New Animal Model of Extrinsic Dental Erosion – Erosive Effect on the Mouse Molar Teeth

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A running title:

Dental Erosion in an Animal Model

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Abstract

Objective: Consumption of acidic food and drinks is considered as important risk factor for development of dental erosion. There are several *in vitro* and *in situ* studies focusing on the risk indicators and preventive treatment, however, the need for a standardized animal model has been emphasised for many years. The aim was to establish an animal model of extrinsic dental erosion, which may serve as a standard for future studies to improve our understanding of the erosion.

Design: Two acidic drinks, sports drink and cola drink, were given to young mice for six weeks. Experimental and control (water) molars and incisors were dissected out and observed by scanning electron microscopy (SEM). Mandibular first molars were subsequently ground transversely and observed again by SEM. The tooth height and enamel thickness were measured on the SEM images.

Results: The lingual surface of the mandibular molars was most eroded after consumption of acidic drinks. The cola drink exhibited higher erosive effect on mandibular molars compared to sports drink. The lingual tooth height, compared to control, was about 34% and 18% lower in the cola drink and sports drink molars, respectively. Compared to the control molars, the lingual enamel was about 23% thinner in the sports drink molars and totally eroded on the certain lingual areas of the cola drink molars.

Conclusions: This new animal model of extrinsic dental erosion and the presented method with ground molars observed in SEM are suitable for further studies, which will gain deeper insights into the erosive disease.

Keywords: Acidic drinks; Animal model; Dental erosive wear; Dental enamel; Scanning electron microscopy

Introduction

Several chemical and mechanical impacts contribute to the wear of the dentition throughout life. The manifestation of dental erosion, acid induced dental substance loss, has generally been accepted to be a multifactorial condition caused by various extrinsic and intrinsic acid sources (Lussi & Carvalho, 2014). There are indications that the prevalence of erosive tooth wear is increasing, especially in younger people, partly due to a change in nutritional habits and lifestyle (Jaeggi & Lussi, 2014; Mulic, Vidnes-Kopperud, Skaare, Tveit, & Young, 2012). A recent review and meta-analysis estimated the prevalence among children and adolescents to be on average 30% (Salas, Nascimento, Huysmans, & Demarco, 2015). Extrinsic acids are mainly acidic drinks and food. Therefore, individuals consuming such products frequently are at risk for this type of dental hard tissue destruction. However, clinicians are still observing that dental erosion may occur or be absent regardless of these factors. The reason for that is still elusive, but it has been suggested that the individual`s susceptibility to dental erosion is influenced by genetic variation (Chadwick et al., 2005; Sovik, Skudutyte-Rysstad, Tveit, Sandvik, & Mulic, 2015; Uhlen, Stenhagen, et al., 2016), as well as by factors in the oral environment (Chadwick et al., 2005; Uhlen, Mulic, Holme, Tveit, & Stenhagen, 2016).

Both the salivary flow rate and the composition of saliva are important factors that could have impact on its protective properties, and saliva has been considered as the most important biological factor in the prevention of dental erosion (Buzalaf, Hannas, & Kato, 2012; Hara & Zero, 2014). It has been suggested that the flow rate may be the best clinical indicator of the protective properties of saliva (Tenovuo, 1997). As far as we know, there are only a few studies investigating the association of low salivary flow rate and the occurrence of dental erosions (Aldosari et al., 2018; Jarvinen, Rytomaa, & Heinonen, 1991; Jensdottir, Buchwald, Nauntofte, Hansen, & Bardow, 2013; A. K. Johansson, Norring, Unell, & Johansson, 2012; Mulic, Tveit, Songe, Sivertsen, & Skaare, 2012). Furthermore, different fluoride treatments in high

concentrations are recommended as part of preventive treatment for individuals with risk for dental erosion. Conventional fluorides offer some, but limited protection against erosion (Magalhaes, Wiegand, Rios, Buzalaf, & Lussi, 2011). Therefore, the interest has grown into fluoride compounds containing polyvalent metal cations such as stannous fluoride (SnF_2) and titanium tetrafluoride (TiF_4). These agents have shown a protective, anti-erosion effect *in situ* (Schlueter, Klimek, & Ganss, 2009; Stenhagen, Hove, Holme, & Tveit, 2013). It has been concluded that tin-containing fluoride product might provide the best protection (Magalhaes et al., 2011).

Although there are a number of *in vitro* and *in situ* studies focusing on the risk indicators and preventive treatment of dental erosion, there are only few studies that have investigated the influence of certain risk indicators related to dental erosion in animal models (Aldosari et al., 2018; Sorvari, 1989; Sorvari & Kiviranta, 1988; Sorvari, Kiviranta, & Luoma, 1988; Sorvari, Pelttari, & Meurman, 1996). Standardized *in vivo* models, which compared to *in vitro* and *in situ* studies without the saliva and soft tissue interactions, are suitable for further studies that may gain deeper insights into the salivary influence on development of dental erosive lesions. An advantage of an animal model, compared with human studies, is that the experimental procedures may be performed under more controlled conditions. Furthermore, human *in vivo* experiments are considered as unethical because of the irreversible loss of dental hard tissues. In the animal models used previously, the methods with limited possibility to study the details did not allow a registration of small erosive lesions and their depths (Higo et al., 2009; Sorvari & Kiviranta, 1988).

The need for a standardized animal model for studying dental erosion has been emphasised for many years (Curzon & Hefferren, 2001). A new animal model where dental lesions of different severity can be created and analysed with sensitive methods is therefore warranted. The aim of the present study was to create an animal model of extrinsic dental

erosion that will improve our understanding of erosive dental disease and serve as an appropriate model for future studies. For this purpose, experimental dental erosion was induced in mouse, and the erosive effect of products containing both citric (sports drink) and phosphoric (cola drink) acid on their dentition was studied in detail. We hypothesise that acidic drinks induce dental erosion in mouse teeth, and that comprehensive measurements of enamel loss and reduction in the tooth height may be recorded by SEM.

Materials and methods

Animal model

Ninety phenotypical, young female mice (CD-1 strain, 7 weeks old, 30±5 g body wt) were selected for the study. Prior to experimental use, the animals were given standard laboratory fodder and water *ad libitum*, and they were maintained on a 12 h light: dark cycle, at 21 ºC with a relative humidity of 65%. The animals were kept in accordance with Norwegian regulation and legislation (Norwegian Regulation on Animal Experimentation of 2015 based on EU directive on the Protection of Animals used for Scientific Purposes 2010/63/EU and Norwegian Animal Welfare Act of 2009). The experiment was approved by Norwegian Food Safety Authority (FOTS ID 12710).

Before the experimental erosive procedures, the wire cages with solid bottom and bedding were prepared in order to reduce the wear of the dentition by attrition. The cages were carefully inspected before the mice were transferred into them. Any hard objects such as wooden sticks and plastic wheels were removed from the cages, and the animals were only supplied with paper boxes and paper ribbons as a part of environment enrichment. All cages were replaced two times per week, and the animals were monitored daily. Moreover, in order to reduce attrition of the teeth during the experiment, the standard laboratory fodder was softened prior to feeding. Fifty pieces of Teklad Global 18% Protein Rodent Diet (Envigo

Teklad, Madison, WI, USA) were soaked with 165 ml of cold tap water, sealed in a plastic bag and left for softening overnight.

The mice were randomly distributed into three experimental groups, which were provided with distilled water (control), Red Bull sugar free sports drink (citric acid, pH=3.39), and Coca Cola drink (phosphoric acid, pH=2.27), respectively. Each group (n=30 animals), was further divided into triplicate subgroups, i.e. ten animals per cage. Two 250 ml bottles with drinks were placed into each cage, and the bottles were replaced three times per week. Prior to the experiment, the changes in pH of both sports drink and cola drink were monitored over a period of three days, and the results showed no changes in pH. During the experiment, all animals were provided with softened laboratory fodder and drinks *ad libitum*, and the consumption of drinks in each cage was recorded. After the experimental period of six weeks, all animals were sacrificed by cervical dislocation, and their heads were fixed in 70% ethanol. All animals were weighted at the start and at the end of the experiment.

Scanning electron microscopy

The maxillary and mandibular molars and incisors were dissected out and fixed in 70% ethanol. The isolated teeth were thoroughly cleaned by dissection and by gentle brushing under running tap water. The specimens were air-dried overnight and mounted on brass cylinders with cyanoacrylate glue, sputter-coated with 30 nm platinum and observed in a Philips XL30 ESEM (Philips, FEI, Netherlands) operated at 12 kV.

The jaw segments containing all three molars were thereafter embedded in Epon and ground transversely. The grinding was performed under a stereo-microscope using grits 800 and 1200 3 M waterproof silicone carbide paper (3 M, St. Paul, MN, USA) in a specially designed apparatus (Risnes, 1985). The ground surfaces were then polished by grinding the specimens against the backside of the 3 M waterproof silicone carbide paper with 0.05 μm

particle size alumina powder (Buehler Micropolish, Buehler, Lake Bluff, IL, USA) in water. After careful brushing under running tap water and removal of excess water, the teeth were etched for 45 s in 1% nitric acid, air-dried overnight, sputter-coated with 30 nm platinum and observed in scanning electron microscopy (SEM). For the transversely ground molars the whole procedure (grinding, polishing, etching, air-drying, sputter-coating, and observing in SEM) was repeated, creating two transversely ground planes for observation. The first plane (T1) was positioned on the mesial aspect of buccal cusp B2 and lingual cusp L2 where the tip of the cusps exhibited enamel-free areas (Lyngstadaas, Moinichen, & Risnes, 1998). The subsequent plane (T2) was ground further in distal direction ending on the distal aspect of buccal cusp B2 and lingual cusp L2 where the tip of the cusps was covered with enamel. The T1 and T2 planes were positioned in an area where the occurrence of dental erosions on the first molars were noted when the whole teeth were observed in the SEM.

Measurements and statistical analysis

SEM images of the transversely ground and etched plane T1 were used for measurements of tooth height and enamel thickness in distilled water (control), sports drink, and cola drink mandibular first molars. Mean values and standard deviations were calculated using Microsoft Excel Worksheet (Microsoft Office Excel, 2017). The step initiation at the eroded lingual enamel in sports drink and cola drink molars, was calculated by measuring the distance between the horizontal line at the level of the step and the corresponding line at the level of the enamel-cementum junction (illustrated in Fig. 5). Measurement data were tabulated and analyzed using the Statistical Package for Social Sciences 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test, and independent *t*-tests were used for the evaluation of data. P-values < 0.05 were considered statistically significant.

Results

Animal wellness and behavior

During the experimental period of six weeks, the animals exhibited normal behavior. Only one animal from the control group showed signs of behavioral disorder and illness four weeks after the start of the experiment, and was consequently excluded from the study. Daily consumption of drinks was about 50 ml per cage, and by the end of the experiment the mean weight gain of the animals was 3.71 g, 3.52 g, and 3.40 g in the control, sports drink, and cola drink group, respectively.

Tooth morphology and enamel distribution in control and experimental mice

The maxillary and mandibular jaw segments were observed from the occlusal, buccal, and lingual aspects. All three molars, in both the control and experimental mice were erupted and in occlusion (Fig. 1a, g, m & Fig. 2a, d, g). In the control mice, the molar enamel was unaffected with a smooth surface covering all sides of the teeth and forming a cuspal complex surrounding a common dentin surface at the tip of the cusps (Fig. 1a-f & Fig. 2a, b). The experimental mice exhibited various degrees of erosion mainly restricted to the lingual and occlusal part of the molars, being more evident in mandibular (Fig. 1g-r) compared to maxillary (Fig. 2d, e, g, h) molars. No morphological differences and effects of acid were observed between the control and the experimental incisors (Fig. 2c, f, i).

A specific erosion pattern was observed in both sports drink (Fig. 1g-l, Fig. 2d, e) and cola drink (Fig. 1m-r, Fig. 2g, h) molars, the latter being considerably more affected. There were no significant differences between molars on the right and left side of the mouth. In the mandibular sports drink molars, a distinct step on the lingual side was observed on all three molars, representing the border between the unaffected cervical and affected occlusal part of the teeth (Fig. 1g, i). The enamel on the buccal aspect of the teeth was unaffected (Fig. 1k, l).

Both lingual and buccal cusps were reduced in height and showed a blunter appearance compared to control molars (Fig. 1i-l). The lingual enamel was eroded; however, it covered the dentin at all aspects of the cusps except for the enamel-free areas at the top (Fig. 1g-l). In the maxillary sports drink molars, a distinct erosion step on the lingual side was observed only on the first molar (Fig. 2d), particularly on the mesial half of the tooth including the mesial central cusp (Fig. 2e).

In the mandibular cola drink molars, severe erosion on the lingual side was observed on all three molars (Fig. 1m, o). The enamel was completely eroded on the lingual aspect of all lingual cusps with exposed and partially eroded dentin (Fig. 1m-p). The cervical part of the lingual enamel under the erosion step and the enamel on the buccal aspect of the teeth was not affected (Fig. 1o-r). Both lingual and buccal cusps were dramatically reduced in height, and the cusps exhibited a rounded appearance (Fig. 1m-r). On the occlusal aspect of the teeth, erosion had increased the size of the enamel-free areas, which were continuous with the lingual dentin (Fig. 1n). In the maxillary cola drink molars, an erosion step on the lingual side was observed on the first and second molar (Fig. 2g). The enamel was slightly eroded on the lingual aspect of the second molar (Fig. 2g) and totally eroded on the lingual aspect of the first molar including the mesial central cusp (Fig. 2h).

Erosive effect on the dental enamel

Based on the SEM observations of the whole teeth (Figs. 1 & 2), the mandibular first molar was chosen as a model for further studies of dental erosion. Accordingly, two transversely grinded planes for observation (T1 and T2) were prepared, as described in Materials and methods. With the present tooth sections, detailed loss of enamel layers in experimental molars compared to control molars were recorded (Figs. 3-5, Table 1).

In Figure 3, images from the transversely ground plane T1 of the mandibular first molars from the control, sports drink, and cola drink mice are presented. At this position, enamel-free areas at the tip of the cusps are normal morphological features (Fig. 3a, d, g). The unaffected enamel at the lingual aspect of control molars (Fig. 3a-c) and at the cervical part of the lingual aspect of experimental molars (Fig. 3d, e, g, h) exhibited a normal basic structure in both the control and experimental molars. The characteristic four layers: 1) an inner prism-free layer, 2) an inner enamel with prism decussation, 3) an outer enamel with parallel prisms, and 4) a superficial prism-free layer were observed. At about 274 μm, as measured from the enamel-cementum junction, erosive step was evident in the sports drink molars (Fig. 3d, e; Fig. 5j; Table 1). Going from this step in an occlusal direction, the enamel was gradually lost, with complete loss of superficial enamel and partial loss of outer enamel (Fig. 3e, f). At this level, the enamel was about 23% thinner (52 μm vs 68 μm) in the sports drink molars compared to the control molars (Fig. 3b, c, e, f; Fig. 5g, h; Table 1). The lingual tooth height was about 18% lower (637 μm vs 781 μm) in the sports drink molars compared to the control molars (Fig 5a, b; Table 1). The enamel on the buccal aspect was unaffected, and the buccal tooth height was only slightly reduced (Fig. 5d, e; Table 1). Further distally, as shown in the transversely ground plane T2 (Fig. 4), the loss of enamel as observed at the level of the erosive step is even more steep, with initial loss of the superficial and part of the outer enamel (Fig. 4d, e). Immediately thereafter, a complete loss of outer enamel is evident (Fig. 4e, f). At this level, the cusps are normally covered with enamel (Fig. 4a, d).

The transversely ground planes of the first mandibular cola drink molars revealed a similar erosive pattern as observed in the sports drink molars, i.e. the lingual enamel being most affected (Figs. 3 & 4). However, the erosive effects in the cola drink molars were more severe, and more extended in the cervical direction (Fig. 3g; Fig. 5k). The unaffected cervical enamel exhibited a normal basic four-layered structure (Fig. 3h). At T1, about 183 μm, as measured

from the enamel-cementum junction, an erosive step was evident in the cola drink molars (Fig. 3g, h; Fig. 5k; Table 1). Going from this step in an occlusal direction, the enamel was rapidly lost, with complete loss of superficial and outer enamel, and gradual loss of inner enamel (Fig. 3h, i). Further occlusally, the complete loss of enamel was evident (Fig. 3j, k). At this level, dentin erosion was also observed, as judged by the lingual outline of the dentin surface (Fig. 3g; Fig. 5). The tooth height was about 34% lower (512 μm vs 781 μm) in the cola drink molars compared to the control molars (Fig. 5a, c; Table 1). The enamel structure and thickness on the buccal aspect was unaffected (Figs. 3-5), however, the buccal tooth height was reduced with about 12% (513 μm vs 580 μm) (Fig. 5d, f; Table 1). Further distally, as shown in the transversely ground plane T2 (Fig. 4g-k), the loss of enamel exhibits the same patterns as observed at T1, but here the level of the erosive step is steeper, resembling the findings of the sports drink molars (Fig. 3h; Fig. 4i).

Discussion

Many dietary drinks include different types of acids, such as citric acid, phosphoric acid, carbonic acid, ascorbic acid, and malic acid, which has an impact on the erosive effect (Barbour & Lussi, 2014; Shellis, Featherstone, & Lussi, 2014; Zero & Lussi, 2005). Lifestyle, drinking habits, and the method of consuming drinks differ between individuals and are accordingly important for how long acids are retained in the mouth before swallowing. It has also been shown that the drinking technique, as well as amount are of consequence for the risk of developing erosive lesion (A. K. Johansson, Lingstrom, Imfeld, & Birkhed, 2004; Sovik et al., 2015). In the present animal study, two popular drinks among both adults and adolescents were chosen, i.e. sports drink (citric acid, pH=3.39) and cola drink (phosphoric acid, pH=2.27). The mice were served these drinks as the only drink for six weeks and, thereafter, the erosive effect on the whole dentition was studied. The results demonstrated a specific erosion pattern, similar

to lesions seen in patients, in both the sports drink and cola drink molars, the latter being considerably more affected. The findings showed, as well as in previous animal studies (Gortner, Restarski, & et al., 1945; Sorvari et al., 1988; Spencer & Ellis, 1950), small individual variation in the erosive effects between mice within the same experimental group. This variation may be due to different drinking habits and the amount of drink consumption, which were not possible to control during the experiment. However, by observing the mice daily during the experimental period of six weeks, no special differences could be observed in drinking frequency. Furthermore, these small differences within the same group of mice may be due to other individual factors, e.g. salivary flow and saliva buffering function.

In accordance with previous studies in rats (Sorvari & Kiviranta, 1988; Sorvari et al., 1988; Sorvari et al., 1996) the lingual surface of mouse mandibular molars exhibited the strongest erosive effect after consumption of acidic drinks (Figs. 1 & 2). In addition, no significant differences between the first and the second and third mandibular molars (Fig. 1) were demonstrated. In the maxillary molars, which in general were less affected, the first molars were more affected compared to the second and third molars (Fig. 2). This is also reported in several clinical studies (Arnadottir et al., 2010; Ganss, Klimek, & Giese, 2001).

Previously, first rat mandibular molars have been preferred for studying erosion in animal models, mainly because of their size (Aldosari et al., 2018; Sorvari & Kiviranta, 1988; Sorvari et al., 1988; Sorvari et al., 1996). So far, few methods, with limited possibility to study the details, have been used in order to study the effects of acidic drinks on dental erosion. Already in 1988, the erosive effect on the rat molar teeth of a sport drink mixture with and without addition of fluoride and magnesium was studied (Sorvari et al., 1988). In that study, the lingual surfaces of the first mandibular molars were investigated and drawn under a stereomicroscope equipped with a drawing tube. Thereafter, the teeth were photographed with a camera, and then the total surface areas, intact areas, and eroded enamel were scored by a

grading system (Sorvari et al., 1988). In a subsequent study by Sorvari and Kiviranta the method for erosion recoding was semi-quantitative, based on the subjective evaluation resulting in numerical data of the intact and eroded surface areas (Sorvari & Kiviranta, 1988). With this method, compared to the grading system used earlier, it was possible to compare total surface areas, which reflected tooth wear, especially occlusal wear. The measured size of the eroded area and the estimated area of occlusal wear together gave a more complete idea of the effects of an erosive agent than the methods used before. Ten years later, surface ultrastructure, particularly of dentin, was studied with SEM in rat molars after experimentally induced erosion and attrition (Sorvari et al., 1996). A recent study on rats was performed with the intention to investigate the susceptibility to dental erosion in partially desalivated animals. The loss of dental hard tissue was measured by use of micro CT (Aldosari et al., 2018). However, the present study is the first of its kind to investigate and standardize the effects of induced experimental erosion in mice, which allows comprehensive measurements of enamel thickness and tooth size. In addition, the erosive effect on the enamel layers in different experimental groups was measured. In excessive tooth tissue loss where erosion, attrition, and abrasion occur simultaneously the original causes may be difficult to distinguish (Eccles, 1982; Shellis & Addy, 2014) and especially erosion may be overlooked in favor of attrition (Lewis & Smith, 1973). It is known that commercial pellets for laboratory animals are abrasive and increase tooth wear when compared with powdered diet, and the faster rate of attrition is not only due to abrasives in the hard pellets but also due to increased time of chewing (Teaford & Oyen, 1989). Additionally, a previous study has shown that different diets given to the rats for six weeks caused morphological surface changes, which were observed by SEM (Sorvari et al., 1996). Rough pellet food caused severe attrition on the lingual surface of the first mandibular molar; the surface was worn with the presence of scratches and small pits when compared to the teeth of rats given soft powdered diet. Studies have reported that erosion increases the susceptibility

of the tooth surface to attrition and abrasion (Lewis & Smith, 1973; Shellis & Addy, 2014; Smith, 1975), and that erosive sport drink together with rough pellet food caused greatest tooth surface loss (A. Johansson, Haraldson, Omar, Kiliaridis, & Carlsson, 1993; Lewis & Smith, 1973; Sorvari & Kiviranta, 1988). In animal studies, abrasion can be excluded and, thus, the mechanical wear is mainly due to attrition. In our study, in order to reduce attrition of the teeth during the experiment, standard laboratory fodder was softened with water prior to feeding. However, a minor reduction of cuspal height due to occlusal wear was observed even in mice that were exposed only to water (Fig. 1a-f), as judged by cuspal complex surrounding a common dentin surface at the tip of the cusps, when compared to just erupted and non-worn mouse molars (Lyngstadaas et al., 1998). It is likely that some of this occlusal wear occurred before the start of the experiment at the age of seven weeks, since all molars in mice reach the occlusion at an age of 35 days (Lyngstadaas et al., 1998). Furthermore, some of the wear may have occurred during the time of the experiment, despite of exposure only to softened food. Importantly, all animals in this study, in both control and experimental groups, were given the same fodder, both before and during the experiment. Although, we cannot exclude a small influence of attrition on the occlusal aspects of the sports drink and cola drink molars, the findings observed in the enamel on the lingual part of the tooth, are predominantly due to erosion.

It is also worth mentioning, that in our model, rapid destruction of the tooth surface was also due to the exaggerative conditions, where the mice were exposed to acidic drinks continuously for six weeks. Therefore, extrapolating findings from the present study to humans must be taken carefully. Rodent and human enamel exhibits the same basic structural elements, prism and interprism, however, the spatial arrangement of the prisms, i.e. the prism pattern, is considerably different (Warshawsky, Josephsen, Thylstrup, & Fejerskov, 1981). Other differences include the speed at which enamel formation occurs (Risnes, 1979) and the

incorporation of iron in the superficial enamel layer of rodent incisors (Warshawsky & Smith, 1974). However, compared to several previously published *in vitro* and *in situ* studies, animal models are highly advantageous due to the salivary influence and soft tissue interactions, resembling the human oral environment.

The present results showed that the mandibular molars exhibited the strongest erosive effect after consumption of acidic drinks (Fig. 1). The maxillary molars were only slightly affected, especially the first molar, and the incisors were unaffected (Fig. 2). This is also reported in several clinical studies (Mulic, Tveit, & Skaare, 2013; Sovik et al., 2015). Mandibular molars, compared to maxillary molars, were probably more affected due to anatomical relations where the acid is present for a longer time in the sublingual compared to palatal area of the oral cavity. However, it is remarkable that when subjected to the cola drink, mandibular molars, which exhibited severe erosive defects lingually and occlusally, showed no effects on the buccal surface (Figs 1, 3 & 4). A likely explanation for this is that the acid did not remain in the mucobuccal fold long enough to induce erosion on the buccal aspect of the molars, in the same way as the acid in the sublingual area induced erosion on the lingual aspect of the same teeth. As expected, the mouse incisors were not affected, probably due to two main reasons. Firstly, it is not expected that the erupted part of the continuously growing mouse incisor, which exhibits enamel only on the labial aspect of the tooth (Sidaly, Risnes, Khan, Stiris, & Sehic, 2015), is in contact with a substantial amount of acid for a long enough time. Secondly, the enamel of mouse incisors, especially maxillary incisors, exhibits yellow-brown iron pigmentation, which is more resistant to acid (Moinichen, Lyngstadaas, & Risnes, 1996).

The results also demonstrated a very distinct step in the enamel on the lingual aspect representing the border between the unaffected cervical part and the affected occlusal part of the molars (Figs. 3-5). It was striking that this cervical part of the enamel was not affected in the cola drink molars where the adjacent enamel was eroded in total (Fig. 4g-k). Previous

observations have shown the same erosive pattern in rat molars (Sorvari et al., 1988). Gingiva covers a part of this cervical enamel, which may serve as a protection, however, a steep erosive gradient as observed in the cola drink molars (Fig. 3 & 4) is still elusive. One would expect the enamel layers to be gradually eroded along the whole surface that was exposed to acid.

The loss of enamel layers on the lingual aspect of the tooth is best observed in transversely ground planes through the tooth. Achieving ideal transversal ground plane of mouse molars through the correct cusps is technically difficult due to their small size, and consequently, the position of transversal sections may have varied slightly. However, it was considered unlikely that this slight variation would mask the significant differences in enamel thickness between the groups. In order to minimize contributions from random errors, samples from 30 mice including biological and technical triplicates were used for each group. It is also likely that there is some variation in the tooth size between the mice. However, previous studies from our lab have shown that these are minor in mice where the body weight is not significantly different (Lyngstadaas et al., 1998; Sehic, Nirvani, & Risnes, 2013; Sehic, Peterkova, Lesot, & Risnes, 2009; Sehic, Risnes, Khuu, Khan, & Osmundsen, 2011). Therefore, using CD-1 strain where the morphology and size of the mouse molars have previously been thoroughly described (Lyngstadaas et al., 1998), and including water as a control, we assume that this may not have affected the accuracy of the measurements significantly.

In the present study, two popular beverages were chosen, one sugar free (sports drink) and the other containing sugar (cola drink). It may be speculated that sugar may have had some influence on the results; however, we did not observe by SEM any caries in the molars of the cola drink mice. Based on these results, it was evident that cola drink exhibited higher erosive potential compared to sports drink. The rate of dissolution of dental minerals, which is crucial to the progression of erosion, is influenced by solubility and also by other factors (Shellis et al., 2014). It is influenced strongly by type of acid, physical factors (temperature, flow rate) and

chemical factors (degree of saturation, presence of inhibitors, buffering, pH, fluoride) (Shellis et al., 2014). Citric acid has a greater erosive potential than phosphoric acid, presumably related to its ability to form chelating complexes and due to its high buffering capacity (Zero & Lussi, 2005). Chelation is a process widely discussed in relation to erosive tooth wear, and is referred to as the ability of certain ions, particularly citrate, to bind calcium and form calcium complexes, and thereby favoring demineralization by increasing the degree of undersaturation (Zero & Lussi, 2005). Therefore, one would expect that the erosive effect of sports drink was higher compared to cola drink, however, the present results showed the opposite trend. It is difficult to isolate the chelating effect from the other parameters; thus, the connection is still uncertain. A recent study demonstrated that under flowing conditions, chelating effects of citric acid seems to be of negligible relevance with respect to enamel erosion (Azadi-Schossig, Becker, & Attin, 2016). The chemistry of dental erosion is complicated and involves several factors; however, it seems that the low pH of cola drink was an important factor for developing dental erosion in mouse molars.

Conclusions

Human experiments on dental erosion are unethical because of the irreversible loss of dental hard tissues when exposed to acids. Therefore, studies using animal models are of high importance since they represent clinical situations. Based on the present results it can be concluded that cola drink (phosphoric acid, pH=2.27) exhibited higher erosive effect on mouse mandibular molars compared to sports drink (citric acid, pH=3.39). This new animal model of extrinsic dental erosion where lesions of different severity can be created is suitable for further studies that will improve our understanding of the disease. The present method with transversely ground molars observed in SEM allows a registration of erosive lesions and lesion depths in small teeth like mouse molars. Both studies on the salivary influence (e.g. by using

knockout mice) and the studies on the effect of fluoride on development of erosive lesions are warranted.

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Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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Legends to Figures

Figure 1. Scanning electron microscopy images of *mandibular* molars from control (a-f), sports drink (g-l), and cola drink (m-r) mouse.

Occlusal view (a, b, g, h, m, n), lingual view (c, d, i, j, o, p), and buccal view (e, f, k, l, q, r). (b, h, n) Higher magnification of occlusal view of mandibular first molar in panels a, g, m, respectively. (d, j, p) Higher magnification of lingual view of mandibular first molar in panels c, i, o, respectively. (f, l, r) Higher magnification of buccal view of mandibular first molar in panels e, k, q, respectively. The white arrows in panel b point to the unaffected and intact lingual surface of the first mandibular control molar. The black arrows in panels h, n, i, o, j, p point to the affected enamel on the lingual surfaces of the mandibular sports drink and cola drink molars. The step indicates the border between the unaffected cervical part and the affected occlusal part of the tooth. The bar represents 500 μm in panels a, g, m, c, i, o, e, k, q, and 200 μm in panels b, h, n, d, j, p, f, l, r. E = enamel, D = dentin, Ab = alveolar bone, B = buccal side, L = lingual side.

Figure 2. Scanning electron microscopy images of *maxillary* molars and incisor from control (a-c), sports drink (d-f), and cola drink (g-i) mouse.

(a, d, g) Occlusal view of all three maxillary molars from control, sports drink, and cola drink mice, respectively. (b, e, h) Higher magnification of occlusal view of mandibular first molar in panels a, d, g, respectively. The white arrows in panel b point to the unaffected and intact lingual surface of the first maxillary control molar. The black arrows in panels e and h point to the affected enamel on the lingual surface of the first maxillary sports drink and cola drink molars. The step indicates the border between the unaffected cervical part and the affected occlusal part of the tooth. (c, f, i) Maxillary incisors from control, sports drink, and cola drink mouse,

respectively. The bar represents 500 μm in panels a, d, g, 200 μm in panels b, e, h, and 500 μm in panels c, f, i. E = enamel, D = dentin, Ab = alveolar bone, B = buccal side, L = lingual side.

Figure 3. Scanning electron microscopy images of transversely ground planes of *mandibular* first molar from control (a-c), sports drink (d-f), and cola drink (g-k) mouse. The top picture shows a representation of a mandibular first molar, indicating the position of the transversely ground plane T1. The white arrows in panel a, d, g point to the lingual enamel, which is unaffected in panel a and affected in panel d and g. (b, c) (e, f) (h-k) Higher magnification of enamel at the position of white arrows in panel a, d, g, respectively. In the control molar the lingual enamel exhibits full thickness and normal basic enamel structure with four layers (b, c). In the sports drink molar, the lingual enamel exhibits a distinct step indicating the start position of enamel erosion (d, e). In the cola drink molar, the lingual enamel shows a distinct step with dramatic erosion of enamel in an occlusal direction (g-i), and thereafter a complete loss of enamel (j, k). The bar represents 200 μm in panels a, d, g, and 20 μm in panels b, c, e, f, h-k. E = enamel, D = dentin, P = pulp, R = resin, B = buccal side, L = lingual side, IPL = inner prism-free layer, IE = inner enamel, OE = outer enamel, SE = superficial enamel, p = prism.

Figure 4. Scanning electron microscopy images of transversely ground planes of *mandibular* first molar from control (a-c), sports drink (d-f), and cola drink (g-k) mouse. The top picture shows a representation of a mandibular first molar, indicating the position of the transversely ground plane T2, which is situated further distally compared to T1. The white arrows in panel a, d, g point to the lingual enamel, which is unaffected in panel a and affected in panel d and g. (b, c) (e, f) (h-k) Higher magnification of enamel at the position of white arrows in panel a, d, g, respectively. In the control molar the lingual enamel exhibits full

thickness and normal basic enamel structure with four layers (b, c). In the sports drink molar, the lingual enamel exhibits a distinct step indicating the start position of enamel erosion (d, e). The superficial and outer enamel is completely eroded (f). In the cola drink molar, the lingual enamel shows a distinct step with dramatic erosion of enamel in an occlusal direction (h, i), and thereafter a complete loss of enamel (g, k). The bar represents 200 μm in panels a, d, g, 20 μm in panels b, c, e, f, h, i, and 10 μm in panels j and k. E = enamel, D = dentin, P = pulp, R = resin, B = buccal side, L = lingual side, IPL = inner prism-free layer, IE = inner enamel, OE = outer enamel, SE = superficial enamel, p = prism.

Figure 5. Schematic representation of a transversely ground planes of *mandibular* first molar from control (A), sports drink (B), and cola drink (C) mouse.

The top picture shows a separate schematic presentation of transversely ground planes (T1 in Fig. 3) of mandibular first molar from each group (A-C). At the bottom is a collective presentation of ground molar planes from all three groups (A-C). Lingual tooth height: Control (a), sports drink (b), and cola drink (c). Buccal tooth height: Control (d), sports drink (e), and cola drink (f). The thickness of lingual enamel: control (g) and sports drink (h). The thickness of buccal enamel in all groups: (i). The distance of step initiation as measured from enamel-cementum junction in sports drink (j) and cola drink (k) molars. E = enamel, D = dentin, P = pulp. Dimensions are presented in Table 1.

Table 1. Dimensions of tooth height and enamel thickness

	Control	Sports drink	Cola drink
Lingual tooth height	781 ± 11 (a)	637 ± 9 (b)*	512 ± 12 (c)*
Buccal tooth height	580 ± 7 (d)	539 ± 6 (e)	513 ± 9 (f)*
Lingual enamel thickness	68 ± 3 (g)	52 ± 2 (h)*	-
Buccal enamel thickness	75 ± 2 (i)	74 ± 2 (i)	75 ± 3 (i)
Erosive step	-	274 ± 8 (j)	183± 6 (k)

Measured dimensions (mean ± SD, μm) of mandibular first molar tooth height and enamel thickness are presented. Letters in parentheses refer to the letters in Fig. 5. The values represent measurements taken from SEM images of transversely ground plane T1 (Fig. 3).

(-) Not applicable; (*) Significant difference, $p < 0.05$