM, and several randomised clinical trials have shown that the continuous use of sulfisoxazole, trimetoprim-sulfamethoxazole, amoxicillin or ampicillin can prevent AOM by comparison with placebo treatment, since the proportion of children suffering at least one AOM episode decreased by 30-81% and AOM incidence density by 43-88% (172-175). There have also been a few trials that have shown no statistically significant difference in the occurrence of AOM between children receiving antimicrobial prophylaxis relative to a placebo (176,177). The overall effect of antimicrobial prophylaxis has been estimated in two meta-analyses, which both showed it to be effective. That published by Williams et al. (178) included nine randomised clinical trials performed during 1972-1991, with a total of 958 subjects and gave an absolute rate difference (RD) in the incidence density of AOM of 0.11 (CI 0.03-0.19) episodes per patient-month, with an NNT of 9, i.e. 9 children have to be treated for one month to prevent a single AOM episode. The other meta-analysis, by Rosenfeld (12), covered altogether thirteen randomised clinical trials performed during 1972-1997, with a total of 1193 subjects, and gave an RD for the incidence density of AOM of 0.12 (CI 0.08-0.16) episodes per patient-month and an NNT of about 8, which is very similar to the results arrived at by Williams et al. The greatest benefit of antimicrobial prophylaxis has been

observed when sulfisoxazole has been used and the children in the placebo group have had high incidence density of AOM (172). In addition to placebo-controlled trials, once-weekly azithromycin has been shown to be more effective for prevention of AOM than once-daily amoxicillin (179).

The main disadvantage of continuous antimicrobial prophylaxis is the induction of antimicrobial-resistant bacteria (180). If the duration of antimicrobial prophylaxis could be shortened, this effect would be limited, in addition to which compliance might also be better. Since AOM develops as a consequence of URI, antimicrobial prophylaxis restricted to the duration of URI symptoms could be ideal for preventing AOM. Intermittent prophylaxis with penicillin during URI proved promising in that it reduced the occurrence of AOM by 50% compared with placebo treatment in a study by Prellner et al. (181), but in two subsequent trials the administration of amoxicillin-clavunate (17) or penicillin (182) after the appearance of URI respiratory symptoms failed to prevent AOM. Thus only continuous antimicrobial prophylaxis can be said to be effective in preventing AOM.

Since URI predisposes children to AOM, it would be logical to treat the preceding viral infection in order to prevent AOM. The treatment of URI symptoms with an antihistamine-decongestant mixture is not effective in preventing AOM in children (183), nor is restriction of the inflammatory response to URI by means of intranasal fluticasone propionate (184). The specific viral drugs active against influenza A and B, zanamivir and oseltamivir, reduce the severity of influenza in children when treatment is started after the appearance of symptoms (185,186). Oral oseltamivir commencing within 48 hours of the onset of influenza reduced the occurrence of new AOM episodes in children by 44% (186), which is a notable finding even though the effect was limited to the influenza season. No therapy against other respiratory viruses is yet available.

2.3.3 Surgery

Tympanostomy tube insertion and adenoidectomy are in common use for the prevention of AOM. Approximately 15% of Finnish children attending day-care centres receive tympanostomy tubes, and almost every third child undergoes adenoidectomy (165). Despite the massive use of preventive surgery, patient selection for such operations is problematic. In a retrospective cohort study of 2512 Finnish children, 60% of those operated on for recurrent AOM had relatively few AOM episodes, while only 10% of those with actual recurrent AOM were operated on (187).

Tympanostomy tube insertion is an effective treatment for chronic OME and is necessary in severe cases with pronounced hearing loss, since spontaneous resolution of the condition is rare (188). The efficacy of tympanostomy tube insertion for the prevention of recurrent AOM has not been demonstrated as convincingly as in the treatment of OME. In two randomised trials that included children with and without OME at entry the proportion of children with at least one AOM episode decreased by 44% (CI 27-58%) (189) and 41% (CI 7-69%) (177) relative to those receiving no therapy or a placebo. These studies may have overestimated the true effectiveness of the procedure for treating recurrent AOM, since the outcome for patients with OME upon

entering the latter study was significantly better than for those without effusion at entry (177). The only study which included only children with recurrent AOM but without OME at entry showed no difference in the average rate of new episodes of AOM between those in whom tympanostomy tubes were installed and those receiving a placebo for prophylaxis (175), although even here the average time spent with AOM or OME during the follow-up was significantly shorter in tympanostomy group than in either the placebo group or the prophylactic amoxicillin group. Insertion of tympanostomy tubes may not reduce new episodes of AOM effectively, but it may still be beneficial for children with recurrent AOM in order to shorten the duration of MEE and alleviate hearing loss.

The efficacy of adenoidectomy as a treatment for chronic OME has been demonstrated (188,190), but the evidence supporting its use for preventing recurrent AOM without OME is limited. There are two randomised trials evaluating the efficacy of adenoidectomy or adenotonsillectomy. In a randomised study of 99 children aged 3 to 15 years with previously inserted tympanostomy tubes, adenoidectomy reduced the number of new AOM episodes by 28% in the first year of follow-up and 35% in the second year while the duration of otitis decreased by 47% and 37% (191). In a following study 304 children aged 3 to 15 years who had not been treated previously with tympanostomy tubes were randomised to receive adenoidectomy, adenotonsillectomy or no surgery (192). In this latter case adenoidectomy alone did not reduce the occurrence of AOM in the first follow-up year and the mean rate of episodes of AOM was higher in the adenoidectomy subjects than in the control group in the second year. The adenotonsillectomy group had a 33% lower occurrence of AOM during the first follow-up year, but the difference disappeared during the second and third years. In conclusion, there is some evidence to support the use of adenoidectomy for preventing AOM after the insertion of tympanostomy tubes, but adenoidectomy alone seems to be ineffective. Evidence on the effect of adenoidectomy or adenotonsillectomy in children younger than 3 years is still lacking.

2.3.4 Immunisation

Vaccines would be an ideal long-term way of preventing AOM without the threat of antimicrobial resistance or the risk of complications of surgery. The development of an effective vaccine against AOM is a challenging task, since several viruses and bacteria can caused AOM.

Both bacterial and viral vaccines have been used to prevent AOM. The first pneumococcal polysaccharide vaccine reduced pneumococcal AOM only within the first six months after vaccination by 58% (193) and had low immunogenicity and efficacy among children younger than 2 years, which limited its clinical usefulness (194,195). A novel conjugate pneumococcal vaccine against seven of the most frequent otitis media serotypes, which account for 70-80% of pneumococcal AOM, is immunogenic also among infants as it contains a carrier protein for capsular polysaccharide. In a randomised trial with about 38 000 children in Northern California the number of AOM episodes diagnosed by primary care physicians decreased by 6.4% (CI 3.9-8.7%) in the children who received the pneumococcal conjugate vaccine relative to those vaccinated with a

meningococcal conjugate vaccine (196). The effect of the pneumococcal conjugate vaccine in prevention of AOM was more thoroughly evaluated in a randomised trial of 1662 infants in Finland (197). The accuracy of the AOM diagnoses was good, as trained physicians diagnosed all the AOM events, and a MEF culture was obtained in most cases. The reduction in AOM episodes achieved by the seven serotypes included in the vaccine was 57% (CI 44-67%). The pneumococcal conjugate vaccine failed to prevent AOM of any cause, however, as the total number of episodes decreased only by 6% (CI -4 to 16%), which was not statistically significant. Vaccines using virulence proteins of *S. pneumoniae* are being developed, and these could prove to be protective against all pneumococci (198).

Influenza virus vaccines have been more effective, although their efficacy is limited to the influenza season. Heikkinen *et al.* and Clements *et al.* observed the occurrence of AOM in children to decrease by 36% (CI 7-64%) and 31% (CI 2-51%) respectively during an influenza season when an inactivated influenza virus vaccine was used (199,200). The inconvenience of the intramuscular injections before every influenza season is a constraint on the widespread use of an inactivated influenza virus vaccine in children. Intranasal live attenuated influenza virus vaccine could be more suitable for children and has been shown to be effective in reducing febrile AOM episodes by 30% (201).

Vaccines against RSV, *H. influenzae* and *M. catarrhalis* could substantially reduce the occurrence of AOM. The first inactivated RSV vaccines introduced in the 1960's predisposed infants previously uninfected by RSV to a more severe disease when they were later infected (202,203). Live RSV vaccines are now being developed, but the most promising candidates still need further modification in order to be safe for use with infants (204). The development of vaccines against *H. influenzae* and *M. catarrhalis* is in the stage of identification of potential antigens and experiments in animal models (205,206).

Passive immunisation with systemic gammaglobulin has not been effective in preventing AOM (207). Immune globulin prepared by immunizing the donors with bacterial polysaccharide vaccines did not prevent new episodes of AOM, although the mean duration of AOM decreased in subjects receiving hyperimmune human immune globulin (208). Gammaglobulin enriched with RSV antibodies and administered intravenously in high doses significantly reduced the occurrence of AOM (209), but the pure monoclonal antibody against RSV, which can be administered intramuscularly, failed to prevent AOM in high risk infants (210). The improvement achievable in the local mucosal defence could be more effective, as intranasal immunoglobulin with high concentrations of IgA reduced URI episodes significantly in a randomised trial with 40 children (211). Maternal immunisation against RSV or pneumococci could protect the infants through the transport of antibodies across the placenta to the foetus, but this technique still needs further research and evaluation (212,213).

2.3.5 Other possibilities

The normal flora in the nasopharynx provides protection against AOM, as its bacteria, especially alpha-haemolytic streptococci, inhibit the growth of common otopathogens. Most antimicrobials used for AOM disturb the functioning of the normal flora. The balance between the normal flora and otopathogens in otitis-prone children is altered in a more pathogenic direction (214). In a placebo-controlled trial with 130 children suffering from recurrent AOM, those randomised to receive a nasal spray containing alpha-haemolytic streptococci after antimicrobial treatment for AOM had 24% (CI 3-46%) fewer recurrences of AOM than those receiving a placebo spray (215)

The attachment of otopathogens to host cells is mediated through bacterial proteins such as adhesins, which recognize the oligosaccharide structures on human cells. The use of exogenous oligosaccharides reduces bacterial attachment to host cells through competitive inhibition (216). Human milk contains high concentrations of such oligosaccharides, which are thought to protect breastfed infants against infections. A novel synthetic antiadhesive pentasaccharide administered intranasally did not prevent AOM in children as compared with subjects receiving a placebo (217); in fact the rate of AOM episodes was higher in the treatment group than in the placebo group after the three months of follow-up.

Table 1. Approaches to the prevention of acute otitis media (AOM). Absolute and relative decreases in AOM incidence density in randomised trials, with 95 % confidence intervals (CI).

Trial (Reference)	Number of children	Decrease in AOM incidence density AOM/Patient-Years at Risk	Relative decrease	95 % CI†
Risk factor intervention				
Hygiene in day care centres (166)	1522	0.4	27 %	17 to 36 %
Pacifier use (169)	484		29 %	
Antimicrobials •				
Sulfisoxazole (172)	108	1.8	88 %	53 to 124 %
Sulfisoxazole (173)	70	2.2	43 %	28 to 60 %
Amoxicillin (175)	86	0.48	44 %	22 to 66 %
Amoxicillin (176)	194	-0.36	-14 %	-64 to 36 %
Meta-analysis (12)	1190	1.4		#
Antimicrobials during URI				
Penicillin (181)	76	1.8	36 %	14 to 59 %
Amoxicillin-clavunate (17)	104	*	18 %	-50 to 86 %
Penicillin (182)	70	0.08	5 %	-31 to 38 %
Treatment of host response to URI				
Intranasal fluticasone (184)	208	*	-35 %	-78 to 10 %
Treatment of viral infection				
Oral oseltamivir (186)	452	**	44 %	9 to 76 %
Tympanostomy				
(189) §	95	4.1	69 %	50 to 89 %
(177) §	42	2.3	57 %	26 to 88 %
(175)	174	0.06	5 %	-13 to 24 %
Adenoidectomy (192)•				
1 st year	140	0.3	14 %	-6 to 34 %
2 nd year	112	-0.5	-42 %	-6 to -77 %
Vaccines				
Pneumococcal, conjugate (197)	1662	0.08	6 %	-4 to 16 %
Influenza, inactivated (199)	187	**	36 %	7 to 64 %
Influenza, inactivated (200)	186	**	31 %	2 to 51 %
Influenza, live attenuated (201)‡	288	**	30 %	18 to 45 %
Recolonisation by alpha haemolytic streptococci (215)				
Intranasal oligosaccharide (217)	507	***	-27 %	-68 to 5 %

† P<0.05 when the 95 % CI contains no zero; positive values favour the experimental group. • Results of 13 randomised clinical trials with placebo treatment are included in the meta-analysis. # Relative decrease not applicable but the absolute decrease is statistically significant (CI 0.9 to 1.9). * Decrease during URI. ** Decrease during the influenza season. *** Decrease in first episodes of AOM. § Children with and without OME at entry. • Children without prior tympanostomy tubes. ‡ Decrease in febrile AOM episodes.

2.4 Xylitol

Xylitol is a five-carbon sugar alcohol (Figure 1), small amounts of which occur naturally in various fruits and berries (218). It has also been called "birch sugar", as it can be produced from xylan derived from birch wood chips (219). It is equal in sweetness to sucrose, with 1 g yielding 4.06 kcal, and it is commonly used as a sweetener (219). Xylitol is a normal intermediate of human metabolism, and several grammes of it is produced daily by the liver (220). Exogenous xylitol is metabolised to glucose and glucogen or pyruvate and lactate mainly in the liver (220). Slow intestinal absorption of xylitol may result in osmotic diarrhoea when the daily dose exceeds 20-40 g in adults, although even daily doses of over 400 g have been tolerated without side-effects (220). Excessive long-term consumption of xylitol by voluntary subjects at daily doses of 60-70 g for two years did not lead to any adverse events (221). Many bacteria are nevertheless unable to utilize xylitol as an energy source, and its presence is harmful to some bacteria despite the availability of an alternative energy source such as glucose (9,222-224).

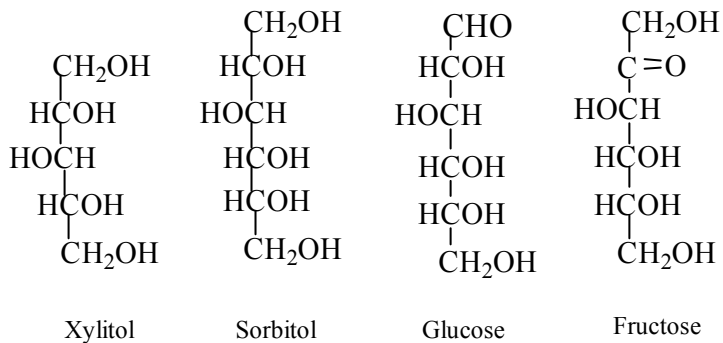


Fig. 1. Structural formulae of five-carbon sugar alcohol xylitol and six-carbon sugar alcohol sorbitol together with the formulae of common six-carbon sugars glucose and fructose.

2.4.1 Effect on bacteria

2.4.1.1 Sugar metabolism of oral bacteria

Bacteria in the oral cavity are transiently exposed to different sugars and live under constantly alternating "feast and famine" conditions. The rapid utilization of available sugars is possible through the phosphotransferase systems (PTS), which transport various sugars in bacterial cells and phosphorylate them (225). The PTS consists of enzyme components, a number of which are specific to sugars. In addition to sugar metabolism, the PTS is a complex protein kinase system regulating many metabolic processes and

gene expression in many Gram-positive and Gram-negative bacteria (226), and its function is significant for oral streptococci such as *Streptococcus mutans* (*S. mutans*), which are dependent on sugars as an energy source (225). The complete genome sequence of *S. pneumoniae* revealed that pneumococci have 21 PTS sugar-specific enzyme complexes, twice as many as in any other sequenced organism relative to genome size, reflecting the importance of sugars for pneumococci (135).

2.4.1.2 Effect on bacterial growth, metabolism and adhesion

It has been shown in several studies that xylitol significantly reduces the growth of *S. mutans* in the presence of glucose or sucrose (222-224,227,228), whereas its effect on other oral bacteria such as *Streptococcus salivarius* and *Streptococcus sanguis* is modest by comparison (228). Xylitol has also been shown to reduce the growth of *Lactobacillus casei* and some strains of *Escherichia coli*, *Saccharomyces cerevisiae* and *Salmonella typhi* and to affect the sugar utilization of *Haemophilus influenzae* (229-232).

The effect of xylitol on the growth of otopathogenic bacteria *in vitro* has been evaluated in an experiment using ten strains of *S. pneumoniae* and *H. influenzae*, five strains of *M. catarrhalis* and nine strains of beta-haemolytic streptococci (9). Xylitol induced a marked inhibition of pneumococcal growth, by 72% in the case of *S. pneumoniae* after exposure to 5% (weight/volume) xylitol and 39% in the presence of 1% xylitol, despite the glucose available in the growth medium. The extent of the effect on pneumococci was similar to that described in *S. mutans* exposed to xylitol (222,223). The growth of beta-haemolytic streptococci was slightly impaired after exposure to 5% xylitol, whereas that of *H. influenzae* and *M. catarrhalis* was not affected at all.

The mechanism of action of xylitol on bacteria has been studied in detail only in *S. mutans* (Figure 2), where the xylitol-induced growth reduction is inhibited in the presence of fructose but not in the presence of other sugars, suggesting that the effect is mediated through a fructose-dependent system (227). Subsequent research showed that xylitol is transported into the *S. mutans* bacteria and phosphorylated through a constitutive fructose PTS (233,234). Since this species is not able to utilize the xylitol phosphate as an energy source, the expulsion of xylitol from the bacterial cell results in an energy-consuming futile xylitol cycle which, together with a harmful intracellular accumulation of xylitol phosphate, results in inhibition of the growth of *S. mutans* (233,235-237). The same futile xylitol cycle also explains the xylitol-induced inhibition of *Lactobacillus casei* growth (238). If the *S. mutans* lacks any constitutive fructose PTS activity, the bacteria become insensitive to xylitol (239).

Since the PTS in bacteria regulates many metabolic processes and the expression of various genes (226), it is likely that in addition to growth retardation, xylitol may also disturb the metabolic processes in the remaining viable bacteria. Xylitol affects polysaccharide synthesis in *S. mutans*, resulting in decreased bacterial adherence (240). The ultrastructure of viable *S. mutans* bacteria is damaged after exposure to even small concentrations of xylitol (241), and their protein synthesis is also disturbed, which implies that xylitol acts as a strong metabolic inhibitor for this species (242).

Since bacteria adhere to host cells through carbohydrate-binding proteins (243), extracellular xylitol may disturb the binding process by acting as a receptor analogue for the host cell, which could result in decreased adherence. Xylitol in a 6 % concentration is capable of reducing the adherence of *S. mutans* (240), and 5 % concentration is sufficient to reduce the adherence of the main otopathogens *S. pneumoniae* and *H. influenzae* to epithelial cells (8).

Xylitol in concentrations exceeding the isotonic level of 4.5 % acts as an osmolyte. The hypertonic solution captures water on mucosal surfaces, which could be beneficial in conditions with abnormally high salt concentrations, since a lowered salt concentration may enhance the activity of antimicrobial substances belonging to the innate immunity system (244).

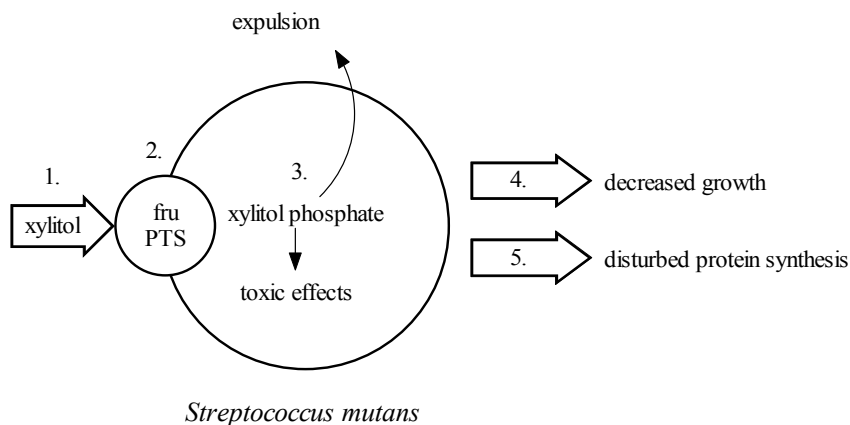


Fig. 2. The effect of xylitol on *S. mutans*. Xylitol (1) enters the cell through a fructose phosphotransferase system (2), producing xylitol phosphate, which is toxic and is expelled from the cell, thereby consuming energy (3). This results in growth reduction and altered cellular functions (4,5). Data from references (233, 235-237, 242).

2.4.2 Prevention of caries

The first observation that xylitol prevents caries was made in animal studies by Mühlemann *et al.* in the 1960's (245), and the first human trials, performed in Turku in the 1970's, showed about an 80% reduction in caries rates among subjects consuming xylitol chewing gum daily compared with subjects receiving sucrose chewing gum (246). All the subsequent open field trials, carried out with a total of 3402 participants in Polynesia, Hungary, Canada, Ylivieska and Estonia, showed caries reduction of about 50-60% in subjects receiving 5-20 g of xylitol daily in chewing gum or sweets compared with those given normal dental hygiene instructions but no xylitol products (247-251). A double-blind cohort study with whole school classes as randomisation units conducted among 1277 school children in Belize to evaluate the effect of different chewing gums (252) showed that when xylitol chewing gum was used five times a day, yielding a daily

xylitol dose of 8.4 g, caries reduction was 73% (CI 64-80 %) relative to subjects receiving no chewing gum and 80% relative to those receiving sucrose chewing gum. The use of sorbitol alone or in combination with xylitol resulted in a less marked decrease in caries rates than with xylitol alone.

The effect of xylitol in preventing caries is based on a reduction in plaque and in the numbers of *S. mutans* in the oral microflora, as observed in clinical trials (253-255), although not all trials have resulted in a permanent decrease in *S. mutans* during xylitol consumption (256). Sorbitol reduces the amount of plaque but not the numbers of *S. mutans* (255). It has been suggested that xylitol enhances the remineralizing process by increasing the calcium content of the plaque, which may cause a reversal of caries (257). Habitual xylitol consumers have been reported to have higher proportions of xylitol-insensitive *S. mutans* strains in their oral microflora than non-consumers (258), but the insensitive strains have been described as less virulent and less cariogenic than the xylitol-sensitive ones, which may lead to a beneficial effect on dental health regardless of any xylitol resistance (259). The acquisition of *S. mutans* can be reduced in infants through maternal xylitol consumption, as xylitol reduces the likelihood of mother-child transmission of *S. mutans* (260).

2.4.3 Prevention of acute otitis media

The prevention of acute otitis media by means of xylitol has been evaluated in two randomised placebo-controlled clinical trials. In the first, where 306 children were randomised to receive five doses of either xylitol or sucrose chewing gum daily, the number experiencing at least one AOM episode was reduced by 42% in the group receiving xylitol chewing gum (Table 2) (10). The second study used a similar set-up but with a liquid xylitol mixture as prophylaxis for children unable to chew gum (11). The proportion of children with at least one AOM episode was again reduced by 40% in the group receiving xylitol chewing gum, and the xylitol mixture was also effective, as the corresponding proportion decreased by 29% (Table 2). Xylitol lozenges also reduced the occurrence of AOM by 20%, but the difference was not statistically significant. The use of xylitol did not affect the occurrence of URI. Compliance was mostly good despite the frequent dosing, and side-effects were infrequent, with only a few children reporting abdominal discomfort, mainly in the xylitol mixture and lozenge groups.

The effect of xylitol in preventing AOM is probably based on the reduced growth of *S. pneumoniae* and the reduced adherence of *S. pneumoniae* and *H. influenzae* (8,9). On the other hand, xylitol did not reduce the nasopharyngeal carriage of *S. pneumoniae* during a two-month clinical trial or in an animal model (10,261). Thus the mechanism of action of xylitol in AOM prevention is not yet fully understood.

Table 2. Effects of xylitol in preventing acute otitis media (AOM) in randomised placebo-controlled clinical trials. The relative decrease in the proportion of children with at least one AOM episode and the decrease in AOM incidence density during the follow-up are presented. All groups except the lozenge group were double-blinded.

Trial (Ref.)	Products (duration)	Xylitol group Proportion of children with at least one AOM episode	Placebo group Proportion of children with at least one AOM episode	Relative decrease (95% CI*)	Absolute decrease in incidence density AOM/PYR (95% CI)	Relative decrease (95 % CI)
(10)	Chewing gum (2 mo)	19/157 (0.12)	31/149 (0.21)	42 % (2-82 %)	0.89 (0.31-1.50)	52 % (18-87 %)
(11)	Chewing gum (3 mo)	29/179 (0.16)	49/178 (0.28)	40 % (10-71 %)	0.65 (0.14-1.16)	39 % (8-69 %)
(11)	Mixture (3 mo)	46/159 (0.29)	68/165 (0.41)	30 % (5-55 %)	1.02 (0.29-1.75)	34 % (10-58 %)
(11)	Lozenge (3 mo)	39/176 (0.22)	49/178 (0.28)	20 % (-13-51 %)	0.36 (-0.17-0.89)	21 % (-10-53 %)

* 95% confidence interval.

3 Aims of the research

The purpose of the present work was to elucidate the mechanism of action of xylitol in preventing AOM (I, II) and to find an optimal regime for its use in clinical practice (III, IV). The specific aims were:

1. To ascertain whether the mechanism of action of xylitol in inhibiting the growth of *S. pneumoniae* is mediated *via* the fructose phosphotransferase system as in the case of *S. mutans* (I).
2. To evaluate the effect of sorbitol on the growth of *S. pneumoniae* (I).
3. To evaluate the effect of xylitol on the ultrastructure of *S. pneumoniae* and *H. influenzae* and on the pneumococcal phenotype (II).
4. To evaluate the pharmacokinetics of the xylitol concentrations in the saliva of children after doses of xylitol equal to those used in previous clinical trials in order to find a more practical schedule for xylitol administration (III).
5. To evaluate whether xylitol prophylaxis is effective if used only during respiratory infections (IV).

4 Subjects, materials and methods

A summary of the subjects, materials and methods employed in papers I-IV is presented in Tables 3 and 4. The subjects for papers III-IV were recruited from day-care centres in the city of Oulu after approval of the protocol by the Ethical Committee of Oulu Municipal Health Centre.

Table 3. Materials and methods used in the microbiological studies (I, II).

Paper	Design	Setting	Xylitol exposure	Number of strains*
I	Experimental	<i>In vitro</i> evaluation of the effect of xylitol on <i>S. pneumoniae</i> growth in the presence of other carbon sources	2.5-5% xylitol alone or combined with 1-5% fructose, glucose, galactose, sucrose or sorbitol	21
II	Experimental	Evaluation of the ultrastructure of <i>S. pneumoniae</i> and <i>H. influenzae</i> by electron microscopy	0.5-5% xylitol for 0.5-2 hours	6

*Including middle ear isolates and ATCC 49619 for *S. pneumoniae* and ATCC 49766 for *H. influenzae*.

Table 4. Subjects and methods used in the clinical studies (III, IV).

Paper	Design	Setting	Intervention	Number of subjects
III	Experimental	<i>In vivo</i> evaluation of xylitol concentrations in saliva	2 pieces of xylitol chewing gum (1.68 g) or 5 mL xylitol mixture (2 g)*	65
IV	Double-blind randomised	Clinical trial in 48 day-care centres in Oulu	Xylitol in chewing gum, mixture or lozenges during URI	1277

* Equal to the doses used in previous clinical trials.

4.1 Effect of fructose on xylitol-induced growth inhibition (I)

Twenty-one strains of *S. pneumoniae* were cultured in brain heart infusion broth (BHI) containing 0.2% glucose supplemented with the sugar or sugar alcohol concentrations shown in Table 5. Each test was carried out in triplicate. The bacterial growth was observed by optical density (O.D.) at a wavelength of 650 nm with an SFM 35 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) and evaluated against the standard medium, measurements being performed every 1 to 2 hours during the logarithmic phase of growth. To confirm the relationship between O.D. and the total number of viable bacterial cells, viability counts were made by the standard dilution method on sheep blood agar plates during all growth phases. The growth of pneumococci in media containing xylitol was compared with that in control media in presence of various other sugars, with special attention paid to the effect of xylitol in the presence of fructose. The effect of another sugar alcohol, sorbitol, was evaluated separately and also in combination with xylitol.

Table 5. Growth media used in series I.

Control medium*	Test medium	Focus of the experiment
BHI only	BHI + 2.5-5% xylitol	Effect of xylitol
BHI + 1-5% fructose	BHI + 1-5% fructose + 5% xylitol	Effect of xylitol in the presence of fructose
BHI + 1-5% glucose/ galactose/ sucrose	BHI+1-5% glucose/ galactose/ sucrose + 5% xylitol	Effect of xylitol in the presence of sugars other than fructose
BHI only	BHI + 1-5% sorbitol	Effect of sorbitol
BHI only	BHI + 2.5% sorbitol + 2.5% xylitol	Effect of sorbitol-xylitol combination

* All concentrations weight/volume.

4.2 Effect of xylitol on the ultrastructure of otopathogens (II)

Five strains of pneumococci and one strain of *H. influenzae* were used at the late exponential phase to ensure sufficient amounts of bacteria for electron microscopy (optical density 0.3-0.4 at 650 nm wavelength). Three hundred microlitres of the suspension was transferred into 3 ml of test medium containing 0.5-5% xylitol. *H. influenzae* bacteria were cultured in BHI containing 10% (vol/vol) FCS and 6 µl of HTM supplement in 3 ml of growth medium up to an optical density count of 0.3-0.4, corresponding to the late exponential phase. Three hundred millilitres of the suspension was transferred into 3 ml of test media containing 5% xylitol. The comparisons were made between bacteria grown in media containing xylitol and bacteria grown in control media without xylitol. The numbers of viable bacteria were estimated after the experiments by plating dilutions of bacterial samples. Bacteria were harvested by centrifugation after 0.5 h to 2 h of xylitol exposure. The bacterial pellet was pre-fixed with 1 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer containing 100 µl of ruthenium red for staining and the bacteria were then incubated overnight, washed in

phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon LX112. Thin sections were cut with a Reichert Ultracut ultramicrotome and examined with a Philips CM100 transmission electron microscope.

For statistical analyses, the proportions of totally damaged and dividing bacteria and of bacteria in diplococci form were calculated based on a minimum of 100 bacteria in each sample. The diameter of a whole bacterium and the thickness of the polysaccharide capsule and bacterial cell wall of a sample of 30 bacteria using ATCC 49619 and ATCC 49766 as seen in electron microscopy were also measured. Morphological changes were described and documented photographically.

4.3 Xylitol concentrations in the saliva of children (III)

A total of 65 normal, healthy 1-6-year-old children were recruited from day-care centres in the city of Oulu with the informed consent of their parents. Xylitol doses equal to one single dose administered in our previous clinical trials were used. The children were given two pieces of chewing gum simultaneously, each containing 0.84 g xylitol with no other sweeteners, and continued chewing them for the whole observation period of up to 15 minutes. Other children received 5 ml of syrup containing 400 mg/ml of xylitol diluted in water, which was administered slowly through a plastic syringe over a period of 5 minutes. The children aged 4-6 years spit samples of saliva out through a funnel into a plastic test tube at different measurement times, while corresponding samples of the saliva of the children aged 1-3 years were collected directly from the mouth using a plastic Pasteur pipette. The collection of saliva took 1-2 minutes through the funnel and 3-5 minutes by pipette. Each child gave one to two samples at different measurement times. These samples were cooled immediately and frozen at -20°C within 3 hours. The volume of saliva needed for the analyses was one ml. Xylitol concentrations were determined enzymatically using the polyol dehydrogenase-based procedure of Boehringer Mannheim (Mannheim, Germany).

4.4 Prevention of AOM with xylitol given only during URI (IV)

This double-blind placebo-controlled randomised trial involved 1366 healthy children aged 10 months to 7 years recruited from 48 day-care centres in the city of Oulu. Written information was given to the parents, after which the purpose of the trial and its protocol were further explained in evening sessions at the centres. Only children whose parents gave their written informed consent were included in the trial. Children receiving antimicrobial prophylaxis or having craniofacial abnormalities were excluded. A personal history and data on risk factors for AOM were collected from the parents by means of a questionnaire. The children were screened with tympanometry (MiniTymp®, Welch Allyn) before starting the trial, and all those who had normal tympanograms (A-curve) were accepted for participation. Those with abnormal tympanograms (other than an A-curve) were checked by pneumatic otoscopy. Any middle ear effusions were treated before starting the trial, and children with current acute respiratory tract infection (ARI)

but healthy ears at screening were re-examined after one week. Altogether 1277 children had normal ear status and were eventually accepted.

The daily doses of control and xylitol products were equal to those used in earlier trials intended to evaluate the efficacy of continuous administration of xylitol. Children unable to chew gum were randomised to receive 5 ml of either a control mixture containing 20 g/L xylitol diluted in water without any other sweeteners or a mixture containing 400 g/L xylitol, in both cases 5 times a day (daily doses of 0.5 g and 10 g of xylitol, respectively). Parents were advised not to give their children any other xylitol preparations during the follow-up. The mixtures were administered after meals with a syringe over a period of 5 minutes in order to maintain a high concentration of xylitol in the oral cavity for as long as practically possible.

A flow chart of the study design is shown in Figure 3. The children who were able to chew gum were randomised to receive control chewing gum (daily dose 0.5 g of xylitol), xylitol chewing gum (daily dose 8.4 g of xylitol) or xylitol lozenges (daily dose 10 g of xylitol). In this way the products were given to 1277 children with normal ear status and without current URI for use during the next URI episode. Compliance was monitored by asking the parents to list the doses actually given on daily symptom sheets, and by counting the unused pieces of chewing gum and lozenges returned at the last appointment and measuring the volume of unused mixture.

Parents were instructed to start giving the product on the first day of ARI, which was defined as the appearance of one or more of the following symptoms: clear or purulent discharge from the nose, congestive nose, cough, conjunctivitis, sore throat, or earache. The children usually had a fever as well (axillary temperature above 38.0°C) and in some cases vomiting. The first visit to the clinic was scheduled within 4 days of the onset of symptoms, or for the same day if the child had earache.

Tympanometry (MiniTymp[®], Welch Allyn) was performed by two trained nurses at the clinic. If the result was normal (A-curve), the child was examined weekly until resolution of the symptoms or for up to 3 weeks, but if it was abnormal (B, C or positive pressure curve) or the child had earache or discharge from the ear, the child was examined by pneumatic otoscopy by a physician engaged in the trial. The final diagnosis of AOM was based on a finding of middle ear effusion in tympanometry (B, C or positive pressure curve) and always confirmed by pneumatic otoscopy. Five AOM cases out of the total of 155 (3%) were not diagnosed at all at the trial clinic and were based only on the family physician's evaluation. These included one case with purulent ear discharge. Dropouts were defined as children who stopped visiting the clinic. Children who prematurely stopped using the product assigned to them but continued to visit the clinic were included in the analysis. Each child participated in the trial for one episode of infection only.

The study was blinded as far as the mixture and chewing gum groups were concerned but open as between the xylitol lozenge and control chewing gum groups. The chewing gums were quite similar in taste, but the xylitol mixture was sweeter than the control mixture. Randomisation was performed in blocks of four in the mixture groups and in blocks of three in the chewing gum and lozenge groups, using a random number table in order to make the proportion of participants in each group about the same at each day-care centre. Each child was given a unique participation number at the time of the initial screening.

The calculations of sample size were based on the occurrence of URI and AOM in previous trials, where 75% of the children suffered at least one URI within a period of 3 months and the occurrence of AOM during the first URI in the control groups was 20% and 13% at ages of 1-3 years and 4-6 years, respectively. It was estimated that if the follow-up time were extended to 4 months, over 90% of the children would experience at least one ARI. Since a 45% reduction in the occurrence of AOM was considered to be clinically important with 0.05 type I error (P-value) and a power of 80%, it was calculated that sample sizes of 203 younger children receiving the mixture and 274 in each group receiving the chewing gum and lozenges were needed (altogether 1220 children).

The mixture groups were analysed separately from the chewing gum and lozenge groups, due to the higher incidence density of AOM among the young children. The children who dropped out (n=24) were excluded from the statistical analysis, but those who prematurely stopped using the products but still visited the clinic (n=35) were included. A normal standard deviate test was used to compare the proportions of children with AOM diagnosed between the control group and the group receiving xylitol. The Kaplan-Meier method was used to analyze the time elapsing before the first AOM during the four-month trial. The children who dropped out contributed days at risk to the cumulative occurrence analysis for as long as they continued to participate. The log rank test was used to test the differences in time elapsing before the first AOM.

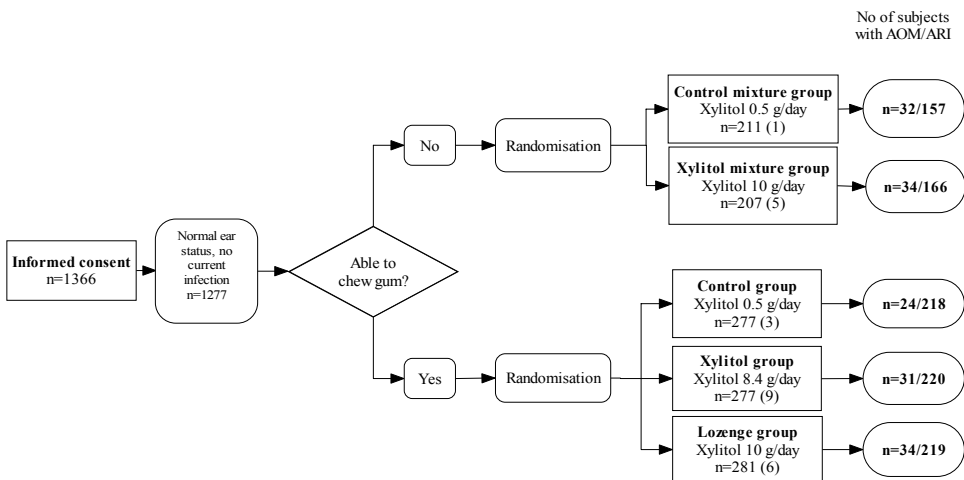


Fig. 3. Study design and main results. Randomisation was performed in two groups depending on the ability to chew gum. Separate analyses were performed for the mixture groups and the chewing gum or lozenge groups due to the higher AOM incidence density among the younger children. The mixture and chewing gum groups were blinded.

5 Results

5.1 Effect of fructose on xylitol-induced growth inhibition (I)

Marked growth inhibition was detected in the presence of 5% xylitol in the basic medium with 0.2% glucose, as in our previous studies (9), but this was prevented by all the fructose concentrations used: 1%, 2.5% or 5% (Figure 4).

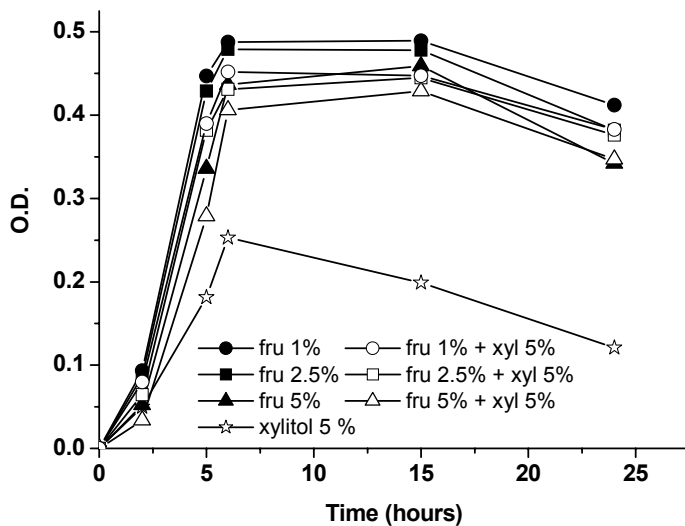


Fig. 4. Mean growth of 11 and 21 *S. pneumoniae* strains measured in terms of O.D. (optical density). Addition of fructose to the growth media eliminated the xylitol-induced growth inhibition.

The addition of 1% glucose, 1% galactose or 1% sucrose did not alter the effect of xylitol, so that growth inhibition was still detected (Figure 2). The mean O.D. of pneumococci in the media containing xylitol at 5 hours was 53% less than in the media with 1% glucose (CI 45-63 %, $p<0.0001$) and 53% less than in the media with 1% galactose (CI 46-65 %, $p<0.0001$). Likewise, the figure at 2 hours was 50% less (CI 31-68 %, $p<0.0001$) than that in 1% sucrose (Figure 5). Xylitol-induced growth inhibition was also observed in the presence of 5% glucose. The mean of O.D. of pneumococci remained lower in all the xylitol media tested than in the corresponding control media throughout the observation period ($p<0.0001$).

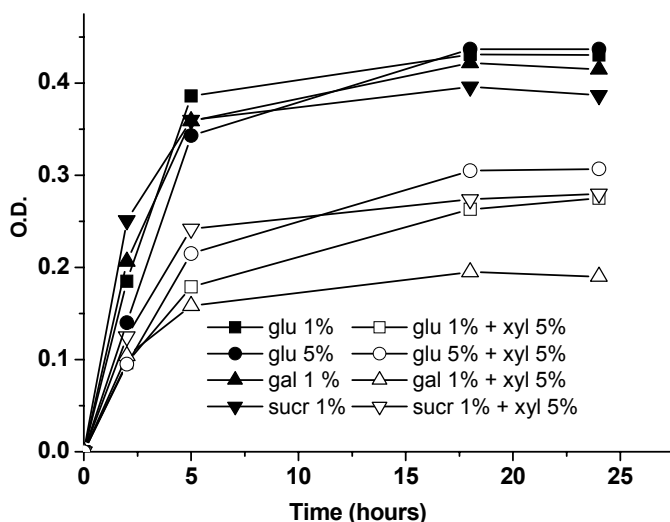


Fig. 5. Mean growth of 11 *S. pneumoniae* strains measured in terms of O.D. (optical density). Addition of glucose, galactose or sucrose did not modify the xylitol-induced growth inhibition of pneumococci, and a significant decrease in growth was still detected.

The xylitol-induced inhibition of pneumococcal growth was seen systematically with the ATCC 49619 strain and with all the isolated pneumococci ($n=21$) except for one, of serotype 7, which was the only strain isolated from a sinus aspirate.

5.2 Effect of sorbitol on growth of *S. pneumoniae* (I)

Sorbitol at concentrations of 1%, 2.5% and 5% had no effect on the growth of pneumococci, and xylitol at a concentration of 2.5% was consequently equally as effective alone as in combination with 2.5% sorbitol (Figure 6), growth inhibitions of 52% (95% CI 35-68%, $p<0.0001$) and 46% (CI 28-60%, $p<0.0001$), respectively, being observed.

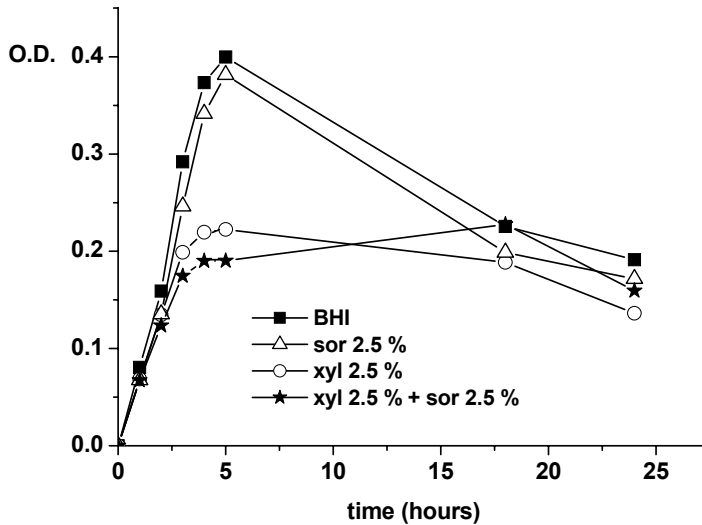


Fig. 6. Mean growth of 11 strains of *S. pneumoniae* measured in terms of O.D. counts. Sorbitol at a concentration of 2.5% did not reduce the growth of pneumococci, nor did it modify the effect of xylitol in the growth medium.

5.3 Effect of xylitol on the ultrastructure of otopathogens (II)

The cell walls of the pneumococci became diffuse and less well defined after exposure to 5% xylitol for two hours (Figure 7) and the normal trilaminated cell wall structure was damaged. These morphological changes were seen in all the serotypes tested, but were most prominent in the 19F serotype (ATCC 49619). The mean diameter of the polysaccharide capsule (ATCC 49619) became wider: 119 nm (SD 44.8) after xylitol exposure as compared with 100 nm (31.4) in the control medium, a difference of 19 nm (95% CI 3.6-34.6, $P=0.008$). Also, the wider polysaccharide capsule was sparse and ragged. Exposure to 0.5% xylitol for 2 hours resulted in similar changes in the bacteria to those seen after exposure to 5% xylitol. The morphology of the pneumococci remained unchanged after exposure to 5% xylitol for 0.5 hours

There was no difference in the proportion of dead bacteria relative to the control media after 30 minutes of xylitol exposure, but after 2 hours of 5% xylitol exposure the proportion of totally damaged or autolyzed bacteria was 13% in the xylitol media as compared with 3% in the control media (CI of the difference 0.07%-12.2%, $P<0.0001$). The proportion of dividing pneumococci was reduced after exposure to 5% xylitol for 30 minutes relative to that in the control medium (26% vs. 53%; CI of the difference 14-37%, $P<0.001$), and the proportion was likewise smaller after 2 hours of exposure (10% vs. 26% in the control media; CI of the difference 12%-19%, $P<0.0001$) and after

exposure to 0.5% xylitol for 2 hours (20% vs. 32%; CI of the difference 0.03%-21%, $P=0.008$). Pneumococci exposed to 5% xylitol for 2 hours were less often seen in the form of diplococci than were the control bacteria, and they failed to form the longer chains comprising 4 to 6 pneumococci at a timethat were seen in the control media.

The ultrastructure of the pneumococci was not damaged after exposure to 5% glucose or 5% fructose, but after sorbitol exposure the cell wall structure became slightly more diffuse and the polysaccharide capsule was wide and ragged. The proportion of totally damaged pneumococci was 6% when exposed to 5% sorbitol, as compared with 1% when grown in BHI (CI of the difference 1%-10%, $P=0.01$) and 23% in the xylitol media (CI of the difference 10%-24%, $P<0.001$).

The general morphology of *H. influenzae* was not altered by 5% xylitol even after 2 hours of exposure, but the mean diameter of the cell wall was thicker than in the control media. The proportion of totally damaged or dead bacteria after exposure to 5% xylitol for 2 hours was 8%, as compared with 0.8% in the control media (CI of the difference 0.02%-12%, $P=0.005$).

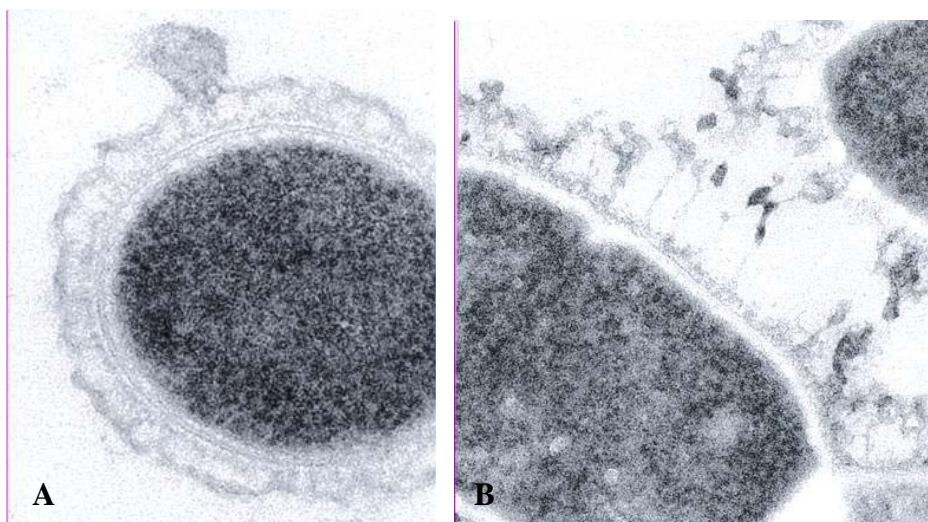


Fig. 7. Ultrastructure of *S. pneumoniae* 49619 in the control medium (A) and after exposure to 5% xylitol for 2 hours, as seen by electron microscopy, magnification x 25 500. The cell wall became more diffuse and the polysaccharide capsule wider and more ragged after xylitol exposure.

5.4 Xylitol concentrations in the saliva of children (III)

The concentration of xylitol in the children's saliva rose to 90 mg/ml immediately after they had been given the two pieces of chewing gum, but it then fell rapidly, and only traces were detected after 15 minutes (Figure 8). When using the xylitol mixture the concentration reached almost the same level as was detected in the chewing gum group, but the maximum was reached later, the highest single value, 260 mg/ml, being detected

after 3 minutes of observation (Figure 8). A concentration of 1%, i.e. sufficient for an antimicrobial effect, was achieved in all the children in the chewing gum group and most of those in the mixture group, but only a few children receiving the mixture had concentrations above this level after 5 minutes.

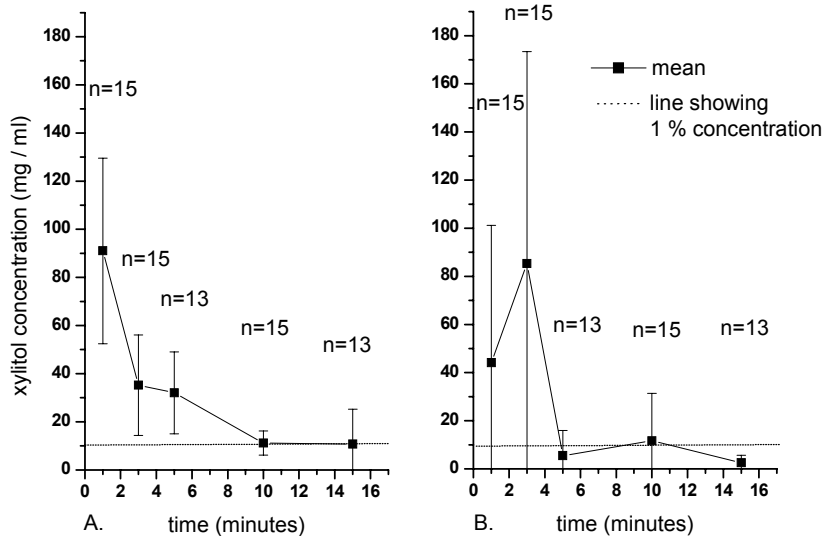


Fig. 8. Xylitol concentrations in the saliva of children aged 4-6 years after starting to chew two pieces of chewing gum (1.68 g xylitol) (A) and in that of children aged 1-3 years after 5 mL xylitol mixture (2 g xylitol) given over 5 minutes through a syringe (B). The dotted line indicates a concentration of 1% (w/v), sufficient for an antimicrobial effect *in vitro*.

5.5 Prevention of AOM with xylitol given only during URI (IV)

Altogether 1277 children were eligible for the trial, i.e. they had normal tympanometry and no current ARI symptoms, and these were randomised to receive either the control mixture (n=212), the xylitol mixture (n=212), the control chewing gum (n=280), the xylitol chewing gum (n=286) or xylitol lozenges (n=287). These groups did not differ in demographic features, known AOM risk factors or AOM history. There were 24 dropouts, which left 1253 children eligible for analysis. Altogether 980 of these (78%) experienced URI and visited the clinic. The proportion of children with URI was similar in all the groups.

AOM occurred in 32 out of 211 children in the control mixture group (15.2%) and 34 out of 207 children in the xylitol mixture group (16.4%), i.e. in 32/157 (20.4%) and 34/166 (20.5%) of the cases of ARI, respectively (Table 6). Among the older children, it was diagnosed in 24 out of the 277 children randomised to receive control chewing gum (8.7%) and 31 out of the 277 in the xylitol chewing gum group (11.2%), implying 24/218

(11.0%) and 31/220 (14.1%) of the corresponding children with URI (Table 7). AOM was diagnosed in 34 out of the 281 children in the xylitol lozenge group (12.1%) and 34/219 (15.5%) among the children with ARI (not shown in the table). None of the differences was statistically significant (Table 6 and 7). The occurrence of AOM during the four-month trial was comparable in time in all the groups and did not differ in its timing during URI.

Table 6. Proportions of children with AOM among those receiving mixtures during acute respiratory tract infection (ARI).

	Control mixture (n=211)	Xylitol mixture (n=207)	Difference	95% CI	P value
Number of children with ARI (%)	157 (74)	166 (80)			
Total number of children with AOM	32	34			
% of children having AOM	15.2	11.2	-1.2	-8.3 to 5.8	0.72
% of children with ARI having AOM	20.4	20.5	-0.1	-8.9 to 8.8	0.98

Table 7. Proportions of children with AOM among those receiving chewing gum during acute respiratory tract infection (ARI).

	Control chewing gum (n=277)	Xylitol chewing gum (n=277)	Difference	95% CI	P value
Number of children with ARI (%)	218 (79)	220 (79)			
Total number of children with AOM	24	31			
% of children having AOM	8.7	11.2	-2.5	-7.6 to 2.5	0.32
% of children with ARI having AOM	11.0	14.1	-3.1	-9.4 to 3.2	0.33

About 80% of the products were administered as intended in all the treatment groups, and only a few children (n= 37) stopped taking them prematurely, except in the group receiving the xylitol lozenges, where 20 out of 219 (9%) did so, mainly because they disliked them or suffered from abdominal discomfort. All but two of the children whose parents stopped giving them the product continued to visit the clinic. In the case of 20% of the URI episodes administration of the products was started later during the infection than had been intended, and in order to test whether the timing of xylitol ingestion could have affected the results, we also performed the analysis separately for those subjects

who received the product on the first day of URI and received at least 80% of the intended doses, but there were no differences between the groups receiving control or xylitol products in this analysis, either.

6 Discussion

6.1 Effect of fructose on xylitol-induced growth inhibition (I)

Most sugars cannot be used as antimicrobial agents, as both microbes and host cells are able to utilize them. Xylitol, which differs from most carbon sources due to its five-carbon polyol structure, is metabolised *via* the pentose pathway in humans, but is unsuitable as an energy source for many bacteria (220,222). Its antimicrobial effect on *S. mutans* is based on its transport and phosphorylation through a fructose phosphotransferase system, which is possible due to its structural analogy to fructose (233,235,253). Unlike mammal cells, *S. mutans* lacks the ability to metabolise xylitol phosphate any further, which leads to an energy-consuming process of xylitol expulsion and intracellular accumulation of xylitol phosphate (233,237), resulting in growth reduction. Fructose is capable of preventing the inhibition of *S. mutans* growth by blocking the entry of xylitol into the bacteria cell. The finding that fructose also prevents the xylitol-induced inhibition of *S. pneumoniae* growth supports the hypothesis that the mechanism of action of xylitol in *S. pneumoniae* is similar to that in *S. mutans* (I). Since phosphotransferase systems play a role not only in sugar phosphorylation but also in the regulation of bacterial functions and gene expression in different nutritional and host environments (225,226), xylitol exposure may disturb the cellular metabolism in *S. pneumoniae* in addition to inhibiting growth.

Xylitol-insensitive *S. mutans* strains, which lack fructose PTS, have been found in habitual xylitol consumers (239,258). Altogether 30 pneumococcal strains from patient samples were tested for xylitol sensitivity in series I here and in an earlier study by Kontiokari et al. (9), and only one strain, of serotype 7, has shown no effect of xylitol on its growth. Although xylitol has been widely used as a sweetener in Finland for two decades, the results of *in vitro* trials (I) (9) and the efficacy of xylitol in clinical trials indicate that most pneumococcal strains are sensitive to it. Marked clinical efficacy is achieved even though the nasopharyngeal carriage of *S. pneumoniae* remains unchanged during xylitol consumption (10,261).

Sorbitol, which is widely used as a sugar substitute and is more common than xylitol due to its lower price, has been shown to have minimal or no effects on the growth of

mutans streptococci in the presence of glucose (222,227) and had no effect here on the growth of pneumococci (I). Sorbitol alone is also less effective than xylitol for caries prevention (252,262). It may enhance the inhibitory potential of xylitol in mutans streptococci by alternating with the intracellular metabolism of xylitol (263), but it neither enhanced nor inhibited the effect of xylitol on pneumococcal growth. It is unlikely that the combination of xylitol and sorbitol in chewing gums would provide any clinical benefit for the prevention of otitis media relative to pure xylitol chewing gums. The dose of xylitol needed to prevent acute otitis media is in any case quite high, so that its partial replacement with sorbitol would be an illogical approach.

6.2 Effect of xylitol on the ultrastructure of otopathogens (II)

Growth studies comparing xylitol media with others (I) by means of optical density measurements show that xylitol has antimicrobial activity with respect to *S. pneumoniae*. Unlike the usual antimicrobial agents, it restored the pneumococci to a viable state in spite of their impaired growth (I), which is in accordance with the observation that xylitol does not reduce the nasopharyngeal carriage of pneumococci (8,10). The experiments reported in paper II were designed to elucidate the mechanism of action of xylitol in preventing AOM, as it is effective despite the unchanged carrier rate.

The electron microscopy findings confirmed that the number of pneumococci decreases after xylitol exposure, as the number of dividing pneumococci is less and the number of dead or autolysing bacteria greater (II). Also, chain formation was disturbed in the xylitol media and more single bacteria were seen. The remaining viable bacteria had an altered ultrastructure, as the cell wall became diffuse and the polysaccharide capsule became wider and ragged in appearance. Interestingly, the morphology of the pneumococci in xylitol exposure was identical to the ultrastructure described for the transparent phenotypic variant of *S. pneumoniae* (136).

Pneumococci undergo spontaneous phase variation, which alters their ability to cause pneumococcal diseases (136,137,264). The transparent variants of *S. pneumoniae* are associated with the nasopharyngeal carriage of pneumococci, whereas the opaque variants are more likely to cause invasive diseases and also more capable of causing AOM during experimental URI (138). The transparent variants also produce hydrogen peroxide to compete with other bacteria in the nasopharynx, which may result in a decreased number of *H. influenzae* there (265). A possible phenotypic variation in *S. pneumoniae* towards the transparent form (II) could explain the good clinical efficacy of xylitol in AOM prevention despite the unchanged nasopharyngeal carriage of *S. pneumoniae*.

The effect of xylitol on *H. influenzae* was less significant, with a slightly thicker cell wall and an increased proportion of damaged bacteria (II). A fructose PTS has been detected in some strains of *H. influenzae*, and xylitol is capable of blocking the system, leading to disturbed utilization of many sugars other than glucose (232). It is thus possible that xylitol may also affect the cellular functions of *H. influenzae*, leading to changes in morphology and virulence (266) and possibly decreased growth in the presence of sugars other than xylitol, since xylitol has not been shown to reduce the growth of *H. influenzae* in the presence of glucose in previous studies (9).

6.3 Xylitol concentrations in the saliva of children (III)

The measurement of xylitol in saliva is a reasonable approach for evaluating the pharmacokinetics of xylitol, as its effect is most likely to be a local one. Consuming 1 g xylitol per kilogramme of body weight in one dose yields a serum concentration of 0.015% (w/v) (220), which is much lower than the concentration of 1% required for antimicrobial activity *in vitro*. Saliva concentrations also provide an estimate of xylitol concentrations in the nasopharynx, which is the critical site for development of AOM. The experiments in series III confirmed that xylitol concentrations high enough for antimicrobial activity were attained in the saliva by giving xylitol in a chewing gum or mixture in an amount equal to the single doses used in previous clinical trials (10,11). Even so, the high xylitol concentrations disappeared rapidly, within 15 minutes.

The fact that high peak concentrations of xylitol are effective in preventing AOM despite the minimal duration of concentrations exceeding the level needed for antimicrobial activity may be explained by the mechanism of action involved. Pneumococci take up xylitol rapidly within minutes of exposure (Kontiokari *et al.*, unpublished observation), most probably through a fructose PTS (I). Like oral streptococci, they are probably not able to utilize the metabolite, xylitol phosphate, which results in a toxic effect on these bacteria (233,235). Xylitol disturbs the protein synthesis of mutans streptococci (242), and the time needed for the bacteria to recover after the toxic effect and metabolic damage is probably longer than the duration of xylitol exposure. This mechanism of action resembles that of antimicrobials, which disturb protein synthesis. Aminoglycosides act in this manner, and their effect persists even after their levels have fallen below the minimum inhibitory concentration. This enables them to be used once daily in clinical practice (267). The results presented in series III here indicate that high single doses administered less frequently than was the case in our earlier clinical trials could be effective in preventing AOM episodes in children and should be tested in further clinical trial. This is supported by the finding that a regimen of xylitol administration three times a day is effective in preventing dental caries (252).

6.4 Prevention of AOM with xylitol given only during URI (IV)

The randomised clinical trial reported in paper IV was conducted in order to find a more practical way to implement xylitol prophylaxis in clinical practice. It had previously been found that five doses of xylitol per day given regularly prevented AOM effectively, but as this regime may constitute a constraint on the use of xylitol in clinical practice, the two main alternative approaches to the problem were either to reduce the number of doses per day or to focus the prophylaxis on the times of greatest AOM risk (i.e. ARI episodes). As we thought that it would be most convenient for the families to give their children xylitol only during ARI episodes, we decided to test this option. The trial nevertheless demonstrated that such a regime was ineffective in preventing AOM.

Our finding is analogous to that regarding antimicrobial prophylaxis, which is effective in preventing AOM when applied continuously but ineffective when applied intermittently during ARI (17,178,182). Bacterial adherence to pharyngeal cells is

enhanced during viral infection and the incidence of otitis media pathogens in the nasopharynx is increased at such times (94,95,116,129). Viral infection leads to changes in bacterial adherence from the first day of inoculation onwards, i.e. 1 to 3 days before the appearance of symptoms (116,268), which may be the explanation for the ineffectiveness of intermittent prophylaxis, since preventive measures initiated in response to symptoms of ARI miss the incubation period and may thus be too late.

Since it is also possible that the results could have been influenced by parents' hesitation in starting the prophylaxis in response to minor or gradually developing symptoms of ARI, separate analyses were performed on the subgroup of children for whom the timing of the xylitol prophylaxis was optimal according to the symptom diary sheets. The prophylaxis could still not be shown to be effective, however. There were no clear differences between the control and xylitol groups in abdominal discomfort or in the number of children who stopped taking the product, with the exception of the xylitol lozenge group, where abdominal discomfort and dislike of the product was common. The xylitol dosing schedule was accepted well by the parents and compliance during the trial was good and comparable to that achieved in the trials where xylitol was used regularly (10,11). Therefore the lack of efficacy was not due to problems of compliance.

The consumption of xylitol products must be continuous in order to be effective in preventing AOM. Continuous administration of xylitol at frequent doses has been criticised as being difficult, since a total of 5600 pieces of xylitol chewing gum are required to prevent a single AOM episode in children (269). Although less frequent dosing would be ideal, the intensive regime may not be such a constraint for children, as they like the sweet taste of the xylitol mixture and xylitol chewing gum and accept xylitol products after meals with pleasure. The widespread use of xylitol for caries prevention has made it familiar, and xylitol chewing gum is almost a routine in many day-care centres. Thus xylitol is the only available method that can be used easily in combination with risk factor intervention to prevent AOM in unselected child populations.

7 Conclusions

1. The effect of xylitol in inhibiting the growth of *S.pneumoniae* may be mediated *via* a fructose phosphotransferase system in a similar manner as in *S. mutans*, since fructose prevents this inhibition.
2. Sorbitol does not inhibit the growth of *S.pneumoniae*. Partial replacement of xylitol by sorbitol in products used for the prevention of AOM is unlikely to provide any clinical benefit.
3. The ultrastructure of *S.pneumoniae* was altered after xylitol exposure. Dead and autolysing bacteria were abundant in electron microscopy samples, and the remaining viable bacteria were identical in appearance to the transparent phenotypic variant with decreased virulence in AOM pathogenesis. The properties of the transparent variant could explain the clinical efficacy of xylitol in spite of the unchanged nasopharyngeal carriage of pneumococci.
4. The xylitol concentrations in the saliva of children after doses equal to those used in previous clinical trials exceeded the level needed for antimicrobial activity *in vitro*. The high peak concentrations of xylitol disappeared rapidly, implying that they are more important as such for the efficacy of prevention than is the time for which xylitol concentrations exceed the antimicrobial level. Thus, a regime with less frequent dosing could be effective and should be tested in further clinical trials.
5. The administration of xylitol during respiratory infections only was ineffective in preventing AOM. Thus xylitol should be administered continuously for this purpose.

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