DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
233

RIINA RUNNEL

Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
233

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
233

RIINA RUNNEL

Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries

UNIVERSITY OF TARTU
PRESS
1632

Department of Stomatology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy (Medicine) on 21 January, 2015 by the Council of the Faculty of Medicine, University of Tartu, Tartu, Estonia.

Supervisor: Professor Mare Saag, MD, PhD
Department of Stomatology, University of Tartu, Estonia

Supervisor: Professor Eino Honkala, DDS, DDPH, MSc, PhD
Faculty of Dentistry, Kuwait University, Kuwait

Supervisor: Professor Kauko K. Mäkinen, BSc, PhD
Institute of Dentistry, University of Turku, Finland

Reviewed by: Professor Mihkel Zilmer, MD, PhD
Department of Biochemistry, University of Tartu, Estonia

Professor Reet Mändar, MD, PhD
Department of Microbiology, University of Tartu, Estonia

Opponent: Professor emeritus Heikki Murtomaa, MD, PhD
Institute of Dentistry, University of Helsinki

Commencement: May 27, 2015

The publication and the commencement of this dissertation is granted by the European Union through the European Social Fund.

This research (ClinicalTrials.gov identifier: NCT01062633) was supported by Cargill R&D Centre Europe (Vilvoorde, Belgium).

ISSN 1024-395X
ISBN 978-9949-32-784-3 (print)
ISBN 978-9949-32-785-0 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I Runnel R, Honkala S, Honkala E, Olak J, Nõmmela R, Vahlberg T, Mäkinen KK, Saag M. Caries experience in the permanent dentition among first- and second-grade schoolchildren in southeastern Estonia. Acta Odontologica Scandinavica 2013; 71: 410–5.

II Honkala E, Runnel R, Honkala S, Olak J, Vahlberg T, Saag M, Mäkinen KK. Measuring Dental Caries in the Mixed Dentition by ICDAS. International Journal of Dentistry 2011; doi:150424.

III Honkala S, Runnel R, Saag M, Olak J, Nõmmela R, Russak S, Mäkinen PL, Vahlberg T, Falony G, Mäkinen K, Honkala E. Effect of Erythritol and Xylitol on Dental Caries Prevention in Children. Caries Research 2014; 48:482–90.

IV Runnel R*, Mäkinen KK*, Honkala S, Olak J, Mäkinen PL, Nõmmela R, Vahlberg T, Honkala E, Saag M. Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables. Journal of Dentistry 2013; 41:1236–44.
*Authors contributed equally to this work.

Author's contribution:

I Clinical examination, plaque samples collection and evaluation, writing the manuscript.

II Clinical examination, plaque samples collection and evaluation, co-author of the manuscript.

III Clinical examination, plaque samples collection and evaluation, an additional study group preparation, co-author of the manuscript.

IV Clinical examination, plaque samples collection and evaluation, an additional study group preparation, writing the manuscript.

LIST OF ABBREVIATIONS

dmfs	Number of decayed, missing and/or filled surfaces of primary teeth
DMFS	Number of decayed, missing and/or filled surfaces of permanent teeth
dmft	Number of decayed, missing and/or filled primary teeth
DMFT	Number of decayed, missing and/or filled permanent teeth
HPLC	High performance liquid chromatography
ICDAS	International Caries Detection and Assessment System
LB	Lactobacilli
MS	Mutans streptococci
WHO	World Health Organization

1. INTRODUCTION

Dental caries is a globally occurring disease, which has exhibited some signs of decreasing but still causes problems at the individual as well as the national public health level. The caries index in Estonia, especially among children, is still higher than in Scandinavia, where governments have contributed to caries prevention programmes and the awareness of people is high.

Caries is a dynamic process, which is hidden before the damage also becomes visible for people without special education and culminates at the end of a negative scenario with the formation of a cavity. It is a prolonged process, requiring a couple of years, and therefore, needs long-term caries prevention methods.

Contemporary theories support the role of sugar consumption in the development of caries, and therefore, the replacement of cariogenic sugars with non-cariogenic substances, like xylitol or other less cariogenic sugar alcohols, is one way to reduce the risk of caries.

Although the history of polyols as non-cariogenic sweeteners is long – studies about the effect of xylitol on caries prevention have been carried out over decades and have demonstrated evidence-based success. The preventive effect of polyols has not always been confirmed or approved. The complexity of this is concealed in the numerous factors of its influence, which need a well-designed study and a multifaceted assessment of the results.

However, there is not much data about alternative sugars like erythritol, which has only been researched in short-term studies. The interest of this clinical trial was to investigate and compare the influence and effectiveness of the long-term consumption of different polyols on mixed dentition.

The present study was designed as a double-blind randomised controlled prospective clinical trial with 3-year intervention (polyols consumption) among elementary school level children which included annual clinical examination of the participants in the Department of Stomatology at the University of Tartu.

2. REVIEW OF THE LITERATURE

2.1. Dental caries

2.1.1. Dental caries development

Dental caries is a world-wide public health problem caused by specific oral bacteria that can affect all dental hard tissues (Kidd 2005). In particular, acidogenic bacteria can cause demineralization and eventual devitalisation of the teeth. The cariotic process begins when acid produced by bacterial metabolism of certain dietary fermentable carbohydrates dissolves Ca^{2+} and inorganic phosphate ions present in the hydroxyapatite mineral that is a main inorganic component of enamel (Fejerskov and Kidd 2008). Dissolution of this mineral normally starts when the pH of dental plaque decreases below 5.5. Because the salivary buffer mechanism attempts to repair the damage caused by acids, the counteracting remineralisation process controlled by saliva will set off when the plaque pH returns to the neutral zone (Marsh and Nyvad 2008).

In summary, the pathological factors involved in the caries process include specific cariogenic bacteria, the presence of fermentable carbohydrates in food, and various salivary effects. Dental caries may be regarded as a main cause of tooth loss at younger ages from childhood to adult life.

2.1.2. Formation of dental plaque

The human body is a place for a large number of different micro-organisms. Hundreds of species of transit flora have been detected in the mouth, many of them representing the permanent microbiota. A particular group of bacteria, called mutans streptococci, has been associated with the initiation of dental caries (Tanzer et al. 2001). These organisms include *Streptococcus mutans* and *Streptococcus sobrinus* whose differentiation in dental plaque and saliva has become possible by means of laboratory tests that have turned out to be time-consuming and costly (Seki et al. 2006). Certain aciduric and acidogenic lacto-bacilli and a number of other bacteria also contribute to the overall structure and metabolism of dental plaque (Avila et al. 2009, Takahashi and Nyvad 2011).

The tooth enamel is normally covered by a thin layer of saliva origin. This biofilm, called pellicle, normally serves as the first stage of plaque formation. Electron microscopic studies have shown that the first bacterial cells, following thorough professional oral prophylaxis, normally adhere to the pellicle. Some of the first colonisers include streptococci, notably *S. oralis*, *S. sanguis* and *S. mutans*, which normally comprise less than one per cent of all microbes present in the average dental plaque (Marsh and Martin 2000, Featherstone 2004, Garcia-Godoy and Hicks 2008).

The biofilm community on the tooth surface comprises multiple combi-nations of colonies from different species of microbes, an extracellular matrix,

voids and fluid channels. Dental plaque is a vital, changing unit where variable physic-chemical interaction between bacteria and the substrate take place (Rosan and Lamont 2000).

The specific role of mutans streptococci in dental caries formation is manifested by their ability to convert the glucose molecules into gel-like extracellular dextran – polymers that constitute the immediate outer capsule of those bacterial cells. These dextran molecules are characterised by their ability to bind to the pellicle or sometimes directly to the hydroxyapatite structure (reviewed by Law et al. 2007). The mutans bacteria are able to exploit the glucose and also the sucrose molecules for the dextran formation. Owing to the high chemical energy present in the glycosidic bond of the sucrose molecule, sucrose constitutes a very favourable substrate for plaque dextran formation (Jenkinson and Lamont 1997). The most important caries-associated acids formed by plaque during bacterial metabolism normally include lactic acid, propionic acid and acetic acid (Lamont et al. 2006).

2.1.3. Risk factors of caries

Caries as a multifactorial preventable disease has multiple risk factors including acidogenic bacteria, previous caries, dietary carbohydrates, low fluoride intake, salivary dysfunction, low socioeconomic status and so on (reviewed by Harris et al. 2004).

2.1.3.1. Early childhood caries

Caries among preschool children has been shown to be a risk factor for caries in their permanent dentition (Tinanoff et al. 2002, Olak et al. 2007). The combination of non-cavitated caries lesions in the permanent dentition, cavitated lesions in primary molars and past caries experience variables have been suggested as predictors of caries (Zhang et al. 2006, Sagheri et al. 2009).

2.1.3.2. Carbohydrates

The frequency of the consumption of fermentable carbohydrates is a critical factor in caries activity, as described above. The type of carbohydrate, form of carbohydrate contained in the product, and contact time with the tooth surface are the key factors during the consumption of carbohydrates. Hence, studies have shown the role of glucose in the initial caries development process (Moynihan et al. 2004, 2014), and a positive association between caries and the amount of consumed sugars (Freeman 2014, Sheiham and James 2014).

2.1.3.3. Fluorides

Previous studies, summarized by Petersen and Lennon (2004), have shown that higher intake of fluorides decreases caries. The development of dental caries is strongly related to fluoride levels in drinking water (Vogel 2011, Peckham and Awofeso 2014), the topical application of fluorides (Ijaz et al. 2010, Chestnutt et al. 2012) and/or using fluoridated oral hygiene products (Marinho et al. 2004, Petersen and Lennon 2004, Rugg-Gunn and Banoczy 2013, Cury and Tenuta 2014).

2.1.3.4. Socioeconomic status

A number of researchers have reported that high frequency carbohydrate intake in combination with inadequate oral hygiene is more common in groups with lower socioeconomic status, especially in early childhood (Hallett and O'Rourke 2003, Petersen 2005, Naidu et al. 2013). The lowest income recorded by Statistics Estonia 2010 (Ingel et al. 2010) is in the South-East (Valgamaa, Võrumaa, Põlvamaa), North-East (Ida-Virumaa) and Central Estonia (Jõgevamaa), which partially coincides areas with low fluoride levels in drinking water and a high prevalence of caries.

2.2. Caries registration

2.2.1. Epidemiology of dental caries in Estonia

The most widely used index for assessing caries experience is the DMFT index, where D marks the number of decayed, M missing and F filled permanent teeth (Larmas 2010). The corresponding index for primary teeth is dmft. The DMFT index has been used in dental epidemiology for over 70 years to describe caries experience. The data are collected worldwide by the WHO Data Bank.

In Estonia, various non-representative studies (Wolf et al. 1996, Alanen et al. 2000, Dragheim et al. 2000, Olak et al. 2007) have been carried out to find out caries prevalence and experience in different age groups. The government has funded some limited projects (Saag et al. 1998); other data have been received during the above-mentioned surveys or clinical studies. However, the data are incomplete and do not include the total population and not all age groups.

The prevalence of caries (in of children with dmft>0) in primary dentition among very young children aged 2–4 years, reported by Olak et al. (2007), was 44% for boys, 34% for girls, and as a mean 41.6% (p=0.05). The prevalence of caries varied from 30.3% to 56.2% depending on the region of the study. The higher prevalence was reported in southern areas (Valga, Võru). Early childhood caries (baby bottle caries) has been found to differ significantly between geographical areas – from 14.0% to 31.2% (p=0.01), being extremely

high in Võru. The percentage of children without caries (dmft=0) was 37% at 24–35 months and 18% at 36–47 months (p=0.005) (Olak et al. 2007).

The study in municipal schools in Tartu showed a prevalence of 83.8% (dmfs 7.7) in primary dentition among 7-year-olds compared to 62.1% (dmfs 5.3) in Danish children of the same age (Dragheim et al. 2000). The caries prevalence in Estonia at the age of 12 was 69.5% and increased rapidly to 81.0% among 15-year-olds (Wolf et al. 1996).

The WHO reported the average number of teeth with caries or caries experience in permanent dentition (DMFT) among 12-year-olds in Estonia as having been high – 4.1 until the early 1990s, but this has decreased since regaining independence being 2.7 in 1998 (2.6 by Kõll-Klais et al. 2004). The latter is also the European average (DMFT 2.6), being higher than in Scandinavia but lower than in the other Baltic countries (Whelton 2004, Gudkina et al. 2008, Patel, 2012).

The dmft/DMFT has been shown to vary in different parts of Estonia. The highest indices in permanent dentition (up to 6) among 12-year-olds are in the central-north and in the south-eastern areas. Wolf (1996) reports the mean DMFT among 14 to 17-year-old pupils was 3.7 in Tartu and 7.0 in Tallinn. There could be several reasons for this variation, including risk factors which are described above.

2.2.2. ICDAS II caries registration system

The DMFT index does not include and does not describe the need for the treatment of early caries lesions. Clinical studies, however, require the registration of early caries lesions stages as well. For these purposes, the International Caries Detection and Assessment System (ICDAS II) was developed by a group of international caries research experts. The purpose was described by Ismail et al. (2007) – to integrate several new caries recording systems and to create an evidence-based caries detection and assessment system, which would be oriented at caries prevention.

Multiple studies (Ismail et al. 2007, Kühnisch et al. 2008) have analysed or compared the ICDAS II system in clinical trials. The weaknesses of the ICDAS system include poor visual control of the contact surfaces and occlusal surfaces without radiographs (Diniz et al. 2009), and because it is highly time consuming (Ormond et al. 2010 and Aidara et al. 2011). Some researchers (Bertella et al. 2013) report the necessity of radiographs prior to the final decision for underlying dark shadows (ICDAS code 4) on permanent molars and in occlusal caries.

At early school age, only the first permanent incisors and molars have erupted. The ICDAS system has been declared to diagnose proximal caries of molars provided the proximal surface is opened for visual control (e.g. a primary molar has exfoliated and a permanent premolar has not erupted yet) (Ekstrand et al. 2011). Recent studies (Isamil et al. 2007, Diniz et al. 2009,

Mendes 2010) have shown that pre-cleaned and dried tooth surfaces and the comprehensive training of observers improves caries detection. The testing of the ICDAS II system continues, and researchers emphasize the importance of preliminary training and necessity of further development before using those codes in epidemiological surveys (de Amorim 2012).

The ICDAS II system is appropriate to record and to monitor the progress of the caries lesions during long-term trials both in primary or permanent dentition (Pitts 2009). Hence, it can be argued that combining examination using the ICDAS system with alternative diagnostic methods in clinical work could improve the detection of cariotic lesions (Mitropoulos et al. 2010).

2.3. Caries prevention

The control or elimination of caries risk factors, in addition to regular oral hygiene procedures through different methods makes it possible to prevent and control the cariotic process. Fluorides, salivary calcium, phosphates, proteins and salivary flow have a significant part in de- and re-mineralization processes in enamel.

2.3.1. Fluorides

Fluorides are chemical compounds which contain the element fluorine. The importance of fluorides in caries prevention is due to the higher resistance of fluorapatite − the enamel tissue where fluoride ions replace calcium to tolerate a lower pH.

2.3.1.1. Systemic delivery of fluorides

The systemic, regular long-term intake of fluorides takes place through everyday water consumption (mainly through drinking water). The level of fluorides in the groundwater in Estonia varies extensively from 0 (south-eastern region) to 7 mg/L (in the west, Silurian-Ordovician aquifer system), presented by Karro et al. (2006) and Indermitte et al. (2007). In addition to the south-eastern area where the fluoride level is low (0–0.3 mg/L), there are also two towns (Tallinn and Narva) with relatively low concentrations of fluorides because surface water is used instead of groundwater. This, however, has an effect on the development of caries in children.

The children growing up in areas with optimal (0.8–1.2 mg/L) or high levels (>1.2 mg/L) of fluorides in their drinking water have a higher content of fluorides in the enamel surface and thus higher resistance to the dissolving of calcium and inorganic phosphate ions (pH up to 4.5 against the 5.5 in case of natural enamel). There is a statistically significant correlation between fluoride levels in drinking water and caries development (systemic effect) (McDonagh et

al. 2000, Armfield 2010). Systemic intake of fluorides is prolonged, and therefore, a principle factor in the development of resistant enamel structure before tooth eruption.

The concentration of the fluoride above the optimum level during the tooth developmental phase can affect the normal growth of the teeth and increases the risk of the dental fluorosis. Critical time is from infancy and continues up to 8 years – the period of the active development and mineralization of permanent tooth germs (before eruption). Symptoms of fluorosis, as they were described by Thylstrup and Fejerskov in 1978, vary from the fractional, unnoticeable white opaque areas or stripes (mild form) to severe forms where all tooth surfaces of all teeth are affected – discoloured, mottled (brown strain) areas and pitting of the teeth.

In 2004, 4% of the population of Estonia (nearly 4,000 citizens) had excessive exposure to natural fluoride (over 4 mg/L), mostly in the western and central part of Estonia (Indermitte at al. 2009). Since 2004, the reduction methods have been applied to optimise the content of fluorides in public water supplies, which have been successful due to the implementation of osmosis technology and bore wells have started using new groundwater layers. In 2012, only 380 consumers still had an extremely high exposure to fluorides (Indermitte et al. 2014).

The systemic delivery of fluorides is individual because of the different levels of fluoride in the drinking water in Estonia and the place of residence in childhood always needs specifying to clarify the overall intake of fluorides during the development of teeth.

2.3.1.2. Topical fluoride intervention

Fluoride ions are highly active, and therefore, they have a tendency to fixate on hard tissues, including enamel. The formation of fluorapatite is a result of de- and remineralisation processes during the substitution where the hydroxyl ion in enamel is replaced by a fluoride ion (Kutsch et al. 2013).

Topical intake of fluorides could be professional applications (with fluoride varnishes *etc*.) or individual (mostly daily use of fluoridated toothpaste). Numerous studies in Cochrane systematic reviews have demonstrated the preventive effect of topical methods on permanent teeth while the evidence is weak or moderate and inconsistent in primary dentition (Tubert-Jeannin et al. 2011, Marinho et al. 2002, 2003 and 2013). An extra topical application of fluoride varnish in addition to daily tooth brushing with fluoride toothpaste (1,000 ppm) may have a slight effect in primary dentition only in high-risk groups (Agouropoulos et al. 2014).

The impact of fluorides is complex, and therefore, calculating the actual effect is complicated, depending on concentration, dose, frequency and combination of fluorides, earlier caries severity, oral hygiene skills, supervised tooth brushing and so on (Marinho et al. 2004, Walsh et al. 2010).

2.3.2. Polyols (sugar alcohols, alditols)

The general formula of sugar alcohols alias polyols alias alditols is $H(HCHO)_{n+1}H$ – the difference is in chain length. They are polyalcohols derived by the reduction of an aldose-polyol dehydrogenase ($NADP^+$), which reversibly converts aldoses to alditols. Alditols are naturally occurring sweet substances found in low amounts in plants, micro-organisms and animal bodies. Industrially they are often obtained on a large scale through the hydrogenation of sugars (except the erythritol) (Monedero et al. 2010).

All polyols can be divided into hypo- or non-acidogenic groups, based on the fermentation by oral microbiota (*in vitro*) evident in the pH measured in dental plaque. The plaque pH, measured *in vivo,* is an indicator of the cariogenicity of polyols or food products. If the plaque pH decreases below 5.5 (which is associated with initial caries) after exposure to fermentable sugar alcohols because of the formation of organic acid in dental plaque, this product is classified as cariogenic. In comparison to this, the alditols are low- or non-cariogenic substances. The anti-caries and/or caries preventive characteristics of polyols are of interest in the field of caries prevention.

A systematic review provided by Moynihan and Kelly (2014), based on clinical studies, supports the evidence of the positive effects of the combination of dosage and frequency of fermentable dietary carbohydrates, chiefly sucrose consumption and caries level. This has brought about an increase in the popularity of polyols in food products. The cause for this, however, is the food industry's interest in finding a suitable, less cariogenic and low-caloric replacement for sugars. The most common alternative is sorbitol, but the trials in oral biology research continue testing and comparing it with other alditols-based sugar-free food substances.

Another relevant factor in polyols is the level of sweetness, which varies in the case of different polyols. The relative sweetness of common sugar alcohols is reported in Table 1. In addition, the molecular parameters, which determine properties of sugar alcohols, are significantly different. The metabolism of common sugar alcohols is well studied, and therefore, it is known that alditols are mostly not metabolised by oral microbiota and polyols are only partially absorbed in the small intestine (up to 80% for sorbitol). The absorption of dietary polyols in the gastrointestinal tract can be active or passive depending on the molecular size and the chemical specificities of the sugar alcohols (Table 1). Payne et al. (2012) have reported that this absorption depends on the degree of polymerisation.

When polyols dissolve, it has a cooling effect in the mouth, which is a result of an endothermic process, negative heat from the solution. Xylitol and erythritol have the strongest cooling effect, which can cause discomfort for some people. One way to relieve this is to combine different agents. Polyols are non-reactive, and therefore, they can be mixed with other sweeteners in products.

The toxicity of polyols has been the topic of several studies. However, earlier studies have been based on animal subjects but the metabolism in the human body does not have exactly the same pathways as in animals (Lina et al. 1996). Those experiments often exposed extremely high doses of the test components. Hence, animal tests do not allow us to infer that the toxicity for humans is similar.

The well-known side effect of almost all polyols is gastrointestinal distress via a laxative effect. Tolerance of polyols varies depending on the type of sugar alcohol, the quantity consumed and individual resistance. Side-effects are absent up to a specific amount consumed. Common complaints after taking large doses of polyols include the following intestinal symptoms: bloating, diarrhoea, abdominal pain because of bowel movements (irritable bowel syndrome) and nausea. Mannitol lingers in the intestinum longer than other sugar alcohols, and therefore, causes side-effects more often. One well-tolerated polyol is erythritol. When avoiding the consumption of excessive amounts of products with sugar alcohols, they are perfectly safe.

Allergic reactions against polyols are rare. Only a couple of cases, such as urticarial, have been reported by Hino et al. (2000) and Yunginger et al. (2001). Some other symptoms, such as hives, skin rashes, difficulty breathing, swelling of the mouth and hands, dizziness, vomiting, weakness, and even anaphylactic shock after consuming erythritol or others sugar alcohols have been reported by Shirao et al. in 2013.

The aim of caries prevention using polyols is to replace the carbohydrates after a meal with some less-cariogenic sugar alcohols. The most effective method is exposure to a polyol immediately after eating, indicatively 4–5 times per day. Prolonged contact between the product and the teeth is required – not less than 2–3 minutes.

The most widely used and tested polyols are xylitol (a pentitol type sugar alcohol), sorbitol, mannitol (hexitol), maltitol, lactitol (12-carbon polyols) and mixed products (van Loveren 2004). The newest promising subject is erythritol (tetritol), tested by Kawanabe (1992) and Mäkinen (2001).

Table 1. Properties of sorbitol, xylitol and erythritol (modified from de Cock and Bechert 2002)

Sugar alcohol	Relative sweetness to sucrose	Food energy (kcal/g)	Maximum dose of polyols not causing laxative side effect (g/kg body wt)
Sorbitol	0.5...0.6	2.4 (EUR) … 2.6 (US)	0.17…0.24
Xylitol	0.9...1.0	2.4	0.3
Erythritol	0.7...0.8	< 2	0.6…0.8

2.3.2.1. Sorbitol (glucitol)

Sorbitol is a 6-carbon hexitol type polyol ($C_6H_{14}O_6$). It occurs naturally and is produced synthetically from glucose. Sorbitol is a common ingredient in (oral) hygiene products (e.g. in toothpastes and mouthwashes) as a humectant and thickener or as a sweetener. It occurs naturally in some fruits (especially in prunes) and berries. The caloric energy of sorbitol is 2.6 kcal (versus 4 kcal in sugar) and its sweetness has nearly 50% of the relative sweetness of sugar. The laxative effect of sorbitol (by drawing water into the large intestine and thereby stimulating bowel movement) is higher than with others polyols.

The consumption history of sorbitol is long, its metabolism and side effects are well investigated, and therefore, it is known that absorbed sorbitol can be converted in a human body to glucose, but this process is not efficient. Unabsorbed parts can cause disturbances in the digestive tract. Because the absorption and metabolism of sorbitol in the human body are slow, the cariogenicity of sorbitol is lower than sugar, and sorbitol has sometimes been used as a low cariogenic control substance in clinical trials (Mickenautsch et al. 2012).

2.3.2.2. Xylitol

Xylitol, pentitol type 5-carbon polyol ($C_5H_{12}O_5$), is naturally found in fruits and vegetables. Commercial xylitol is extracted from corn or birch wood or other xylan-containing plants. The sweetness of xylitol is like white, table sugar but the caloric value is 2.4 kcal/g compared to 4 kcal/g in sugar. The human body synthesises 5–15 g xylitol per day, where the xylitol is an intermediate product in carbohydrate metabolism in the clucoronate-xylulose cycle. The body does not require insulin to metabolise xylitol and therefore xylitol has been approved in special dietary foods, e.g. for diabetics.

Xylitol has a long history – it has been used since the early 1970s. Xylitol, as a non-cariogenic and anti-cariogenic replacement for sugar, is popular in dietary products and in oral hygiene articles, especially in the US and in Finland. In the Turku Sugar Study (Scheinin and Mäkinen 1975), the potential of xylitol was thoroughly tested. In addition, numerous clinical trials have been carried out to investigate the effectiveness of xylitol.

Repeated studies over 40 years (Sheinin et al. 1975, 1985, Kandelman and Gagnon 1990, Mäkinen et al. 1995) have confirmed the significant reduction of dental caries on several occasions, both in low- or high-risk groups. Xylitol has been used as a non-cariogenic sweetener, with the sweetness similar to sugar. The oral bacteria are generally not able to metabolize xylitol (metabolism is possible by some mutans streptococci species), and therefore, no acid production follows from the use of it. The study by Twetman et al. (2003) reported that lactic acid concentration reduced significantly in dental plaque after xylitol experience. The increased pH in dental plaque does not take place

and demineralisation is slower or non-existent. This could be one of the most important mechanisms of xylitol for caries prevention.

Xylitol has also been used to reduce the quantity of dental plaque – it prevents the sticking of bacteria to teeth (Mouton et al. 1975, Söderling and Hietala-Lenkkeri 2010) although recent in vitro studies did not confirm this (Giertsen et al. 2011, Decker et al. 2014). Mäkinen et al. (1989) and Isokangas et al. (1991) have shown that xylitol is able to reduce the growth of mutans streptococci in the dental plaque, both after short- and long-term use. Thabuis et al. (2013) demonstrated a similar significant reduction of the growth of three other cariogenic species (*S. sobrinus*, *Actinomyces viscosus* and *Lactobacillus*) in dental plaque. This effect occurs through inhibiting *Streptococcus mutans* metabolism (Trahan 1995, Lingström et al. 1997). This was first discovered in *in vitro* studies (Knuuttila and Mäkinen in 1975) and since then, the mechanism has been repeated in several clinical trials (Miyasawa-Hori et al. 2006).

In the majority of studies chewing gum containing xylitol has been used (Sheinin and Mäkinen 1976, Kandelman and Gagnon 1887, 1990, Wennerholm et al. 1994, Mäkinen et al. 1995, Thabuis et al. 2013, Keukenmeester et al. 2014). Since chewing gum stimulates the secretion of saliva, which is a substantial factor in the process of pH neutralization, this could be an additional impact factor. However, xylitol can reduce dental plaque even in the form of candies (Alanen et al. 2000, Shyama et al. 2006). In addition to the preventive effect, xylitol has been found to promote tooth remineralisation. The inference has been based on the re-hardened caries lesions and negative caries lesions increment (Kandelman and Gagnon 1990). The recommended daily dosage for a caries preventive effect is 6–10 grams of xylitol (in different forms – chewing gum, tooth paste, mouthwash or candy), preferable regularly 5 times every day (Kandelman and Gagnon 1987), although studies using xylitol-sweetened chewing gum three times per day have shown *Streptococcus mutans* inhibitory effects (Autio 2002, Kiet et al. 2006, Holgerson et al. 2007).

The long-term use of xylitol increases the portion of xylitol-resistant mutans streptococci, which lacks the fructose phosphotransferase system. This adaptation mechanism minimises the anti-cariogenic effect of xylitol but does not imply acidity (van Loveren 2004). Trahan (1995) has shown that the percentage of MS does not increase in dental plaque but increases in saliva. One feasible explanation given by Trahan et al. (1992) is more easily shed xylitol-adapted strains. The same could explain the reduction of dental plaque during xylitol intake.

2.3.2.3. Erythritol

Erythritol is a tetritol, 4-carbon polyol ($C_4H_{10}O_4$). It is the first industrially produced polyol from glucose using a fermentation process with yeast, *Moniella pollinis*. Erythritol occurs naturally in some fruits, algae, fungi, lichens and fermented foods. The relative sweetness of erythritol is 60–70% of that of table

sugar (sucrose). The cooling effect is close to xylitol –stronger than with the others alditols. The erythritol differs from others sugar alcohols, mostly because of its small molecular size and its unique digestion pathway.

The first interest from the food industry was to use erythritol as a noncaloric bulk sweetener. The new trend among consumers towards natural components accepts erythritol, which is made naturally by fermentation and satisfies the food safety requirements. Erythritol is well tolerated in the gastrointestinal tract because of its property to be well absorbed (via passive diffusion) in the small intestine but not metabolized (fermented) in the body, and therefore, it is qualified as a non-caloric polyol. Erythritol has an estimated energy value near 0, maximum 0.24 kcal/g (de Cock 1999, 2012, de Cock and Bechert 2002), and it has no effect on blood glucose and insulin levels. Excretion from the blood takes place through the kidneys (about 90%) and non-absorbed erythritol (~10%) that remains unchanged passes to the large intestine and is excreted with the faeces (Storey et al. 2007). This part of erythritol may be fermented by the microbiota of the colon. The laxative effect of erythritol is considerably smaller than after consuming others alditols because 90% is absorbed in the small intestine before moving to the large intestine.

Erythritol has been shown (by Kawanabe et al 1992 and Mäkinen et al. 2005) to be similar to xylitol in its dental plaque reducing effect. The mechanism of this effect has been examined for a shorter period and there are fewer studies about erythritol compared to alditols that have been in use for a longer period like sorbitol and xylitol. The *in vitro* experiment conducted by Kawanabe (1992) has shown that the species of streptococci do not produce either lactic nor other acids from erythritol. Similar test results have been shown by xylitol, but at the same time other widespread sugar alcohols indicated the same acid production. As reported by Söderling and Hietala-Lenkkari in 2010, streptococci were not able to grow and mostly not produce insoluble dextran in plaque from erythritol.

***

In conclusion, both erythritol and xylitol have a clinical effect on caries prevention despite their biochemical mechanisms being different (Mäkinen et al. 2005).

When designing the current study, there were no reports available on comparative long-term surveys of the impact of erythritol and xylitol consumption on dental caries development.

3. AIMS OF THE STUDY

The general aim of the research was to evaluate the experience of caries among elementary school children and to test the impact of long-term polyol consumption on caries prevention.

The purposes of this study were:

1. To identify the prevalence of caries in permanent teeth and mixed dentition among first and the second grade schoolchildren in south-eastern Estonia.
2. To determine the associations of the number of caries lesions between permanent and primary molars using ICDAS in the mixed dentition.
3. To test and to compare the clinical effects of long-term intervention using polyols (erythritol, xylitol and sorbitol as a positive control) on the development of dental caries in mixed dentition.
4. To compare the effects of interventions using erythritol, xylitol and sorbitol on saliva and dental plaque.

4. SUBJECTS AND METHODS

4.1. Selection of the schools and subjects

The 3-year intervention study was a double-blind, randomized, placebo-controlled prospective clinical trial. The study was conducted in south-eastern Estonia, where the level of fluorides in the drinking water is extremely low (near 0) and the DMFT index is the highest in Estonia (Ingel et al. 2010). The number of schools in this region, which includes 4 counties, was 102 in 2008, and the combined number of first and second grade pupils was 4,150 (Ingel et al. 2010). Schools stratified according to localisation and size were selected randomly from all municipal schools in this area (10%). One urban school, four regional centre schools, four rural schools and one elementary school, based on the proportional distribution, were selected. The invitation was sent via email. When the school refused (n=1) or did not respond (n=10), another school from the same strata (location, size of the school) was invited. The schools were not requested to state the reason for their refusal.

When the management of the school agreed to participate in the study all the first and second grade children were included. Two to five first and second year classes per school were involved. The estimated sample size required for the three-year trial was 151 children in each intervention group where the expected drop out rate was 25%, according to the Belize study (Mäkinen et al. 1998). All first year classes in the schools were divided into three different study groups randomly using computer-generated numbers. The second year classes were distributed into different groups as appropriate to reduce a school bias.

The written informed consent forms (n=522; Figure 1) were signed by the parents or guardians before the survey. The Research Ethics Committee of the University of Tartu approved the clinical trial (the study protocol 166/T-7). The clinical trial was registered with the US National Institute of Health register, identifier number NCT01062633.

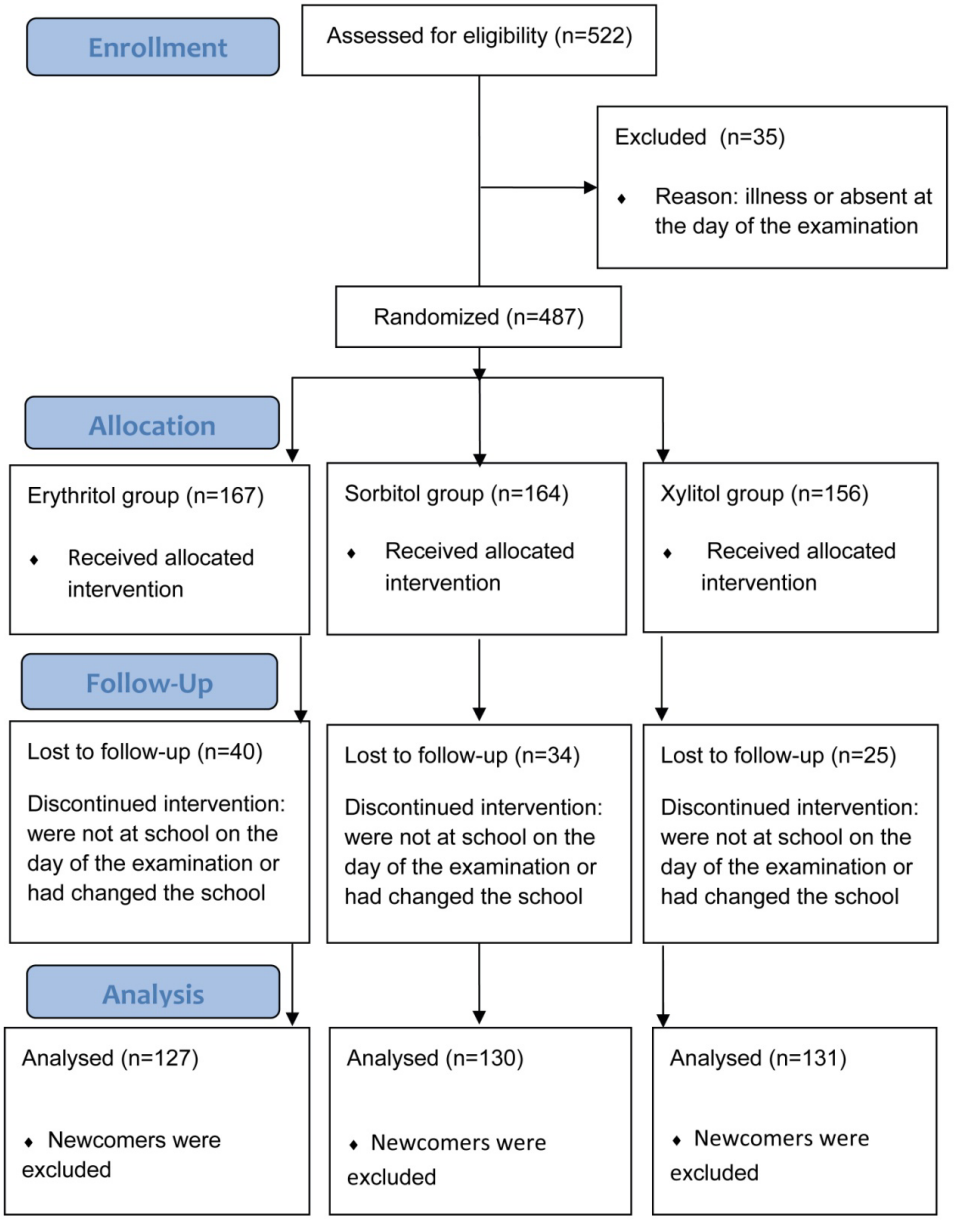

Figure 1. Flow chart of the clinical trial

4.2. Clinical examinations

The clinical examinations were carried out in the Department of Stomatology (University of Tartu) at baseline and once a year (exactly at 12, 24 and 36 months) during the trial. The examination included caries status registration, and plaque and saliva sample collection. Altogether, 485 children (45.6% boys and 54.4% girls) were observed in the standard dental units at baseline. The mean age of children in the first grade at baseline was 7.8 years (SD=0.35) and in the second grade 8.8 years (0.38). On the day of the examination 7.1% (n=37) of the children were absent. According to the teachers, the main reason for the absence was illness.

The double-blind clinical examinations using ICDAS II criteria were completed by four examiners. The computer-based 90-minute e-learning programme, training and calibration which included examination, discussion and re-assessment of the same objects and inter- and intra-examinations of investigators were carried out two days before every annual examination. This process was guided by a senior clinical researcher. At first, eight children, not participating in the study, were examined by all of the four team members. All caries findings and disagreements were discussed and resolved after re-assessment of the lesions. On the second day, 25 children were studied twice by one of the three examiners and by the senior examiner. During the study the scheduled 10 pupils were examined twice by each examiner and another 10 by the senior examiner. The inter- and intra-examiner consistency of the examiners was high (surface and tooth-based weighted kappa >0.9).

4.2.1. Saliva sample collection

The children were instructed not to brush their teeth on the morning of the examination day. The regular daily diet (breakfast and light lunch, if needed) was allowed. The pupils were blindly assigned to one of the four examiners. Dental plaque samples were taken for the Orion Diagnostica (Espoo, Finland) Site Strips Test from each quadrant by means of disposable Quick-Stick® microbrushes (Dentonova AB, Huddinge, Sweden) by gently rubbing and rotating near the gingiva between the teeth, from the interdental area of the mesial surface of the first permanent molars.

Dental plaque – from all available, free surfaces of the teeth – was collected during three minutes using a dental probe. The plaque was immediately weighed, suspended in sterile 0.9% NaCl, frozen in dry ice and stored at –80 °C for further chemical analyses at a laboratory.

The level of *Streptococcus mutans* (SM) and lactobacilli (LB) in the saliva were ascertained using industrially manufactured Dentocult® SM and Dentocult® LM test kits from the Orion Diagnostica company (Espoo, Finland) respectively. The tests were used to evaluate the counts of mutans streptococci (MS) in the saliva and in the (interdental) plaque of each quadrant and

aerobically cultured aciduric micro-organisms (mostly representing lactobacilli) from the saliva.

To gather saliva streptococci, a spatula was rotated in a closed mouth 4–5 times, touched the tongue and then gently removed through the subject's gently closed lips. The interdental plaque and saliva sample spatulas were cultivated in the same tube according to the manufacturer's prescriptions for two days (48 hours at 37°C). The quantity of mutans streptococci and other acidogenic micro-organisms was valued by three investigators – scored 0, 1, 2 or 3, based on the manufacturer's instructions – the guidelines from the Orion Diagnostica Dentocult® SM test. The mode of three examiners was used in the analyses. Scores 0 and 1 were <100,000 bacteria/ml saliva; score 3 was >1,000,000 bacteria/ml saliva and score 2 was the parameter between the scores 1 and 3.

Stimulated saliva, formed using paraffin, was collected while chewing a piece of paraffin (1.0 g) and the saliva flow rate – ml per minute – was measured. Some pupils were not able to follow the instruction during the saliva collection and sufficient volume of stimulated saliva for the LB test was then not collected. The same stimulated saliva from children was used for the LB tests (the Dentocult® LB). The slides were incubated for four days at 37°C. The number of lactobacilli and the other aciduric micro-organisms (the species was not distinguished) was counted by three people and scored 0, 3, 4, 5, or 6 per ml in the stimulated saliva, based on the manufacturer´s guidelines and a model chart, where zero marked no growth or a few colonies and six was abundant growth which covered the entire slide area. Again the mode of three assessments was used in the analyses.

4.2.2. Caries recording

Caries status was registered after the procedure described in the last paragraph and two-minutes of tooth brushing. All visible tooth surfaces in the mixed dentition were observed using a dental mirror and a standard light and compressed air from the dental unit. The blunt periodontal probe was used to detect any roughness of enamel surface. No radiographs were taken during the study. All restorations and caries lesions were recorded using the two-digit International Caries Detection and Assessment System (ICDAS II) where the first number denotes the restoration or sealant material and the second number shows the extent of cariotic lesions: early caries lesions in enamel (codes 1–3) and dentinal caries lesions (codes 4–6), maximum 28 teeth and 128 tooth surfaces.

A caries code 0 represents a sound, healthy tooth surface, free from any caries defects. The codes 1 and 2 mark the first visual changes in the enamel: 2 provided the lesion is seen immediately on the wet tooth surface during the examination and 1 if the white spot lesion is visible only after 5 seconds of drying with compressed air from the dental unit equipment. Code 3 denotes superficial defect, localised in the enamel – no dentine is visible. If the defect is

shown as a grey or darker shadow under the intact enamel (without cavitation into dentine) it would be marked as code 4. Both 5 and 6 indicate a caries lesion in the dentine: 5 if the distinct cavity is confined to less than half of the corresponding tooth surface and 6 when the bulk of the dentine of the surface involves caries.

No treatment procedures were carried out during the clinical examination. The written information about the need for restorative or orthodontic treatment was given to the parents. Oral health and diet education for participants took place in a lecture hall every year during the investigation. The basic recommendation was to brush teeth twice a day. A toothbrush and fluoride toothpaste (with 0.24% sodium fluoride) were given to all children twice a year.

4.3. Polyols consumption

All classes were randomly allocated (detailed description above) into three intervention groups – sorbitol (n=164), xylitol (n=156) and erythritol (n=165) group. The number of children dropped during the clinical trial (see Figure 1). The polyols were in the form of hard lozenges, designed for sucking and manufactured for this study by Cargill R&D Centre Europe. The weight of the candies was around 0.7 g and ~90% contained sorbitol, xylitol, or erythritol. Each polyol was consumed three times per day − four candies at a time, 12 daily − in the morning before the beginning of the first lesson, after school lunch (before noon) and at the end of the school day, immediately before leaving school. The candies were distributed and supervised by the class teachers. Daily polyol intake was about 7.5 g for around 200 school days per year.

Annual site-visits by the investigators took place during the study to control the storage of the polyols in schools and observe how the consumption of the candies was organised. A presentation was delivered in classrooms to enhance the motivation and awareness of the participants.

4.4. Chemical procedures

The fresh dental plaque samples, collected using a probe and placed in a disposable weighing cup at the time of the clinical examination within 3 minutes from all free tooth surfaces of the children, were instantly weighed on the analytical scales and dissolved in sterile physiological saline (0.9% NaCl) in an iced water bath. The plaque samples of the fixed group, ten plaque samples in a pool at baseline, were combined for HPLC-based analyses. In later years, the same ten subjects were part of the same collection process or if a child was absent during re-examination, the number of samples in the pool was smaller.

The specimens from the pools were frozen in dry ice and stored at –80°C and transported to the Cargill laboratory (Vilvoorde, Belgium) for chemical

analyses, where the concentration of plaque sugars, organic acids (acetic, propionic and lactic acid) and polyols was assessed using HPLC.

4.5. Data analyses

The final study groups for statistical analyses consisted of 485 pupils for epidemiological studies (Original Papers I and II) and of 374 children to investigate the impact of polyols (Papers III and IV).

All data were entered into an Excel table and were analysed using SPSS (version 18.0 and 19.0; SPSS Inc., Chicago, IL, USA) and/or using the SAS System for Windows (9.2 or higher; SAS Institute Inc., Cary, NC, USA). All the main statistical analyses were conducted at ClinData Services (Turku, Finland) by Tero Vahlberg (responsible statistician).

The ICDAS II caries codes were converted into DMFT indices for statistical analyses to produce descriptive epidemiological data. To assess the changes in caries lesions through the follow up, the ICADS II codes were used. The dynamics of the carious process was evaluated only on those 374 participants who were examined each year during the trial – on baseline and subsequently every year (four times in total) – those data were included to analyse the clinical caries preventive effect of polyols. The following surfaces and teeth were excluded from the analysis because in these cases it was not possible to follow the dynamics of caries: extracted teeth which during a previous examination were recorded as having dentinal caries, surfaces which were restored by a dentist before an investigation, and occlusal surfaces with partial or full sealants (restoration codes 1 and 2).

The scoring procedures for mutans streptococci and lactobacilli have been described above. The mode of the scores for mutans streptococci and lactobacilli, assessed by three investigators, was taken.

5. RESULTS

5.1. Caries experience in permanent teeth (baseline data)

At 7–9 years the first permanent molars have erupted and deciduous incisors replaced with permanent ones. The exfoliation time of primary molars (and canines) is commonly between 9 and 12 years. The permanent premolars rarely erupt in the first school years, except in cases of early loss where the primary molars have been extracted before physiological exfoliation because of pulpal infections. In that case the permanent premolars can replace the extracted deciduous molars earlier than happens normally.

In the case of the upper incisors, when recently erupted, even enamel caries damage was rare. In this case the proximal, labial and/or palatal surfaces of all upper incisors were involved. Only a couple of white spot lesions on the lower incisors were found during the examination.

The permanent molars appear to the end of the tooth arch at the age of 6 without any replacement of teeth and can remain unnoticed by parents who believe them to be primary teeth. The first permanent molars were the most affected teeth in the permanent dentition. According to the data from the examination (Paper I, Table 3), caries damage was found mainly in fissures (occlusal surfaces, buccal fissures in the lower and palatal fissures in the upper molars). Restorative treatment of dental decay had been carried out in 10.9% of the upper molars (teeth 16 and 26) and in 21.0% of the lower molars (36 and 46). The number of sealants was lower than expected, only 2.4% among the first graders' and 3.9% among the second grade children's fissures of the first permanent molars, which were partially or totally covered by sealants to prevent occlusal caries.

Caries was recorded annually during the investigation using the ICDAS II coding system separated as early stage caries lesions or caries defects in the enamel (ICDAS II codes 1–3) and dentinal caries – codes 4–6 as caries in the dentine is equal to the D (decay) component of the DMFT index. Enamel caries lesions are not considered for the DMFT index. The prevalence of caries – the proportion of children with $D_{4-6}MFT>0$ – in permanent dentition was 36.2% among first grade pupils and 48.3% among second grade pupils.

Most caries indices in permanent dentition were predominantly higher among the older, the second grade children, The mean $D_{4-6}MFT$ indices were 0.8 (SE=0.11) among the first graders and 1.1 (0.10) among the second graders (Figure 2). The mean $D_{4-6}MFS$ (caries or its treatment or complication by surface) indices coincided, respectively 1.6 (0.31) and 1.6 (0.17). There were no statistically significant differences in the caries experience between the genders in the mean caries indices: DMFT and DMFS indices, $D_{1-3}T$, $D_{1-3}S$ (enamel caries lesions), $D_{4-6}T$, $D_{4-6}S$ (dentinal caries lesions), and FT, FS (restorations) indices.

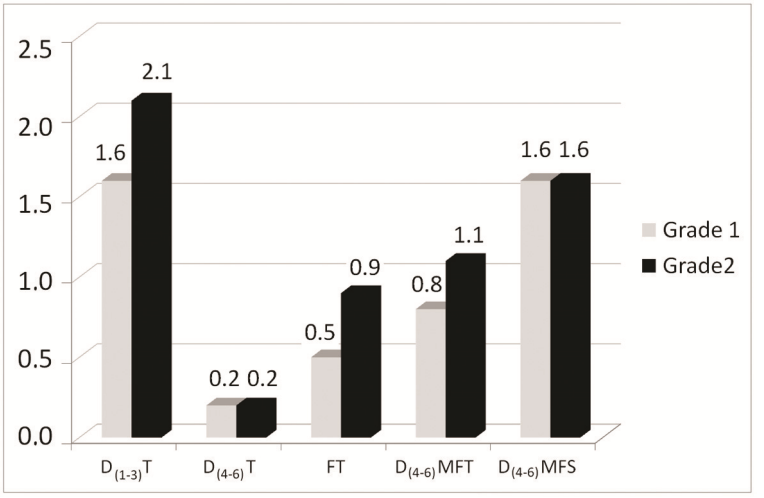

Figure 2. Mean caries indices (enamel and dentine) by grade

The mean number of enamel caries lesions ($D_{1–3}T$ and $D_{1–3}S$) and restorations (FT and FS) were significantly lower for the first graders than the second graders. The mean of enamel caries lesions by tooth ($D_{1–3}T$) in first graders was 1.6 (0.10) and the mean number of enamel caries lesions by surface, ($D_{1–3}S$) 2.2 (0.16). Among second graders, the same figures were respectively 2.1 (0.11) and 3.0 (0.18). At the same time, there was no statistically significant difference between the grades in the number of dentinal caries lesions: the mean $D_{4–6}T$ in both groups was 0.2 (SE=0.06/0.03). The $D_{4–6}S$ (dentinal caries by surface) for first grader children was 0.4 (0.12) and among second graders 0.2 (0.04).

5.2. ICDAS II codes in mixed dentition

During the trials all caries lesions were registered according to the ICDAS II two-digit system.

Teeth are more susceptible to damage immediately after eruption, generally during the first year in the mouth. Therefore, it was expected to find that caries lesions were more prevalent in the early stage (ICDAS II codes 1–3) in permanent molars, mainly on the occlusal surfaces of both lower and upper permanent molars but also on the buccal surfaces of the lower molars (Table 2). The teeth most affected by dentinal caries (codes 4–6) were predominantly the second lower primary molars.

Table 2. The mean percentages of ICDAS codes in the upper and lower first permanent molars and second primary molars according to tooth surfaces

	ICDAS codes								*ICDAS codes*						
	0	**1**	**2**	**3**	**4**	**5**	**6**		**0**	**1**	**2**	**3**	**4**	**5**	**6**
16/26								**36/46**							
M	97.7	0.3	0.4	0.3	0.0	0.2	0.0	**M**	94.6	0.3	3.6	0.4	0.0	0.2	0.2
O	62.5	12.4	17.0	4.8	0.8	1.4	0.0	**O**	68.8	7.5	13.6	6.4	1.1	1.5	0.3
D	99.0	0.0	0.0	0.0	0.0	0.0	0.0	**D**	99.1	0.0	0.0	0.1	0.0	0.0	0.1
B	91.5	2.2	4.8	0.3	0.0	0.1	0.0	**B**	64.5	9,4	16.3	7.3	0.6	0.9	0.2
L	83.4	4.7	9.0	1.0	0.3	0.5	0.0	**L**	97.3	0.5	1.3	0.0	0.0	0.0	0.1
55/65								**75/85**							
M	81.8	0.4	1.5	1.0	1.4	4.3	5.4	**M**	72.9	0.9	5.0	3.0	0.5	3.7	5.6
O	69.4	5.1	10.2	3.5	1.0	2.9	2.3	**O**	66.6	4.1	8.9	3.5	1.0	2.2	5.3
D	93.4	0.1	0.1	0.7	0.3	1.2	4.0	**D**	82.9	0.3	1.0	0.0	0.0	1.2	6.0
B	90.9	0.6	1.4	0.3	0.1	0.4	2.1	**B**	68.0	3,3	13.1	2.0	0.1	0.5	3.5
L	83.9	2.2	3.1	2.1	0.5	0.8	3.3	**L**	85.0	0.2	0.8	0.1	0.1	0.4	4.9

The most common finding was code 2 – first visible changes on the wet tooth surface in the enamel, when the dentine was not yet involved – in fissures on the occlusal surfaces of the upper permanent molars (17.0%) and on the buccal (16.3%) and occlusal (13.6%) surfaces of lower permanent molars. Codes 2, 3 (defects still only in the enamel), and 5 (limited visible lesion through the enamel in the dentin) per child dominated in their mixed dentition, while code 4 (shadow under enamel) was the least recorded. The highest mean number of affected surfaces per child were ICDAS II codes 2 (2.67) and 6 (extensive cavitation into the dentine; 1.96), and the lowest once again code 4 (0.68).

The distribution of ICDAS II codes between the first permanent molars and the second primary molars showed a significant correlation on the lingual surfaces of the upper molars and on the buccal surfaces of the lower molars (Paper II, Table 3).

The codes in mixed dentition by age and by grades varied (Paper II, Table 4) and were less informative because of physiological exfoliation and extractions of primary molars, which were extensively damaged by caries.

5.3. Clinical effects of erythritol and xylitol on caries prevention

At the start of the study, the mean age of the children in the erythritol group was 8.6 years (SD=0.5). In other groups the age was lower, 8.2 (0.5) in the xylitol and 8.1 (0.6) years in the control (sorbitol) groups ($p<0.001$). The percentage of boys was a slightly higher in all study groups: 57.0% in the erythritol, 54.5% in the xylitol and 51.8% in the control group ($p=0.645$).

There were no differences between the study groups in terms of basic caries indicators in their mixed dentition before the intervention, at the baseline. After one year of alditols consumption, the main caries indices were not statistically significantly different. In the second year of the study the numbers and percentage of teeth ($Dd_{4-6}Tt$) and surfaces ($Dd_{4-6}Ss$) with dentinal caries in mixed dentition were lower in the erythritol intervention group than in the xylitol group: Relative Risk (RR) for caries was 1.96, (95% CI 1.24–3.10) and 2.33 (95% CI 1.37–3.98), respectively. Similar changes were found in the third year when there was a higher number of dentinal caries on tooth surfaces ($Dd_{4-6}Ss$) (RR=1.93, 95% CI 1.12–3.33) in the xylitol group.
The development time of the caries lesions (both caries in enamel and dentin) was considerably slower over the three years ($p<0.001$) in the erythritol group than in the other groups (Paper III, Figure 2). The cariotic process affected 4.6% of the tooth surfaces of the erythritol group against 5.8% in the xylitol group and 5.5% in the sorbitol group (Table 3). At the same time, there were no statistically significant differences between the groups analysed by gender, age or school.

Table 3. Number and percentage of surfaces according to the analysis of caries development

Analysis	Erythritol	Sorbitol	Xylitol	p value[a]	Erythritol vs. sorbitol, p value[b]	Xylitol vs. sorbitol, p value[b]
Enamel/dentin caries development	860/18,763 (4.6%)	1,022/18,596 (5.5%)	948/16,414 (5.8%)	<0.001	<0.001	0.265
Dentin caries development	248/19,513 (1.3%)	333/19,406 (1.7%)	342/17,178 (2.0%)	<0.001	<0.001	0.052
Increase in caries score	1,046/19,645 (5.3%)	1,202/19,577 (6.1%)	1,163/17,366 (6.7%)	<0.001	<0.001	0.028
Decrease in caries score	401/1,313 (30.5%)	456/1,531 (29.8%)	449/1,584 (28.3%)	0.415	0.682	0.385

[a] χ^2 test. [b] Fisher's exact test (two-tailed).

Looking at the data separately, there were less tooth surfaces with enamel caries that had developed into dentinal caries within three years in the erythritol group (1.3%) than in the other groups. The percentage of caries lesions extending to dentin was noticeably higher ($p=0.003$) in the xylitol (2.0%) and in the sorbitol (1.7%) groups (Paper III, Table 4). The results were similar in respect to the time it took for the caries process to extend into the dentin – it was significantly longer in the erythritol group compared to the other (xylitol and sorbitol) groups ($p=0.009$; Paper III, Figure 3). During the intervention caries risk was higher among the xylitol and sorbitol groups, younger children and among boys than among erythritol groups, older children and girls.

A decrease in all caries codes over the trial took place in all study groups (28.3–30.5%; Paper III, Table 4) and was markedly lower ($p=0.006$) according to the surfaces in the erythritol group compared to the xylitol and sorbitol (as a control) groups.

5.4. Salivary and plaque indicators

5.4.1. Salivary SM and LB levels

At baseline there were no differences in LB or SM levels in saliva or dental plaque between the study groups. Statistically significant differences occurred during the third year in the SM scores. The SM counts in saliva and in plaque on the upper teeth were significantly higher in the sorbitol (as a control) group compared to the erythritol group ($p<0.05$). Both positive intervention (erythritol and xylitol) groups had statistically significant differences ($p<0.01$) within the groups (except in LB counts), where xylitol did not reduce the SM level.

5.4.2. Salivary flow rate

The stimulated salivary volume was measured during each examination. As expected, the volume increased ($p<0.01$) in all groups because the children were growing. The mean rates were 1.1 ml/min at baseline, 1.5, 1.8 and 2.0 ml/min respectively in the first, the second and the third year. No differences were found between the groups during the study.

5.4.3. Plaque weight

There were no significant differences in the weight of the fresh, just collected plaque between the groups at baseline. The lowest values and reduction of collected plaque were seen during the trial in the erythritol group ($p<0.05$). In the other (sorbitol and xylitol) groups no changes were observed during the study (Figure 3).

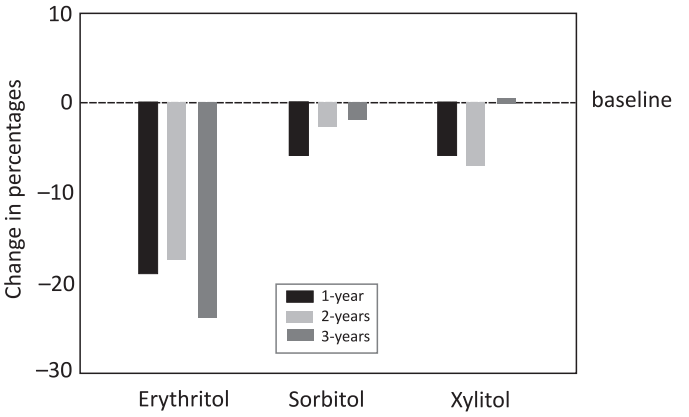

Figure 3. The reduction of mean plaque fresh weights during intervention, compared with baseline values

5.4.4. Chemical analyses of plaque

The concentration of acetic, propionic and lactic acids were calculated within the clinical trial. The lowest findings were registered after the third year in the erythritol group. The statistically significant ($p \leq 0.05$), detectable reduction of acetic and propionic acids were observed among all study groups. Any statistically significant differences were not recorded during the clinical study in the xylitol group.

The concentration of erythritol and xylitol did not differ between groups in any of the years. The sorbitol level in individual plaque varied from 0 to 700 µg per gram of dental plaque, but it did not differ significantly between the investigation groups.

6. DISCUSSION

6.1. Methodological consideration

The study consisted of two elements: the first part was an epidemiological study among elementary school level children (Original papers I and II), and the second, an evaluation of the clinical effect of the long-term consumption of polyols on caries prevention in mixed dentition (Papers III and IV).

This survey was the first long-term clinical trial to compare the role of different polyols in caries prevention. The randomly selected investigation group covered around 10% of all schools and schoolchildren in this region, and provides reliable information about caries prevalence and experience. The size of the study groups was sufficient to get statistically significant results. The co-operation with the schools, teachers and children was good throughout the study.

6.1.1. Study subjects

Participants were from randomly selected schools in south-eastern Estonia. The initial number of returned written agreements, signed by parents or guardians, was larger (n=522) than expected but the final size of the groups (n=485) was formatted by the baseline examination; respectively 164 in the sorbitol group, 156 in the xylitol group, and 165 in the erythritol group, which was close to the planned 151 children for each group. The predicted percentage of participant dropout was 25% over the three years; however, the actual decrease was lower (mean of 22.9%), being higher in the erythritol group (26.6%) and lower in the sorbitol group (23.2%), and in the xylitol group (19.2%). The main reason for the absences during the examination was illness and the main reason of interruptions to the study was children changing their place of residence and school (reported by the class teachers).

6.1.2. Study design

The hypothesis of the clinical trial was that erythritol and xylitol would have a similar effect, as previously shown from a 6-month study (Mäkinen et al. 2005).

This study was the first randomized clinical trial where the efficacy of erythritol and xylitol in caries prevention were compared with sorbitol during long-term daily intake. At the same time it was the longest follow up study of erythritol consumption. The study was carried out over four years: a baseline examination and three-year polyols consumption with annual examinations.

All children were examined annually at the university clinic by four investigators. The clinical examinations took place once a year during one week at the same time each year. The sequence of classes and the timetable were (with small changes) the same for each examination. The purpose of keeping

the timeframe was to minimize the differences of the chemical ingredients in the dental plaque, which could have been the result of time differences between the meal/polyols candies consumption and taking plaque samples.

Responsible teachers were remunerated for extra work, which consisted of daily candy distribution and monitoring. The clinical examinations were also combined with bonuses (free transport and visits to museums) and gifts (free fluoridated toothpaste and a toothbrush twice a year) for children, which probably motivated and ensured lasting participation.

The supervision of polyols consumption in schools was organized on-site by the class teachers. The annual site-visits confirmed that the products were being stored appropriately as described by the manufacturer, and the consumption of polyols on schooldays was managed as required by the study design. Verbal communication with the teachers confirmed the children's good tolerance of polyols intake. The motivation among the participants was high, and throughout the trial all newcomers were included in the study via a signed consent form by their parent/guardian.

6.1.3. ICDAS II coding system in clinical examination

Caries findings were recorded using the new ICDAS II system, described by Ismail et al. (2007) and Pitts et al. (2009). The calibration was carried out following the instructions of the developers of this system. The ICDAS II caries recording system needs thorough training. The 90-minute e-learning programme is available on the web for self-instruction and even an experienced person that has used this system before is recommended to investigate the programme before the trial and for calibration.

Even though the reliability of the inter- and intra-examiners during the calibration of examiners was very high (Cicchetti and Allisson, 1971; the weighted kappa was >0.9), each child was examined and dental plaque was collected by the same examiner each time in order to reduce possible variation due to human factors. A good light (of the dental unit) and clean, dry teeth are obligatory for adequate caries detection. Proximal caries diagnosis was questionable at later ages in permanent dentition, where in case of doubt additional radiographic diagnosing is necessary. In our study, the average time per child for registering their dental status was around 3…5 minutes, depending mainly on the number of cariotic lesions, although Braga et al. (2009) reports that an examination using the ICDAS II system takes twice as long as DMFT index recording according to WHO criteria.

The visual assessment of caries is well tolerated even in younger children and caused less discomfort compared with other caries diagnostic methods (Novaes et al. 2012). In our study only a couple of children refused the dental examination at such an early age because of dental anxiety. At the same time, they were not afraid of the saliva tests, which were taken.

The main difference between the DMFT index, which has been used as the main epidemiological caries indicator for more than 70 years, and the newer ICDAS II system is the registration of caries at an early stage. It gives the opportunity to monitor the evolution of caries lesions during the clinical investigation study. The ability to convert recorded caries codes from ICDAS II for entry in the DMFT index has been approved in recent studies (Iranzo-Cortes et al. 2013, de Amorim et al. 2012) with some difficulties in classifying codes 3 and 4 as concluded in a review of the literature (Clara et al. 2012). Thorough calibration and following the guidelines of the ICDAS Committee provides reliable recorded data.

6.2. Dental caries

During the 1990s, the DMFT indices among 12-years-olds in Estonia decreased rapidly from 4.1 in 1992 to 2.7 in 1998, according to the electronic Oral Health Database (2007). The reason for this could be the re-establishment of independence in Estonia when the transition from the socialist system to the capitalist system was taking place. People's attitude to oral hygiene and healthy teeth changed – healthy teeth and a bright smile became a characteristic sign of a higher social and economic status. The open borders made access to fluoridated toothpaste possible, which is an important factor in caries prevention.

The mean DMFT among 12-year-olds is still higher in Latvia (3.4 in 2004) and in Lithuania (3.7 in 2005; Oral Health Database 2007) than in Estonia. In Nordic countries the national programmes for caries prevention have led to a significant rapid decrease in caries. In Finland DMFT among 12-year-olds declined from 6.9 in 1975 to 0.7 in 2009, in Denmark from 5.2 in 1975 to 0.6 in 2012, and in Sweden from 6.3 in 1977 to 0.8 in 2011. The caries risk among the same age Estonian schoolchildren is moderate – the mean DMFT in Estonia is near the European average (2.6). Earlier studies have shown high caries indices (up to 5.1 in Võrumaa and up to 5.6 in Põlvamaa) in the south-eastern region versus the Estonian average (2.7) among 12-year-olds. The expectation before the baseline data collection was high caries scores among the younger group (7–8-year-olds).

In our study the DMFT+dmft indices in mixed dentition were higher at the baseline and lower later because of the normal exfoliation of primary teeth. The most damaged teeth were primary molars, and for the fourth and fifth grades, the extensive foliation process of primary molars was taking place producing lower caries indices in later examinations.

6.3. Prevention of caries

6.3.1. Professional procedures

The high caries indices in south-eastern Estonia clearly demonstrate the need for caries prevention in this region, but the obtained data showed low prevalence of fissure sealants in elementary level schoolchildren in the present study. The official guideline on caries prevention in children – prepared by order of the Health Insurance Fund of Estonia – provides professional directions for dentists and includes methods like local fluoride application after every 6 months and covering fissures and pits in primary and/or permanent molars with sealants if an individual need for this has been assessed. In this study, the written information about treatment need and verbal instructions for the proper oral hygiene and dietary recommendations were given to pupils, but no prescription for professional prevention procedures. Subsequent contact with the child's dentist for treatment was voluntary and the child or his/her guardian could refuse or ignore those directions. Based on the study by Olak et al. (2013), children from low-income families probably stayed away because of low motivation of oral health and/or dental anxiety.

During annual examinations in the Department of Dentistry at the University of Tartu, children attended courses on oral health education, including brushing instruction and diet recommendations.

6.3.2. Fluorides

All children were equipped with 0.24% sodium fluoride toothpaste – one tube was given biannually, after every 6 months – during the trial and, furthermore, they were instructed to brush their teeth with this twice a day, which would ensure the required daily dose of topical fluorides on the prevention of caries.
The fluoride level in the drinking water in Estonia varies greatly. The level of fluorides in the groundwater in this area has been reported to be lower than the Estonian average. Some randomly selected schools were located in territories where the fluoride level was optimal (from 0.8 to 1.2 mg/L) or even higher (one school in Tartu where the concentration of fluorides in tap water was over 1.2 mg/L).

Additional analyses of data on the basis of the level of fluorides in the water supply (<0.5 versus >0.8 mg/L) showed statistically significant differences in the occurrence of caries lesions. In areas, including south Estonia, where the level of fluorides in drinking water is below optimal (<0.5mg/L), preventive programmes are urgently needed.

6.3.3. Effect of polyols

DMF indices in mixed dentition decreased throughout the trial because of the physiological exfoliation of primary teeth, especially in deciduous molars, which were the most affected teeth in the mouth according to assessments at the baseline. In order to minimize this effect, only teeth which were registered during the two consecutive examinations were involved in the statistical analyses.

6.3.3.1. Xylitol

The most unexpected outcome of the trial was that no differences were detected between the xylitol group (as an intervention) and the sorbitol group (which was the control). The majority of studies have demonstrated the priority of xylitol, and the lack of any effect during the study is difficult to explain. One of the most possible reasons could be a discrepancy in dosing frequency and intervals. The recommendation to achieve the maximum effect for caries prevention is to distribute portions of xylitol evenly throughout the whole day (Kandelman and Gagnon 1987, 1990, Söderling 2009). Schooldays at elementary school level are short and could have already ended at noon for the first grades and around 3 pm in the higher grades. Therefore, the use of the test items was limited to a relatively short period of about 5 hours considering the recommendation that xylitol should ideally be used throughout the day. There were only three exposures per day, which is definitely too few. Unfortunately, the need for the controlled intake of polyols could not allow the self-consumption of polyols later, at home. Therefore, the consumption did not cover the whole day and the weekends and school holidays (around 150 days in total). Consequently, the intervals between the last dosage and the first dosage of the following day were longer than required and this could reduce the anticipated positive effect of xylitol.

On the other hand, xylitol has shown the prolonged caries prevention effect to be significantly higher when xylitol has been used for a couple of years immediately after tooth eruption (Hujoel et al. 1999, Hayes 2001). So we could have the expected similar effect in the mixed dentition in the present study as well.

The second explanation of the inconsistency could be a fact which appeared during the personal conversation with teachers at the time of site visits in the schools. Teachers were instructed not to force children, so when children occasionally complained about the excess of sweets (it was not possible to designate the type of polyols because of the double-blind study design), teachers suggested children take less polyols lozenges than suggested. Therefore, the daily dose was low. The consumption was also omitted when the pupils were ill. The tolerance of the taste of polyols during the study was not asked, but some children complained about the taste of the product, and this

would have required additional investigation. One possible solution to reduce the sweetness of polyols could be a mixed combination of polyols, but another similar long-term trial has recently been carried out where xylitol and erythritol, both in combination with maltitol were tested (Lenkkeri et al. 2012), during which no caries-preventive effects were discovered.

6.3.3.2. Erythritol

The main finding of this study of long-term polyols consumption was lower incidence of caries lesions in dentin by tooth and surface in mixed dentition in the erythritol group compared with the xylitol and sorbitol groups. The first statistically significant differences between the groups were recorded at 24 and 36 months of polyols intake. No differences were recognized at 12 months – this is an anticipated outcome because caries formation is a dynamic process with multiple de- and remineralisation cycles, which can be stopped, and caries development from superficial damage to visible cariotic cavities takes some years. Warren et al. (2006) reported that about 30% of white spot lesions of fissures in primary (and later in mixed) dentition progressed to cavities during four years. On the smooth surfaces (e.g. near the gingiva), these figures are even lower – only 5% of all demineralised enamel lesions were filled after four years.

6.3.3.3. Supplemental impact

Most xylitol studies have used xylitol in the form of a chewing gum (see literature review by Mäkinen, 2010), which have accompanied the effect of chewing, and thereby, increased saliva production as an additional preventive factor. Only a couple of studies have used polyols in other forms (Honkala et al. 2006, Taipale et al. 2007, Milgrom et al. 2009). Duane (2011) and Lenkkeri et al. (2012) reported the use of polyols in combination in the form of lozenges, and there were no statistically significant differences on caries prevention. In a systematic review of non-chewable xylitol candies and lozenges, Antonio et al. (2011) concluded that although an overall reduction of caries took place, the effect was higher on the free surfaces and was not expressed on the closed, proximal surfaces.

One important explanation could be annually delivered dietary and tooth cleaning instructions to the participants. Cooper et al (2013), in their systematic review, could not find sufficient evidence of improvements from the same habits during school-based behavioural intervention among children aged 4–12 years. In our study, an extremely low amount of new caries lesions occurred during the intervention in all study groups, which could be credited to improved oral hygiene and the overall reduction of sugar consumption between meals. This was obviously due to the increased attention drawn towards dental health by the teachers, parents and examiners.

6.4. Saliva and plaque parameters

6.4.1. Bacterial content

The thickness and bacterial content of dental plaque have a strong correlation with dental caries. The thicker layer of plaque includes a potentially higher number of cariogenic bacteria which are able to rapidly metabolize dietary carbohydrates into acid (Kashket et al. 1996). The neutralizing of this pH decrease, caused by acidic excretes of bacteria in plaque, may be hindered and slowed if the plaque contains voluminous mass.

Biological studies have shown that erythritol could have a similar effect to xylitol, and therefore, may influence plaque quantity, adherence, and the growth of streptococci in dental plaque. In our study, all biological parameters associated with caries development – plaque weight and MS counts in salivary and in plaque, all assigned during the present clinical trial – were ultimately lower in the erythritol group compared to the xylitol and sorbitol groups. This result is compatible with the lower dentinal caries numbers in the erythritol group in the present study and with earlier short-term trials (Mäkinen et al. 2001, 2005), which also demonstrated reduced levels of MS in saliva and in dental plaque.

The majority of biological studies have shown clear distinctive differences between xylitol and sorbitol containing products where xylitol had reduced the amount of plaque and inhibited the growth of MS on the tooth surfaces. The latter is also reflected in the SM level of saliva. The expected positive effect on caries prevention in the xylitol group was not confirmed in this study.

The overall LB level in the present case was relatively high, which coincided with the previous study, where a high level of LB in saliva was reported by Kõll-Klais et al. (2004). At the same time, there was no statistical difference in the LB counts between the study groups, although the long-term programmes with xylitol chewing gum in schools in Belize and China demonstrated salivary LB reduction (Mäkinen et al. 1995, 1996, 2008). The overall level of teeth with dentinal caries among pupils was relatively low during the trial and this may be reflected in the stability of the LB counts.

6.4.2. Chemical properties

The growth of aciduric organisms accelerates during the metabolism of dietary carbohydrates, and the replacement of sugars with polyols should decrease the growth of this type of microbiota. In our study, the reduction of organic acids, which were measured in dental plaque during the trail as surrogate markers of caries – acetic acid, propionic acid and lactic acid – occurred only in the erythritol group. One historical research (Carr and Krantz 1945) has shown that acetic acid bacteria do not metabolize erythritol, and the same could characterize lactic acid bacteria.

As it was presented in the results, the sorbitol level in plaque varied markedly between subjects and increased throughout the study. During the clinical trial there was no opportunity to eliminate all other polyols except the intervention substance. Sorbitol is an especially common sweetener in ordinary food, sweets and also in toothpastes, which were given to the children during the present study. We can speculate at the role other polyols consumed at the same time may have played. The answers may be found from studies, which test polyols in a mixed form.

The success of erythritol may be concealed in the differences of the physical properties between the polyols. Erythritol as a four-carbon tetritol type polyol has a smaller molar mass (122.1 g/mol) compared with xylitol (152.1 g/mol) and sorbitol (182.2 g/mol), which allows higher mobility and is permeable in the organism.

CONCLUSIONS

1. The prevalence and experience of caries among elementary school children in south-eastern Estonia is high, which matches previous studies. The prevalence increases rapidly in this age group – there is a statistically significant difference between first and the second graders. This age group needs intensive caries prevention programmes.
2. ICDAS recording gives appropriate information about the occurrence of caries lesions. Untreated caries lesions in mixed dentition increase caries risk for permanent teeth.
3. Lower second primary molars and the first permanent molars are the most frequently affected teeth in mixed dentition. The first molars are the first affected permanent teeth. Dental caries starts in fissures, predominantly shortly after the eruption of the teeth which may be prevented using sealants, but the number of sealants among the examined children is extremely low.
4. Erythritol demonstrates a delaying of caries − caries progress was slower in the erythritol group compared to the sorbitol and xylitol groups and the effect was apparent after 2 years of erythritol lozenge consumption. We can conclude that erythritol is a well tolerated sweetener which has high potential in caries reduction.
5. Erythritol consistently reduces the amount of dental plaque, the levels of acids in dental plaque and the counts of *Streptococcus mutans* compared with sorbitol and xylitol lozenges. Therefore, erythritol is effective in caries prevention through controlling plaque quality and quantity.
6. As previous studies have proven the caries decreasing effect of xylitol, the modest role of xylitol in this study is difficult to explain. One probable explanation could be insufficient consumption per day – 3 times per day against 4–5, which has been generally recommended. The regular, daily intake of polyols is recommended.

   Based on the results obtained, erythritol based lozenges could be recommended to replace sugar containing candies to prevent dental caries in children.

REFERENCES

Agouropoulos A, Twetman S, Pandis N, Kavvadia K, Papagiannoulis L. Caries-preventive effectiveness of fluoride varnish as adjunct to oral health promotion and supervised tooth brushing in preschool children: A double-blind randomized controlled trial. J Dent. 2014; 42:1277–83.

Aidara AW, Pitts N, Markowska N, Bourgeois D. Quality of data gathered with International Caries Detection and Assessment System (ICDAS) assessment and dentists' perceptions of completion of dental records. Int Dent J. 2011; 61:314–20.

Alanen P, Isokangas P, Gutmann K. Xylitol candies in caries prevention: results of a field study in Estonian children. Community Dent Oral Epidemiol. 2000; 28:218–24.

Armfield JM. Community effectiveness of public water fluoridation in reducing children's dental disease. Public Health Rep. 2010; 125:655–64.

Antonio AG, Pierro VS, Maia LC. Caries preventive effects of xylitol-based candies and lozenges: a systematic review. J Public Health Dent. 2011; 71:117–24.

Autio JT. Effect of xylitol chewing gum on salivary Streptococcus mutans in preschool children. ASDC J Dent Child. 2002; 69:81–6, 13.

Avila M, Ojcius DM, Yilmaz Ö. The Oral Microbiota: Living with a Permanent Guest. DNA Cell Biol. 2009; 28: 405–11.

Bertella N, Moura dos S, Alves LS, Damé-Teixeira N, Fontanella V, Maltz M. Clinical and radiographic diagnosis of underlying dark shadow from dentin (ICDAS 4) in permanent molars. Caries Res. 2013; 47:429–32.

Braga MM, Oliveira LB, Bonini GA, Bönecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization criteria. Caries Res. 2009; 43:245–9.

Carr CJ, Krantz JC. Metabolism of the sugar alcohols. Adv Carbohydr Chem 1945; 1:175–92.

Chestnutt IG, Chadwick BL, Hutchings S, Playle R, Pickles T, Lisles C, Kirkby N, Morgan MZ, Hunter L, Hodell C, Withers B, Murphy S, Morgan-Trimmer S, Fitzsimmons D, Phillips C, Nuttall J, Hood K. Protocol for "Seal or Varnish?" (SoV) trial: a randomised controlled trial to measure the relative cost and effectiveness of pit and fissure sealants and fluoride varnish in preventing dental decay. BMC Oral Health. 2012; 12:51.

Clara J, Bourgeois D, Muller-Bolla M. DMF from WHO basic methods to ICDAS II advanced methods: a systematic review of literature. Odontostomatol Trop. 2012; 35:5–11.

Cooper AM, O'Malley LA, Elison SN, Armstrong R, Burnside G, Adair P, Dugdill L, Pine C. Primary school-based behavioural interventions for preventing caries. Cochrane Database Syst Rev. 2013; 5:CD009378.

Cury JA, Tenuta LM. Evidence-based recommendation on toothpaste use. Braz Oral Res. 2014; 28:1–7.

Cicchetti D, Allison T. A new procedure for assessing reliability of scoring EEG sleep recordings. Am J EEG Techn, 1971; 11:101–9.

de Amorim RG, Figueiredo MJ, Leal SC, Mulder J, Frencken JE. Caries experience in a child population in a deprived area of Brazil, using ICDAS II. Clin Oral Investig. 2012; 16:513–20.

Decker EM, Klein C, Schwindt D, von Ohle C. Metabolic activity of Streptococcus mutans biofilms and gene expression during exposure to xylitol and sucrose. Int J Oral Sci. 2014. doi: 10.1038/ijos.2014.38.
de Cock P, Bechert CL. Erythritol.Funktionality in noncaloric functional beverages. Pure Appl Chem. 2002; 74:1281–9.
de Cock P. Erythritol: a novel noncaloric sweetener ingredient. World Rev Nutr Diet. 1999; 85:110–6.
De Cock P. Erythritol, Chapter 10 in: O'Donnell K, Malcolm WK (eds.) Sweeteners and Sugar Alternatives in Food Technology, 2nd Ed; 2012
Diniz MB, Rodrigues JA, Hug I, Cordeiro Rde C, Lussi A. Reproducibility and accuracy of the ICDAS-II for occlusal caries detection. Community Dent Oral Epidemiol. 2009; 37:399–404.
Dragheim E, Petersen PE, Kalo I, Saag M. Dental caries in schoolchildren of an Estonian and a Danish municipality. Int J Paediatr Dent. 2000; 10:271–7.
Duane BG. No evidence of caries reduction found in a school xylitol and erythritol lozenge programme. Evid Based Dent. 2011; 12:102–3.
Ekstrand KR, Luna LE, Promisiero L, Cortes A, Cuevas S, Reyes JF, Torres CE, Martignon S. The reliability and accuracy of two methods for proximal caries detection and depth on directly visible proximal surfaces: an in vitro study. Caries Res. 2011; 45:93–9.
Featherstone JD. The continuum of dental caries-evidence for a dynamic disease process. J Dent Res. 2004; 83:39–42.
Fejerskov O, Kidd E. Dental Caries: The Disease and Its Clinical Management, 2nd Ed. Oxford Blackwell Munksgaard Ltd; 2008.
Freeman R. Moderate evidence support a relationship between sugar intake and dental caries. Evid Based Dent. 2014; 15:98–9.
García-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: the role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc. 2008; 139:25–34.
Giertsen E, Arthur RA, Guggenheim B. Effects of xylitol on survival of mutans streptococci in mixed-six-species in vitro biofilms modelling supragingival plaque. Caries Res. 2011; 45:31–9.
Gudkina J, Brinkmane A. Caries experience in relation to oral hygiene, salivary cariogenic microflora, buffer capacity and secretion rate in 6-year olds and 12 year olds in Riga. Stomatologija. 2008; 10:76–80.
Hallett KB, O'Rourke PK. Social and behavioural determinants of early childhood caries Australian Dental Journal 2003; 48:27–33.
Harris R, Nicoll AD, Adair PM, Pine CM. Risk factors for dental caries in young children: a systematic review of the literature. Community Dent Health. 2004; 21(Suppl 1):71–85.
Hayes C. The effect of non-cariogenic sweeteners on the prevention of dental caries: a review of the evidence. J Dent Educ. 2001; 65:1106–9.
Hino H, Kasai S, Hattori N, Kenjo K. A case of allergic urticaria caused by erythritol. J Dermatol. 2000; 27:163–5.
Holgerson PL, Sjöström I, Twetman S. Decreased salivary uptake of [14C]-xylitol after a four-week xylitol chewing gum regimen. Oral Health Prev Dent. 2007; 5:313–9.
Honkala E, Honkala S, Shyama M, Al-Mutawa SA. Field trial on caries prevention with xylitol candies among disabled school children. Caries Res 2006; 40:508–13.

Hujoel PP, Mäkinen KK, Bennett CA, Isotupa KP, Isokangas PJ, Allen P, Mäkinen PL. The optimum time to initiate habitual xylitol gum-chewing for obtaining long-term caries prevention. J Dent Res. 1999; 78:797–803.
Ijaz S, Croucher RE, Marinho VC. Systematic reviews of topical fluorides for dental caries: a review of reporting practice. Caries Res. 2010; 44:579–92.
Indermitte E, Karro E, Saava A. Tap water fluoride levels in Estonia. Fluoride 2007; 40:244–7.
Indermitte E, Saava A, Karro E. Exposure to high fluoride drinking water and risk of dental fluorosis in Estonia. nt J Environ Res Public Health. 2009; 6:710–21.
Indermitte E, Saava A, Karro E. Reducing exposure to high fluoride drinking water in Estonia-a countrywide study. Int J Environ Res Public Health. 2014; 11:3132–42.
Ingel I, Kerner R, Krusell S, Põder K, Servinski M, Soiela M. Statistical Yearbook of Estonia 2010. Tallinn: Statistics Estonia 2010; 71–3.
Iranzo-Cortés JE, Montiel-Company JM, Almerich-Silla JM. Caries diagnosis: agreement between WHO and ICDAS II criteria in epidemiological surveys. Community Dent Health. 2013; 30:108–11.
Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol. 2007; 35:170–8.
Isokangas P, Tenovuo J, Söderling E, Männistö H, Mäkinen KK. Dental caries and mutans streptococci in the proximal areas of molars affected by the habitual use of xylitol chewing gum. Caries Res. 1991; 25:444–8.
Jenkinson HF, Lamont RJ. Streptococcal adhesion and colonization. Crit Rev Oral Biol Med. 1997; 8:175–200.
Kandelman D, Gagnon G. Clinical results after 12 months from a study of the incidence and progression of dental caries in relation to consumption of chewing-gum containing xylitol in school preventive programs. J Dent Res. 1987; 66:1407–11.
Kandelman D, Gagnon G. A 24-month clinical study of the incidence and progression of dental caries in relation to consumption of chewing gum containing xylitol in school preventive programs. J Dent Res. 1990; 69:1771–5.
Karro E, Indermitte E, Saava A, Haamer K, Marandi A. Fluoride occurrence in publicly supplied drinking water in Estonia. Environ Geol. 2006; 50: 389–96.
Kashket S, Zhang J, Van Houte J. Accumulation of fermentable sugars and metabolic acids in food particles that become entrapped on the dentition. J Dent Res. 1996; 75:1885–91.
Kawanabe J, Hirasawa M, Takeuchi T, Oda T, Ikeda T. Noncariogenicity of erythritol as a substrate. Caries Res. 1992; 26:358–62.
Keukenmeester RS, Slot DE, Rosema NA, Van Loveren C, Van der Weijden GA. Effects of sugar-free chewing gum sweetened with xylitol or maltitol on the development of gingivitis and plaque: a randomized clinical trial. Int J Dent Hyg. 2014; 12:238–44.
Kidd E. Essentials of dental caries: the disease and its management, 3rd Ed. Oxford University Press; 2005.
Knuuttila ML, Mäkinen K. Effect of xylitol on the growth and metabolism of Streptococcus mutans. Caries Res. 1975; 9:177–89.
Kutsch VK, Chaiyabutr Y, Milicich G. Reconsidering remineralization strategies to include nanoparticle hydroxyapatite. Compend Contin Educ Dent. 2013; 34:170–6.
Kõll-Klais P, Mändar R, Leibur E, Kjaeldgaard M. High levels of salivary lactobacilli in Estonian schoolchildren. Eur J Paediatr Dent. 2004; 5:107–9.

Kühnisch J, Berger S, Goddon I, Senkel H, Pitts N, Heinrich-Weltzien R. Occlusal caries detection in permanent molars according to WHO basic methods, ICDAS II and laser fluorescence measurements. Community Dent Oral Epidemiol. 2008; 36:475–84.

Laitala ML, Alanen P, Isokangas P, Söderling E, Pienihäkkinen K. Long-term effects of maternal prevention on children's dental decay and need for restorative treatment. Community Dent Oral Epidemiol. 2013; 41:534–40.

Lamont RJ, Burne RA, Lantz MS, LeBlanc DJ. Oral Microbiology and Immunology. American Society for Microbiology Press; 2006.

Larmas M. Has dental caries prevalence some connection with caries index values in adults? Caries Res. 2010; 44:81–4.

Lenkkeri AM, Pienihäkkinen K, Hurme S, Alanen P. The caries-preventive effect of xylitol/maltitol and erythritol/maltitol lozenges: results of a double-blinded, cluster-randomized clinical trial in an area of natural fluoridation. Int J Paediatr Dent. 2012; 22:180–90.

Lina BA, Bos-Kuijpers MH, Til HP, Bär A. Chronic toxicity and carcinogenicity study of erythritol in rats. Regul Toxicol Pharmacol. 1996; 24:S264–79.

Lingström P, Lundgren F, Birkhed D, Takazoe I, Frostell G. Effects of frequent mouthrinses with palatinose and xylitol on dental plaque. Eur J Oral Sci. 1997; 105:162–9.

Ly KA, Milgrom P, Roberts MC, Yamaguchi DK, Rothen M, Mueller G. Linear response of mutans streptococci to increasing frequency of xylitol chewing gum use: a randomized controlled trial [ISRCTN43479664]. BMC Oral Health. 2006; 6:6.

Marsh PD, Martin MV. Oral microbiology. 4th Ed. MPG Books Ltd; 2000.

Marsh, PD, Nyvad, PDB. The oral microflora and biofilms on teeth. In: Fejerskov O.and Kidd E. (eds.). Dental Caries: the Disease and its Clinical Management. 2nd Ed. Oxford Blackwell Munksgaard Ltd; 2008

Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2002; 2:CD002280.

Marinho VC, Higgins JP, Logan S, Sheiham A. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2003; 4:CD002782.

Marinho VC, Higgins JP, Sheiham A, Logan S. Combinations of topical fluoride (toothpastes, mouthrinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2004; 1:CD002781.

Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2013; 7:CD002279.

McDonagh MS, Whiting PF, Wilson PM, Sutton AJ, Chestnutt I, Cooper J, Misso K, Bradley M, Treasure E, Kleijnen J. Systematic review of water fluoridation. BMJ. 2000; 321:855–9.

Mendes FM, Braga MM, Oliveira LB, Antunes JL, Ardenghi TM, Bönecker M. Discriminant validity of the International Caries Detection and Assessment System (ICDAS) and comparability with World Health Organization criteria in a cross-sectional study. Community Dent Oral Epidemiol. 2010; 38(5):398–407.

Mickenautsch S, Yengopal V. Effect of xylitol versus sorbitol: a quantitative systematic review of clinical trials. Int Dent J. 2012; 62:175–88.

Milgrom, P, Zero DT, Tanzer JM. An examination of the advances in science and technology of prevention of tooth decay in young children since the Surgeon General's Report on Oral Health. Acad Pediatr 2009; 9:404–9.

Mitropoulos P, Rahiotis C, Stamatakis H, Kakaboura A. Diagnostic performance of the visual caries classification system ICDAS II versus radiography and micro-computed tomography for proximal caries detection: an in vitro study. J Dent. 2010; 38:859–67.

Miyasawa-Hori H, Aizawa S, Takahashi N. Difference in the xylitol sensitivity of acid production among Streptococcus mutans strains and the biochemical mechanism. Oral Microbiol Immunol. 2006; 21:201–5.

Monedero V, Pérez-Martínez G, Yebra MJ. Perspectives of engineering lactic acid bacteria for biotechnological polyol production. Appl Microbiol Biotechnol. 2010; 86:1003–15.

Mouton C, Scheinin A, Mäkinen KK. Effect of a xylitol chewing gum on plaque quantity and quality. Acta Odontol Scand. 1975; 33:251–7.

Moynihan P, Petersen PE. Diet, nutrition and the prevention of dental diseases. Public Health Nutr. 2004; 7:201–26.

Moynihan PJ, Kelly SA. Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines. J Dent Res. 2014; 93:8–18.

Mäkinen KK. Sugar alcohols, caries incidence, and remineralization of caries lesions: a literature review. Int J Dent. 2010; 981072.

Mäkinen KK, Alanen P, Isokangas P, Isotupa K, Söderling E, Mäkinen PL, Wenhui W, Weijian W, Xiaochi C, Yi W, Boxue Z. Thirty-nine-month xylitol chewing-gum programme in initially 8-year-old school children: a feasibility study focusing on mutans streptococci and lactobacilli. Int Dent J. 2008; 58:41–50.

Mäkinen KK, Bennett CA, Hujoel PP, Isokangas PJ, Isotupa KP, Pape HR Jr, Mäkinen PL. Xylitol chewing gums and caries rates: a 40-month cohort study. J Dent Res. 1995; 74:1904–13.

Mäkinen KK, Chen CY, Mäkinen PL, Bennett CA, Isokangas PJ, Isotupa KP, Pape HR Jr. Properties of whole saliva and dental plaque in relation to 40-month consumption of chewing gums containing xylitol, sorbitol of sucrose. Caries Res. 1996; 30:180–8.

Mäkinen KK, Hujoel PP, Bennett CA, Isokangas P, Isotupa K, Pape HR Jr, Mäkinen PL. A descriptive report of the effects of a 16-month xylitol chewing-gum programme subsequent to a 40-month sucrose gum programme. Caries Res. 1998; 32:107–12.

Mäkinen KK, Isotupa KP, Kivilompolo T, Mäkinen PL, Toivanen J, Söderling E. Comparison of erythritol and xylitol saliva stimulants in the control of dental plaque and mutans streptococci. Caries Res. 2001; 35:129–35.

Mäkinen KK, Saag M, Isotupa KP, Olak J, Nõmmela R, Söderling E, Mäkinen PL. Similarity of the effects of erythritol and xylitol on some risk factors of dental caries. Caries Res. 2005; 39:207–15.

Mäkinen KK, Söderling E, Isokangas P, Tenovuo J, Tiekso J. Oral biochemical status and depression of Streptococcus mutans in children during 24- to 36-month use of xylitol chewing gum. Caries Res. 1989; 23:261–7.

Naidu R, Nunn J, Kelly A. Socio-behavioural factors and early childhood caries: a cross-sectional study of preschool children in central Trinidad. BMC Oral Health. 2013; 13:30.

Novaes TF, Matos R, Raggio DP, Imparato JC, Braga MM, Mendes FM. Influence of the discomfort reported by children on the performance of approximal caries detection methods. Caries Res. 2012; 44:465–71.
Olak J, Mändar R, Karjalainen S, Söderling E, Saag M. Dental health and oral mutans streptococci in 2–4-year-old Estonian children. Int J Paediatr Dent. 2007; 17:92–7.
Olak J, Saag M, Honkala S, Nõmmela R, Runnel R, Honkala E, Karjalainen S. Children's dental fear in relation to dental health and parental dental fear. Stomatologija. 2013; 15:26–31.
Ormond C, Douglas G, Pitts N. The use of the International Caries Detection and Assessment System (ICDAS) in a National Health Service general dental practice as part of an oral health assessment. Prim Dent Care. 2010; 17:153–9.
Patel, R. The state of Oral Health in Europe. 2012.
Payne AN, Chassard C, Lacroix C. Gut microbial adaptation to dietary consumption of fructose, artificial sweeteners and sugar alcohols: implications for host-microbe interactions contributing to obesity. Obes Rev. 2012; 13:799–809.
Peckham S, Awofeso N. Water fluoridation: a critical review of the physiological effects of ingested fluoride as a public health intervention. Sci World J. 2014; 293019.
Petersen PE. Sociobehavioural risk factors in dental caries – international perspectives Community Dent Oral Epidemiol 2005; 33: 274–9.
Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dent Oral Epidemiol. 2004; 32:319–21.
Pitts N. Detection, Assessment, Diagnosis and Monitoring of Caries. Karger, Monogr Oral Sci. 2009; 21.
Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect. 2000; 2:1599–607.
Rugg-Gunn A, Bánóczy J. Fluoride toothpastes and fluoride mouthrinses for home use. Acta Med Acad. 2013; 42:168–78.
Saag M, Russak S, Vasar R, Olak J. Dental caries situatsion in Estonia. Finn Dent J. 1998; 18:1010–1.
Sagheri D, Wassmer G, Hahn P, McLoughlin J. Dental caries experience of school-children of two different oral health care delivery systems. Int Dent J. 2009; 59:161–7.
Scheinin A, Mäkinen KK, Tammisalo E, Rekola M. Turku sugar studies XVIII. Incidence of dental caries in relation to 1-year consumption of xylitol chewing gum. Acta Odontol Scand. 1975; 33:269–78.
Seki M, Yamashita Y, Shibata Y, Torigoe H, Tsuda H, Maeno M. Effect of mixed mutans streptococci colonization on caries development. Oral Microbiol Immunol. 2006; 21:47–52.
Sheiham A, James WP. A reappraisal of the quantitative relationship between sugar intake and dental caries: the need for new criteria for developing goals for sugar intake. BMC Public Health. 2014; 14:863.
Shirao K, Inoue M, Tokuda R, Nagao M, Yamaguchi M, Okahata H, Fujisawa T. "Bitter sweet": a child case of erythritol-induced anaphylaxis. Allergol Int. 2013; 62:269–71.
Shyama M, Honkala E, Honkala S, Al-Mutawa SA. Effect of xylitol candies on plaque and gingival indices in physically disabled school pupils. J Clin Dent. 2006; 17:17–21.
Soames JV, Southam JC. Oral pathology. 3rd Ed. Oxford University Press; 2001.

Storey D, Lee A, Bornet F, Brouns F. Gastrointestinal tolerance of erythritol and xylitol ingested in a liquid. Eur J Clin Nutr. 2007; 61:349–54.
Söderling E. Controversies around Xylitol. Eur J Dent. 2009; 3:81–2.
Söderling EM, Hietala-Lenkkeri AM. Xylitol and erythritol decrease adherence of polysaccharide-producing oral streptococci. Curr Microbiol. 2010; 60:25–9.
Zhang Q, van Palenstein Helderman WH. Caries experience variables as indicators in caries risk assessment in 6–7-year-old Chinese children. J Dent. 2006; 34:676–81.
Taipale T, Pienihäkkinen K, Alanen P, Jokela J, Söderling E. Dissolution of xylitol from a food supplement administered with a novel slow-release pacifier: Preliminary results. Eur Arch Paediatr Dent 2007;8:123–5.
Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res. 2011; 90:294–303.
Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ. 2001; 65:1028–37.
Thabuis C, Cheng CY, Wang X, Pochat M, Han A, Miller L, Wils D, Guerin-Deremaux L. Effects of maltitol and xylitol chewing-gums on parameters involved in dental caries development. Eur J Paediatr Dent. 2013; 14: 303–8.
Thylstrup A, Fejerskov O. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Community Dent Oral Epidemiol. 1978; 6:315–28.
Tinanoff N, Kanellis MJ, Vargas CM. Current understanding of the epidemiology mechanisms, and prevention of dental caries in preschool children. Pediatr Dent. 2002; 24:543–51.
Trahan L. Xylitol: a review of its action on mutans streptococci and dental plaque – its clinical significance. Int Dent J. 1995; 45:77–92.
Trahan L, Söderling E, Dréan MF, Chevrier MC, Isokangas P. Effect of xylitol consumption on the plaque-saliva distribution of mutans streptococci and the occurrence and long-term survival of xylitol-resistant strains. J Dent Res. 1992; 71:1785–91.
Tubert-Jeannin S, Auclair C, Amsallem E, Tramini P, Gerbaud L, Ruffieux C, Schulte AG, Koch MJ, Rège-Walther M, Ismail A. Fluoride supplements (tablets, drops, lozenges or chewing gums) for preventing dental caries in children. Cochrane Database Syst Rev. 2011; 12:CD007592.
Twetman S, Stecksén-Blicks C. Effect of xylitol-containing chewing gums on lactic acid production in dental plaque from caries active pre-school children. Oral Health Prev Dent. 2003; 1:195–9.
Van Loveren C. Sugar alcohols: what is the evidence for caries-preventive and caries-therapeutic effects? Caries Res. 2004; 38:286–93.
Vogel GL. Oral fluoride reservoirs and the prevention of dental caries. Monogr Oral Sci. 2011; 22:146–57.
Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2010; 1:CD007868.
Warren JJ, Levy SM, Broffitt B, Kanellis MJ. Longitudinal study of non-cavitated carious lesion progression in the primary dentition. J Public Health Dent. 2006; 66:83–7.
Wennerholm K, Arends J, Birkhed D, Ruben J, Emilson CG, Dijkman AG. Effect of xylitol and sorbitol in chewing-gums on mutans streptococci, plaque pH and mineral loss of enamel. Caries Research. 1994; 28:48–54.
Whelton H. Overview of the impact of changing global patterns of dental caries experience on caries clinical trials. J Dent Res. 2004; 83:C29–34.

Wolf J, Peltola JS, Seedre T, Russak S, Männik A, Vink M, Sirkel M. Dental health in 14- to 17-year-old Estonian schoolchildren in Tartu and Tallinn. Acta Odontol Scand. 1996; 54:242–6.

Yunginger JW, Jones RT, Kita H, Saito K, Hefle SL, Taylor SL. Allergic reactions after ingestion of erythritol-containing foods and beverages. J Allergy Clin Immunol. 2001; 108:650.

Electronic references

Oral Health Database: http://www.mah.se/CAPP/Country-Oral-Health-Profiles/EURO//European-Union-and-European-Economic-Area–/Dental-Caries-for-12-year-olds/

http://www.egohid.eu/Documents/EGOHID.pdf

WHO Data Base: http://www.who.int/oral_health/action/information/surveillance/en/

https://www.icdas.org

SUMMARY IN ESTONIAN

Suutervis algklasside õpilastel ning polüoolide toime hambakaariese ennetusele

Sissejuhatus

Kaaries on multifaktoriaalne haigus, mille peamised määrajad on atsidogeensete bakterite olemasolu ja hulk, eelnevate kaariese kahjustuste esinemine, süsivesikute osakaal toidus ja nende tarvitamise sagedus, fluoriidide tarbimine, sülje hulk, sotsiaalne staatus jpm tegurid.. Suuõõnes on leitud sadu erinevat liiki mikroorganismi ja osa neist kuulub püsikooslusesse. Hambakaariese tekkega seostatakse eeskätt streptokokkide perekonda kuuluvaid baktereid, millest tuntuimad on *Streptococcus mutans* ja *S. sobrinus*. Kaaries algab hamba pinnalt laigu staadiumis kahjustusena kui emailist on talle tugevust andvad mineraalid (kaltsiumi ja fosfori ioonid) bakterite happeliste ainevahetusjääkide toimel välja lahustunud, samas toidukordade vahel ladestuvad mineraalid süljest hambapinda tagasi. Selline protsess, millega kaasneb emaili ja dentiini destruktsioon, on pidev.

Hambaemaili remineraliseerumist soodustab fluoriidide olemasolu joogivees, mis on Eesti erinevates piirkondades väga kõikuv, varieerudes 0 mg/l Kagu-Eestis kuni varasemalt kohati 5–7 mg/l Lääne-Eestis (Indermitte jt 2009, 2014). Kagu-Eesti kõrgemaid kaariese näitajaid seostataksegi fluoriidide vähesusega joogivees. Tänapäevased teooriad tunnustavad suhkrute osa kaariese tekkes ja seetõttu on oluline leida alternatiivseid magusaineid, mis oleksid vähem kariogeensed. Selliseks süsivesikute rühmaks on suhkuralkoholid ehk polüoolid millede metabolism suuõõnebakterite poolt on aeglasem kui suhkrutel. Tuntuimaks esindajaks on ksülitool, mida on uuritud aastakümneid ja mille kaariest ennetav efekt on korduvates katsetes tõestust leidnud. Soomes kuulub ksülitool riiklikult reguleeritud kaariese ennetuse programmi. Uuematest ainetest on esile kerkinud erütritool, mis testituna lühiajalistes katsetes on andnud ksülitooliga sarnaselt häid tulemusi kaariese ennetuses vaatamata nende erinevale toimemehhanismile. Polüoolide ehk suhkuralkoholide regulaarne kasutamine on näidustatud eelkõige riskirühmadel – lastel ajal, mil hambad on kergemini kahjustatavad (umbes aasta jooksul peale lõikumist) ja kaariese riski vähendamiseks toidukordade vahepalades ning maiustustes.

Kaariese ulatust iseloomustakse DMFT indeksiga, mis näitab kaariesest kahjustatud (D), kaariese komplikatsioonide tõttu eemaldatud (M) ja täidistega (F) hammaste arvu uuritava kohta. Eestis on 12-aastaste keskmine DMFT indeks 2,7. See näitaja on lähedane Euroopa keskmisele (WHO järgi 2,6). See on madalam kui teistes Balti riikides, kuid tunduvalt kõrgem kui Skandinaaviamaades. Põhjamaades on saavutatud tervete hammaste osakaalu tõus, kuna riiklikul tasemel on rakendatud ennetusprogramme ja tõstetud inimeste suutervisealast teadlikkust. DMFT indeksi puuduseks on algavate kaarieskahjustuste mittearvestamine – see ei võimalda seda indeksit kasutada kaariese arengu

jälgimiseks. Seetõttu loodi rahvusvahelise kaariese uurijate grupi poolt uus ICDAS süsteem (Ismail jt 2007), mis täiendavalt eristab kaariese varaseid staadiume, võimaldades jälgida dünaamikat, ja mida on kerge teisendada DMFT indeksiks.

Nooremas koolieas toimub intensiivne hammaste vahetumine. Eestis puuduvad kaariese epidemioloogilised andmed selle vanusegrupi osas, kuid Olak jt (2007) on näidanud kõrget kaariesest haaratust väikelastel, seda eriti Kagu-Eesti piirkonnas. Piimahammaste kaaries soodustab omakorda kaariese arengut jäävhammastel. Seetõttu on kaariese preventsioon vahelduvas hammaskonnas väga oluline.

Ajal, mil kliinilist uuringut alustati, puudusid pikaajalised polüoolide toimet võrdlevad uuringud, kuid erütritooli kasutamisel oli saadud positiivseid tulemusi lühiajalistes kliinilistes uuringutes.

Uurimistöö eesmärgid

Peamiseks uuringu ülesandeks oli hinnata kaariese levimust noorema kooliastme õpilaste hulgas ja testida polüoolide pikaajalise kasutamise efektiivsust kaariese ennetuses.

Uurimistöö alaeesmärkideks seati:

1. Saada epidemioloogiline ülevaade kaariese levimusest jäävhammastel Kagu-Eesti noorema kooliastme õpilastel vahelduvas hammaskonnas.
2. Leida seos jäävmolaaride ja ajutiste molaaride kaariese esinemise vahel vahelduvas hammaskonnas.
3. Testida ja võrrelda erinevate polüoolide ehk suhkuralkoholide (erütritool, külitool ning sorbitool positiivse kontrollainena) pikaajalise tarbimise mõju kaariese arengule vahelduvas hammaskonnas.
4. Võrrelda erinevate polüoolide (erütritool, ksülitool ja sorbitool) mõju süljele ja hambakatule.

Metoodika

Tegemist oli kolmeaastase topeltpimeda, randomiseeritud, prospektiivse sekkumisuuringuga. Uuring viidi läbi Kagu-Eestis, kus kaariese näitajad on riigi kõrgeimad. Osales 10 kooli neljast erinevast maakonnast, mis moodustas 10% vastava piirkonna koolide koguarvust. Kõik lapsevanema või hooldaja poolt kirjaliku eelinformeeritud nõusoleku andnud 522 esimese ja teise klassi õpilast kaasati uuringusse. Esimesed klassid jaotati arvuti poolt juhuslikult genereeritud numbrite alusel kolme erinevasse gruppi (erütritooli, ksülitooli ja sorbitooli grupp) ja teised klassid ekvivalentselt nii, et igasse uuringugruppi kuuluks esmaselt vähemalt 151 last. Uuringu toimumiseks oli koolide juhtkonna nõusolek ning Tartu Ülikooli Eetikakomitee luba (protokoll 166/T-7). Kliiniline uuring registreeriti ka Ameerika Ühendriikide Rahvuslikus Tervise Instituudis identifitseerimisnumbriga NCT01062633.

Õpilaste kliiniline läbivaatus toimus Tartu Ülikooli Stomatoloogia kliinikus esmase läbivaatusena ja seejärel 12, 24 ja 36 kuu pärast. Läbivaatus teostati nelja eelkalibreeritud isiku poolt standartsel hambaravitoolil ja sisaldas kaariese-kahjustuste registreerimist ICDAS II süsteemi kasutades ning hambakatu- ja süljenäidiste kogumist. Esmasel kliinilisel läbivaatusel osales 485 õpilast, kelle keskmine vanus oli 7,8 esimese klassi ja 8,8 aastat teise klassi õpilastel. Haiguse tõttu jäi kõrvale 37 õpilast (7,1% uuringus osalejatest).

Uuringu toimumise päeva hommikul oli palutud lastel hambaid mitte pesta. Interdentaalse hambakatu näidised võeti kõigist sektoritest esimese jäävmolaari mesiaalselt küljelt mikroharjakesega, kandes näidised Orion Diagnostica (Espoo, Finland) Site Strips Test'ile. Hambakattu koguti diagnostilist sondi kasutades kolme minuti jooksul kõigilt vabalt juurdepääsetavatelt hamba-pindadelt ja kaaluti koheselt, lahustati steriilses 0,9% NaCl lahuses ning külmutati –80°C juures edasisteks keemilisteks analüüsideks. Streptokokkide hulga määramiseks kasutati Dentocult® SM (Orion Diagnostica company; Espoo, Finland) komplekti kuuluvat spaatlit, mida roteeriti kergelt suletud suus. Lisaks koguti ja mõõdeti stimuleeritud sülje hulk ning kasutati seda laktobatsillide hulga määramise (Dentocult® LM; Orion Diagnostica) testis.

Polüoolide tarbimise alusel moodustus kolm gruppi: erütritooli (n=165), ksülitooli (n=156) ja sorbitooli kui positiivse kontrolli rühm (n=164). Kõik polüoooolid olid disainitud ja toodetud spetsiaalselt uuringu tarbeks Cargill R&D Centre Europe's ning olid imemiseks mõeldud pelletid, mis sisaldasid ~90% toimeainet. Polüoole tarvitati kogu kooliaasta vältel kõigil koolipäevadel (~200 päeval aastas) kolm korda koolipäeva jooksul: kohe kooli saabudes, lõunasöögi järgselt ja enne koolist lahkumist. Pastillide manustamist juhtisid ja kontrollisid klassijuhatajad.

Kõik hambakatu keemilised analüüsid toimusid Belgias, Cargilli laboratooriumis, kus määrati suhkrute, orgaaniliste hapete ja kaltsiumi sisaldus katus.

Statistilisteks analüüsideks kasutati epidemioloogiliste andmete jaoks esmasel läbivaatusel osalenud 485 lapse tulemusi ning kaariese dünaamika jälgimise ja polüoolide mõju hindamiseks kõigil neljal läbivaatustel osalenud 374 õpilase andmeid. Kõik olulisemad statistilised analüüsid tehti Turu Ülikooli ClinData Service osakonna statistiku poolt.

Tulemused ja järeldused

1. Kaariese esinemissagedus ja kaariesenäitajad Kagu-Eesti algklasside õpilaste hulgas on kõrged. Keskmine kaariese indeks ($D_{4-6}MFT$) oli esimese klassi õpilastel 0,8 (SE=0,11) ja 1,1 (0,10) teise klassi õpilastel. Laste keskmine vanus esmasel läbivaatusel oli vastavalt 7,8 (SD=0,35) esimeses ja 8,8 (SD=0,38) teises klassis. Kaariesest haaratus ($D_{4-6}MFT>0$) esimeses klassis on 36.2% ja teises klassis juba 48.3%. Saadud tulemus sobitub eelnevate, teistes vanusegruppides läbi viidud uuringutulemustaga. Kaarieskahjustuste arv tõuseb kiirelt, olles peaaegu kõigi näitajate poolest teise klassi õpilastel

statistiliselt oluliselt kõrgem kui esimese klassi õpilastel. Seega on ennetusprogrammid selles vanuses väga olulised.

2. ICDAS II kaariese registreerimise süsteem sobib kasutamiseks kliinilistes uuringutes, mis nõuavad kaarieskahjustuste dünaamika jälgimist. Leiti oluline seos ($p<0,0001$) jäävmolaaride ja ajutiste molaaride lingvaalsel ja oklusaalsel pinnal paiknevate dentiini ulatuvate kahjustuste vahel. Ravimata piimahambad suurendavad jäävhammaste kaariesest kahjustumise riski.
3. Esimesed alumised jäävmolaarid (eriti nende mälumispinnad) on esimesena kahjustuvad jäävhambad. Täidiseid on 10,9%-l ülemistest ja 21,0%-l alumistest esimestest jäävmolaaridest Kaaries saab alguse enamasti kohe peale hamba lõikumist ja selle ennetusena kasutatakse fissuuride katmist silantidega, kuid nende arv uuritavate hulgas oli üllatavalt madal – 2,4% esimese- ja 3,9% teise klassi õpilastel. Dentiinikaariest (ICDAS koodid 4–6) esines kõige enam teistel piimamolaaridel, mis on ootuspärane, kuna tegemist on kõige kauem suus püsiva, viimasena vahetuva piimahambaga.
4. Võrreldes sorbitooli ja ksülitooli grupiga oli erütritooli grupis kaariese areng aeglasem. Kõige selgemini avaldus see alates teisest uuringuaastast, olles aeglasem ka kolmandal aastal. Hilise avaldumise põhjuseks võib olla aeg, mis karioosse defekti välja kujunemiseks kulub – orienteeruvalt 2 aastat. Erütritooli kaariese kulgu pärssiv omadus laseb seda ainet soovitada ühe alternatiivse suhkruasendajana.
5. Erütritooli grupis oli märgatavalt madalam nii hambakatu hulk ($p<0.05$), uuritavate hapete tase ($p\leq0.05$) kui *Streptococcus mutans´*i hulk võrreldu na sorbitooli ja ksülitooli tarbinutega. See kinnitab erütritooli sobivust kaariese ennetuseks läbi hambakatu hulga ja koostise positiivsete muutuste. Ükski tarvitavatest polüoolidest ei mõjutanud laktobatsillide taset süljes.
6. Sorbitool ja ksülitool selle uuringu jooksul kaariese arengule vahelduvas hammaskonnas mõju ei avaldanud. Eelnevad uuringud on tõestanud ksülitooli kaariest vähendavat mõju, kuid antud uuringus jäi ksülitooli efekt tagasihoidlikuks. Üheks seletuseks võib olla väiksem päevase tarvitamiskordade arv – ametlikult soovitatava 4–5 korra asemel 3 korda päevas. Samuti ei tarbitud polüoole koolivälistel päevadel, kokku ~200 päeva jooksul aastas, mis jätab tarbimisse pika tühimiku.

   Vastavalt saadud uuringutulemustele võib erütritooli sisaldavaid tooteid soovitada suhkruid sisaldavate maiustuste asemel ühe kaariese ennetuse vahendina lastel.

ACKNOWLEDGEMENTS

To launch and conduct a clinical trial to obtain plausible and evidence-based results is a time-consuming and resource-intensive project, which needs the effort and co-operation of the whole research group. I am grateful for the joint effort of the staff of the Department of Stomatology at the University of Tartu, where this study took place from 2008 to 2014.

My greatest thanks go to my magnificent supervisors whose experience and volume of knowledge amazed me again and again.

My greatest acknowledgments go to the head of the Department of Stomatology, University of Tartu, Professor Mare Saag, who coordinated the whole study and encouraged me to start my doctoral studies, and always helped me despite being occupied with numerous administrative duties. She never lost belief in my success and therefore motivated and forced me to continue.

I wish to express my appreciation to my supervisor Professor Eino Honkala for his invaluable advice and support during the trial and writing process and whose position on the area of caries epidemiology and experience in organising clinical trials were noticeable and ensured the achievement of the goals of this study.

I am deeply grateful to my supervisor, Professor Kauko Mäkinen, an outstanding scientist exploring the role of sugar alcohols in the prevention of dental diseases throughout his carreer. He inspired me with his broad mind, kindly shared boundless knowledge of biochemistry and patient guidance during my first steps as a scientist.

Special thanks go to Sisko Honkala, who advised me and so kindly shared her experience as a journal editor and was able to catch all the errors in our manuscripts.

I am grateful to biostatistician Tero Vahlberg, from the Department of Biostatistics of the University of Turku, who has annually conducted numerous statistical analyses for all the manuscripts and reports during this study.

Special thanks go to Jana Olak and Triin Jagomägi who have shared the path of a scientist while writing and defending the thesis. I want to thank my colleagues Rita Nõmmela and Silvia Russak for their contribution to this study.

I appreciate the students and residents for their enthusiastic help during the examination.

I am grateful to Cargill R&D Centre Europe (Vilvoorde, Belgium) where all the chemical laboratory tests of dental plaque took place and where all the test products were manufactured. I wish to thank Peter de Cock who was always ready to advise us concerning polyols.

I am grateful to my reviewers Professor Mihkel Zilmer and especially to Professor Reet Mändar who took time to read my manuscript carefully and cave me directions and suggestions which notably improved the last version of dissertation.

I am also appreciative of Professor emeritus Heikki Murtomaa who kindly agreed to be my opponent during the defence.

Most of all I am grateful to my husband Andres and my sons Andres-Kristjan and Arle-Martin for their patience and managing during my long working days and professional trips. They have always supported me and improved my computer and language skills which facilitated my work.

PUBLICATIONS

CURRICULUM VITAE

Name:	Riina Runnel
Date of birth:	28 October 1972, Pärnu, Estonia
Address:	University of Tartu, Faculty of Medicine, Department of Stomatology Raekoja plats 6, 51003, Tartu, Estonia
Phone:	+372 731 9856
E-mail:	riina.runnel@ut.ee

Education

2009–2015	University of Tartu, Faculty of Medicine, PhD studies
1996–1997	Tartu University Hospital, Clinic of Dentistry, internship
1991–1996	University of Tartu, Faculty of Medicine, Stomatology
1980–1991	Pärnu 4th Secondary School and Senior Secondary School, with extended curricula in biology and medicine

Professional employment

2005–	University of Tartu, Faculty of Medicine, Department of Stomatology, assistant
2002–2004	University of Tartu, Faculty of Medicine, Institute of Anatomy, assistant
2003–	Private practice, Dental Clinic Blendent, dentist
2000–2003	Tartu University Hospital, Clinic of Dentistry, dentist
1997–2003	Dentes Health School, lecturer
1997–2001	Dentes Dental Clinic, dentist
1996–1998	Tartu University Hospital, Clinic of Dentistry, Department of Paediatric Dentistry, dentist

Scientific work

Main fields of research: dental caries diagnostics, caries epidemiology, sugar alcohols in caries prevention.

Publications: Six scientific articles in international peer reviewed journals, 3 in Estonian journals and 18 international conference presentations have been published.

Membership:

2013–	Estonian Dental Association, council member
2010–	International Association for Dental Research
2009–	European Association of Dental Public Health
2008–	Estonian Dental Association

ELULOOKIRJELDUS

Nimi:	Riina Runnel
Sünniaeg:	28. oktoober 1972, Pärnu, Eesti
Aadress:	Tartu Ülikool, Arstiteaduskond, Stomatoloogia kliinik Raekoja plats 6, 51003, Tartu, Eesti
Telefon:	(+372) 731 9856
E-mail:	riina.runnel@ut.ee

Hariduskäik

2009–2015	Tartu Ülikool, Arstiteaduskond, doktorantuur
1996–1997	Tartu Stomatoloogia Polikliinik, internatuur
1991–1996	Tartu Ülikool, Arstiteaduskond, stomatoloogia
1980–1991	Pärnu 4. Keskkool, bioloogia-meditsiini süvaõppega klass

Erialane teenistuskäik

Alates 2005	Tartu Ülikool, Arstiteaduskond, Stomatoloogia kliinik, assistent
Alates 2003	OÜ Blendent, hambaarst
2002–2004	Tartu Ülikool, Arstiteaduskond, Anatoomia instituut, assistent
2000–2003	SA Tartu Ülikooli Kliinikum, Stomatoloogia kliinik, arst-õppejõud
1997–2001	Erakliinik Dentes, hambaarst
1997–1998	Tartu Stomatoloogia kliinik, lastestomatoloogia osakond, hambaarst
1997–2003	Dentes Tervishoiukool, lepinguline lektor
1996–1997	Tartu Stomatoloogia kliinik, nooremarst

Teadustöö

Peamised teadustöö suunad: hambakaariese diagnostika, kaariese epidemioloogia, suhkuralkoholide osa kaariese ennetuses.

Publikatsioonid: avaldatud 6 teadusartiklit eelretsenseeritavates rahvusvahelise levikuga ajakirjades, 3 kodumaistes ajakirjades ja 18 rahvusvahelise konverentsi ettekannet.

Liikmelisus:

2013–	Eesti Hambaarstide Liidu täidesaatva kogu liige; suutervise edendamise vastutusala
2010–	IADR (International Association for Dental Research) liige
2009–	EADPH (the European Association of Dental Public Health) liige
2008–	Eesti Hambaarstide Liidu liige

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.
20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.

21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel.** Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik.** Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk.** Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets.** Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik.** Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi.** Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo.** Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber.** *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla.** Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk.** Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf.** Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv.** Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi.** Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva.** Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin.** Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev.** Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik.** Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto.** Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar.** Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge.** Humoral immune responses to allergens in early childhood. Tartu, 1998.
42. **Heli Grünberg.** The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.

43. **Epp Sepp.** Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots.** Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe.** Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv.** Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan.** Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik.** Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand.** Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi.** Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask.** Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson.** Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar.** Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl.** Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap.** Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg.** *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro.** Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi.** Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann.** Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke.** Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves.** Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda.** Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist.** Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.
64. **Jana Kivastik.** Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.

65. **Karin Kull.** Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos.** Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova.** Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda.** Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner.** *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi.** Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla.** Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas.** Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk.** Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen.** Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar.** Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep.** Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes.** Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu.** Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi.** Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa.** Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.
83. **Helgi Kolk.** Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.

84. **Helena Soomer.** Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin.** Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann.** Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu.** Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas.** Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba.** Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev.** CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja.** Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla.** Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid.** Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter.** Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa.** Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre.** Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev.** Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.
100. **Aune Rehema.** Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet.** Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.

102. **Eduard Maron.** Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona.** *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk.** Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv.** Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun.** Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda.** Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo.** The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila.** Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla.** Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell.** Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme.** Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor.** Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm.** Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite.** Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll.** Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach.** Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.
120. **Katrin Pudersell.** Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako.** Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.

122. **Aare Märtson.** Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld.** Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann.** Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar.** Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson.** Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus.** Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder.** Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru.** Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand.** Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo.** Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo.** The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild.** Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik.** Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller.** Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik.** Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals.** Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus.** Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab.** Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.
141. **Lea Pehme.** Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin.** The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik.** Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.

144. **Tarvo Rajasalu.** Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu.** Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik.** Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets.** The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits.** Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam.** Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots.** Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp.** The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull.** Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre.** Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link.** Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre.** Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak.** Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich.** Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep.** Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk.** Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.
161. **Kaire Heilman.** Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel.** HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller.** Immune markers in major depression and in antidepressive treatment. Tartu, 2009.

164. **Siim Suutre.** The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman.** Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa.** Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org.** Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht.** Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu.** Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand.** Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull.** Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar.** Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky.** Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija.** Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht.** Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram.** The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum.** Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek.** The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer.** The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina.** Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik.** Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.
183. **Maarja Krass.** L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai.** Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.

185. **Tiit Salum.** Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova.** The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi.** Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi.** A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe.** Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm.** Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu.** Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont.** Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt.** Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru.** Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov.** Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg.** Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm.** Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg.** Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal.** Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.
203. **Jana Lass.** Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu.** Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.

205. **Oksana Jagur.** Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk.** Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal.** Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand.** Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma.** Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre.** Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp.** Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum.** Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson.** Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand.** Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma.** Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg.** Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit.** Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees.** Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik.** The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel.** Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna.** The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.
222. **Ingrit Liiv.** Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison.** Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.

224. **Krista Ress.** Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru.** Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu.** Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets.** The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome.** Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo.** Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin.** Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna.** Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase.** Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.