ACTA ODONTOLOGICA SCANDINAVICA
VOLUME 78, 2020
NUMBER 05

Acta Odontologica Scandinavica

ISSN: 0001-6357 (Print) 1502-3850 (Online) Journal homepage: https://www.tandfonline.com/loi/iode20

Dental erosion in mice with impaired salivary gland function

Amela Tulek, Aida Mulic, Kjersti Refsholt Stenhagen, Hilde Kanli Galtung, Muhammad Saeed, Tor Paaske Utheim, Cuong Khuu, Pål Galteland & Amer Sehic

To cite this article: Amela Tulek, Aida Mulic, Kjersti Refsholt Stenhagen, Hilde Kanli Galtung, Muhammad Saeed, Tor Paaske Utheim, Cuong Khuu, Pål Galteland & Amer Sehic (2020) Dental erosion in mice with impaired salivary gland function, Acta Odontologica Scandinavica, 78:5, 390-400, DOI: 10.1080/00016357.2020.1734234

To link to this article: https://doi.org/10.1080/00016357.2020.1734234

Published online: 06 Mar 2020.

ACTA ODONTOLOGICA SCANDINAVICA
2020, VOL. 78, NO. 5, 390–400
https://doi.org/10.1080/00016357.2020.1734234

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Dental erosion in mice with impaired salivary gland function

Amela Tulek[a], Aida Mulic[b], Kjersti Refsholt Stenhagen[c], Hilde Kanli Galtung[a], Muhammad Saeed[a], Tor Paaske Utheim[a,d,e], Cuong Khuu[a], Pål Galteland[e] and Amer Sehic[a,e]

[a]Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway; [b]Nordic Institute of Dental Materials (NIOM AS), Oslo, Norway; [c]Department of Cariology, Institute of Clinical Dentistry, University of Oslo, Oslo, Norway; [d]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway; [e]Department of Maxillofacial Surgery, Oslo University Hospital, Oslo, Norway

ABSTRACT

Objective: Salivary flow rate exerts an essential impact on the development and progression of dental erosion. In this work, the experimental dental erosion in non-obese diabetic (NOD) mice with reduced salivary flow rate was induced, and the erosive effect of acidic drinks on their dentition was studied.

Material and methods: Three acidic drinks (sports drink, cola light drink and sugar containing cola drink) were given to adult NOD mice (groups: $N = 11$) as the only drink for 6 weeks. Two control groups were included; wild type and NOD control (groups: $N = 9$). Experimental and control (water) teeth were dissected out and observed by scanning electron microscopy (SEM). Mandibular first molars were subsequently embedded in Epon, ground transversely, observed again by SEM, and the enamel thickness and tooth height were measured.

Results: Mandibular molars were considerably more eroded than maxillary molars. The erosive process started at the top of the cusps and subsequently extended in the cervical, mesio-distal, and pulpal direction. Erosive lesions were evident in increased succession from sports drink, cola light to cola drink exposed mandibular molars, with the lingual tooth height being approximately 23%, 26%, and 37% lower, respectively, compared to the control. The lingual enamel was approximately 48% thinner in sports drink molars and 62% thinner in cola light molars. In cola drink molars, the lingual enamel was totally eroded, and significant erosion of dentine was evident.

Conclusion: Reduced salivary flow, together with a high consumption of acidic drinks, results in severe erosion of NOD mice molars.

ARTICLE HISTORY
Received 4 December 2019
Revised 12 February 2020
Accepted 19 February 2020

KEYWORDS
Acidic drink; dental enamel; NOD mice; scanning electron microscopy; tooth erosion

Introduction

Dental erosion, or acid-induced dental hard tissue destruction, is a multifactorial condition caused by various extrinsic and intrinsic acid sources [1]. The prevalence of erosive tooth wear is increasing, mainly due to changes in lifestyle and drinking habits [2,3]. Individuals that frequently consume acidic drinks and food exhibit a higher risk for this type of dental substance loss, and the consumption of drinks and food with low pH has increased significantly over the past decades [4–6].

Understanding of dental erosive disease has improved considerably; however, knowledge of the role of factors in the oral environment, such as saliva and dental enamel, remains elusive. Human *in vivo* studies on dental erosion are unethical because of the irreversible acid-induced loss of dental hard tissues. Therefore, studies using animal models, in which the experimental procedures can be performed under controlled conditions, are important. However, only a few studies have focussed on the risk indicators and preventive treatment related to dental erosion in animal models [7–11], and the criteria for the registration of dental erosive lesions were mainly semi-quantitative, and did not allow precise recording of small erosive lesions and their depth [9,12]. Recently, an animal model in which experimental dental erosion was induced in mouse with normal salivary flow was developed, and the erosive effect of products containing both citric and phosphoric acid was studied in detail [13]. The results showed that cola drink (phosphoric acid) exhibited higher erosive effects on mouse mandibular molars compared to sports drink (citric acid), and the presented method, with transversely ground molars observed under a scanning electron microscope (SEM), enabled comprehensive registration of erosive lesions and their depths in small teeth such as mouse molars [13].

Saliva, enamel phenotype, and dietary habits play essential roles in the formation and progression of dental erosion [14]. Saliva is considered the most significant biological factor in the prevention of and in modifying the development of dental erosive wear, and both the saliva composition and salivary flow rate are fundamental factors that may have an effect on its protective properties [15,16]. Saliva directly dilutes, clears, neutralizes, and buffers erosive agents; forms

CONTACT Amer Sehic amer.sehic@odont.uio.no Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway
This article has been republished with minor changes. These changes do not impact the academic content of the article.

a protective membrane covering the enamel surface; reduces the demineralization rate; and enhances the remineralization of eroded enamel and dentine by providing fluoride, phosphate, and calcium [15]. The salivary flow rate has been considered the best clinical indicator of the protective properties of saliva [17]; however, only a few studies have investigated the association between low salivary flow rate and the occurrence of dental erosive lesions [11,18–21].

Non-obese diabetic (NOD) mice are a well-known animal model for studying Sjögren's syndrome, an autoimmune disease characterized by ocular and oral dryness [22,23]. In NOD mice, salivary glands are most frequently affected with lymphocytic infiltrates, which lead to glandular hypofunction [24]. The very first signs of salivary gland infiltration have been observed at 8 weeks of age [25–27]. A study investigating salivary gland function in NOD mice reported significantly reduced salivary flow rate and a moderate presence of inflammatory cells in the salivary gland tissue between week 17 and 24 [28]. Another study reported progressive loss of salivary function between week 16 and 20 [29]. In this work, experimental dental erosion was induced in NOD mice and the erosive effect of products containing both citric (sports drink) and phosphoric (cola/cola light drink) acid on their dentition was studied in detail. The hypothesis was that acidic drinks induce dental erosion in NOD mice and that decreased salivary flow rate plays a role in this development of erosive lesions.

Material and methods

Animal model

Forty-two phenotypical 9 weeks old NOD/MrkTac female mice were purchased from Taconic Biosciences (Ejby, Denmark), and nine young wild-type female mice (CD-1 strain, 9 weeks old) were selected as an additional control. The animals were kept in accordance with Norwegian regulations 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 the Norwegian Animal Welfare Act of 2009). They were maintained on a 12-h light:dark cycle at 21 °C with 65% relative humidity. The study was approved by the Norwegian Food Safety Authority (FOTS ID 16721).

Prior to experimental use, the NOD mice were kept in the facility for approximately 7 weeks for them to reach the age of 16 weeks, the time point at which they develop dysfunction of the salivary glands resulting in significantly reduced salivary flow rate [28,29]. Pilocarpine-stimulated salivary flow was measured in both wild type and NOD mice at 9, 17, and 22 weeks of age. Before the experimental erosive procedures, the animals were given water *ad libitum*. However, in order to exclude attrition of the teeth, both before and 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 cold tap water, sealed in a plastic bag, and left to soften overnight. Furthermore, wire cages with solid bottom and bedding were prepared to reduce wear of the dentition by attrition, as described previously [13]. 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 part of the environment enrichment. All cages were replaced two times per week, and the animals were monitored daily.

At the age of 16 weeks, the NOD mice were randomly distributed into four groups, which were provided with distilled water (control) ($n = 9$), Red Bull sugar-free sports drink (citric acid, pH 3.39) ($n = 11$), Coca Cola light drink (phosphoric acid, pH 3.10) ($n = 11$), and Coca Cola drink (phosphoric acid, pH 2.27) ($n = 11$), respectively (Figure 1). The wild-type mice ($n = 9$), serving as an additional control group, were provided with distilled water (Figure 1). Each group was further divided into triplicate subgroups (cages), and two 150-mL bottles with drinks were placed into each cage. The animals were provided with drinks *ad libitum*, the bottles were replaced three times per week, and the consumption of drinks in each cage was recorded. Prior to the experiment, the changes in pH of both the sports drink and cola drink were monitored over 3 days, and the results showed no changes in pH. After the 6-week experimental period, the animals were sacrificed at the age of 22 weeks by cervical dislocation, and their heads were fixed in 70% ethanol. All animals were weighed at the start and end of the experiment. Figure 1 presents the flow chart of the experiment.

Scanning electron microscopy

The maxillary and mandibular molars and incisors from both wild-type control, NOD control and experimental mice 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 under a GeminiSEM 300 SEM (Zeiss, Oberkochen, Germany), operated at 5 kV.

Thereafter, the jaw segments with all three molars were embedded in Epon and ground transversely. Under a stereomicroscope, grinding was performed using grit 800 and 1200 waterproof silicon carbide paper (3 M, St. Paul, MN, USA) in a specially designed apparatus [30]. When the grinding reached the planned position, the surfaces were polished by grinding specimens against the backside of the silicon carbide paper with 0.05-μm particle size alumina powder (Buehler MicroPolish, Buehler, Lake Bluff, IL, USA) in water. After careful cleaning by 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 again observed under SEM. The whole procedure (grinding, polishing, etching, air-drying, sputter-coating, SEM observation) was repeated, creating three 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 tips of the cusps exhibit enamel-free areas [31]. The subsequent plane (T2) was ground further in the distal direction, ending on the distal aspect of buccal cusp B2 and lingual cusp L2, and the final third plane

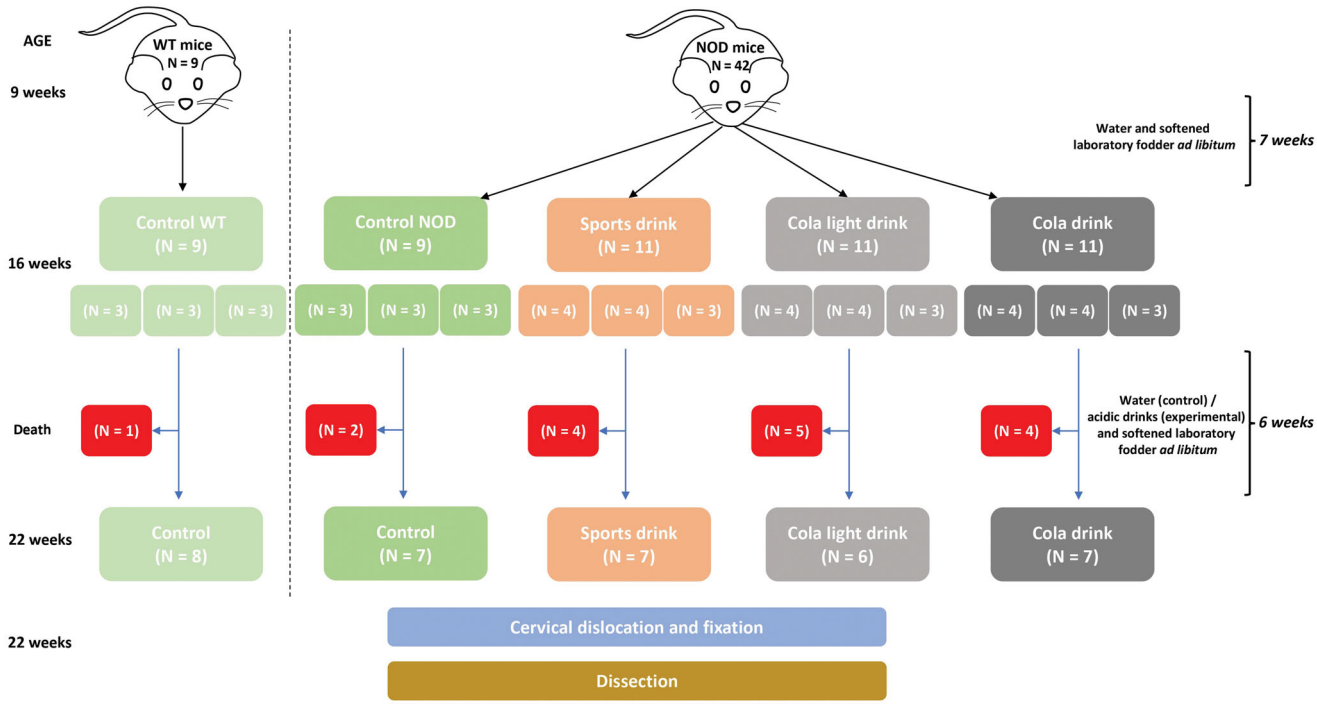

Figure 1. Flow chart of animals enrolled in the study. Forty-two NOD mice and nine wild-type mice, 9 weeks old, were kept for 7 weeks and provided with water and *softened* laboratory fodder *ad libitum*. At the age of 16 weeks, NOD mice were randomly distributed into four groups, i.e. control and three experimental groups. The wild-type group served as an additional control. After the 6-week experimental period, the surviving animals were terminated at the age of 22 weeks by cervical dislocation, and their heads were fixed in 70% ethanol.

(T3) was positioned reaching the mesial aspect of the buccal cusp B3 and lingual cusp L3. Figure 4 shows the position of transversely ground planes T1–T3. Since there were no morphological differences and trace of acid effect on the incisors, as judged by SEM, the incisors were not subject for grinding procedures.

Statistical analysis

SEM images of the transversely ground and etched plane T1 were used for measuring enamel thickness and tooth height in wild-type control, NOD control and experimental mandibular first molars, corresponding to our previous study [13]. Mean values and standard deviations were calculated using Microsoft Excel Worksheet. The manifestation of the step in the eroded lingual enamel in the experimental molars was calculated by measuring the distance between the line representing the step and the corresponding level at the enamel–cementum junction (illustrated in Figure 6(b–d)). Measurement data were tabulated and analysed using Statistical Package for the Social Sciences (SPSS) software program, version 22.0 for Windows (SPSS, Chicago, IL, USA). The data were evaluated using one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test, and independent *t*-tests. *p*-Values $<.05$ were considered statistically significant.

Results

The behaviour and wellness of the NOD mice

During the 6-week experimental period, daily consumption of both water and acidic drinks was approximately 15 mL per cage. Most of the animals exhibited normal behaviour and wellness; however, in total 16 mice (15 NOD and 1 wild type) showed signs of illness and were consequently excluded from the study (Figure 1). One mouse from the cola light group was found dead during the experiment, whereas the remaining 15 mice were terminated during the experimental period due to observed abnormal behaviour, sickness, lethargy, and poor general health and appearance. During the experiment, when NOD mice, including those in the control group, showed signs of abnormal behaviour and sickness, we measured serum glucose levels using a Contour XT glucose test kit (Ascensia Diabetes Care, Parsippany, NJ, USA). The results showed high glucose levels exceeding 30 mmol/L. The similar findings, with glucose levels approximately 30 mmol/L, were observed in both NOD control and experimental animals that have survived. By the end of the experiment, the mean weight of the surviving animals was only slightly altered and not significantly different between the groups. Mean weight gain was 0.4 g in both the control and cola drink group, while mean weight loss was 0.5 g and 0.2 g in the sports drink and cola light drink group, respectively. At 9 weeks of age, the salivary flow rate (μL of saliva/100g body weight/20 min) in NOD mice and wild-type mice were not significantly different, while at 17 and 22 weeks of age, salivary flow in NOD mice decreased significantly in all groups, by approximately 35% and 60%, respectively, compared with that in wild type.

The erosive effect of sports drink on the dental enamel in NOD mice

Compared to the control teeth (Figure 2(e–h)), sports drink mandibular molars had rounded cuspal morphology, with

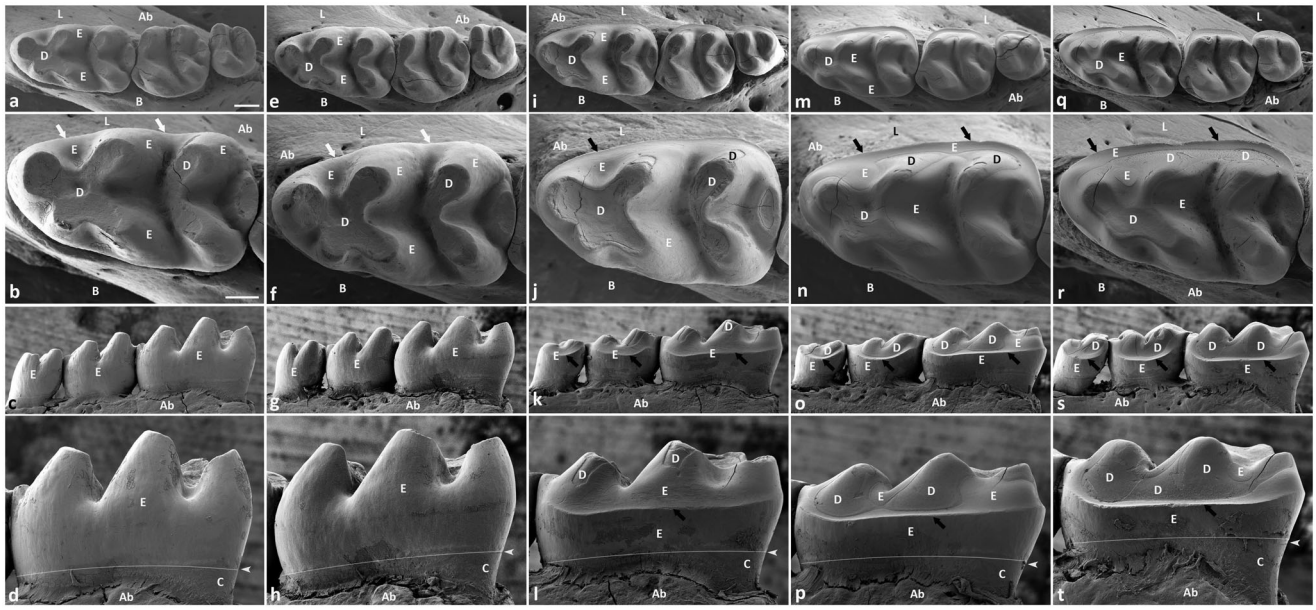

Figure 2. SEM images of mandibular molars from wild type (a–d) and NOD control (e–h) mice, and sports drink (i–l), cola light drink (m–p), and cola drink (q–t) NOD mice. Occlusal view (a, b, e, f, i, j, m, n, q, r) and lingual view (c, d, g, h, k, l, o, p, s, t). (b, f, j, n, r) Higher magnification of occlusal view of mandibular first molar in panels a, e, i, m and q, respectively. (d, h, l, p, t) Higher magnification of lingual view of mandibular first molar in panels c, g, k, o and s, respectively. The white arrows in panels b and f indicate the unaffected and intact lingual surface of the first mandibular wild type and NOD control molar, respectively. The black arrows in panels j, n, r, k, o, s, l, p, and t indicate the affected enamel on the lingual surfaces of the sports drink, cola light drink, and cola drink mandibular NOD molars. The step indicates the border between the unaffected cervical part and the affected occlusal part of the tooth. The white arrowheads and white horizontal lines in panels d, h, l, p and t indicate the enamel–cementum junction. The bar represents 300 μm in panels a, e, i, m, q, c, g, k, o and s, and 200 μm in panels b, f, j, n, r, d, h, l, p and t. E: enamel; D: dentine; C: cementum; Ab: alveolar bone; B: buccal side; L: lingual side.

Table 1. Dimensions of tooth height and enamel thickness.

	Wild-type control	NOD control	NOD Sports drink	NOD Cola light drink	NOD Cola drink
Lingual tooth height	786 ± 12	792 ± 19	613 ± 9*	589 ± 14*	501 ± 19*
Buccal tooth height	579 ± 8	572 ± 9	529 ± 13	523 ± 13	503 ± 11*
Lingual enamel thickness	67 ± 3	66 ± 5	34 ± 4*	25 ± 2*	–
Buccal enamel thickness	74 ± 4	73 ± 3	75 ± 2	76 ± 5	73 ± 2
Erosive step	–	–	232 ± 13	211 ± 15	162 ± 11

Measured dimensions (mean ± SD, μm) of mandibular first molar tooth height and enamel thickness are presented. The values represent measurements taken from SEM images of transversely ground plane T1.
(–) Not applicable.
(*) Significant difference, $p < .05$.

evident distinct erosion on the lingual aspect of the teeth (Figure 2(i–l)). The lingual tooth height of first mandibular sports drink molars was 23% lower (613 μm vs. 792 μm) compared to the control (Table 1). The buccal enamel was unaffected; however, the buccal tooth height was reduced by 7% (529 μm vs. 572 μm) (Table 1). The maxillary molars were only slightly eroded, particularly on the lingual mesial half of the tooth (Figure 3(e,f)), while the incisors were unaffected (results not shown).

A recognized erosion pattern, with a distinct step indicating the border between the unaffected cervical and affected occlusal part of the lingual enamel, was observed on all three sports drink mandibular molars (Figures 2(k,l) and 4(i–l)). The eroded enamel covered the dentine at the lingual aspects of the cusps; however, the dentine on cusp L2 and L3 was exposed (Figures 2(l) and 6(b)). The erosive step in the sports drink first mandibular molars, evident at approximately 232 μm as measured from the enamel–cementum junction (Table 1), was continuous on the whole lingual aspect of the tooth (Figures 2(l) and 4(j–l)). Proceeding this step in an occlusal direction, the enamel was considerably eroded, with complete loss of the superficial prism-free and outer enamel layers (Figure 5(k,l)). The lingual enamel in the sports drink molars, based on the measurements of the transversely ground plane T1, was 48% thinner (34 μm vs. 66 μm) compared to the control molars (Table 1).

Erosive effects of cola light and cola drink on the dental enamel and dentine in NOD mice

In the cola light (Figure 2(m–p)) and cola drink (Figure 2(q–t)) mandibular molars, extensive erosion was observed on the lingual aspect of all three molars. In the cola light first mandibular molars, the lingual enamel was almost completely eroded, and the lingual dentine was exposed. Small areas with remaining enamel were only observed in the pits between cusps L1 and L2 and between L2 and L3 (Figures 2(p) and 4(n–p)). However, in the cola drink molars, the erosive outcome was more extended, and the exposed dentine appeared as a continuous layer (Figures 2(t) and 4(r–t)).

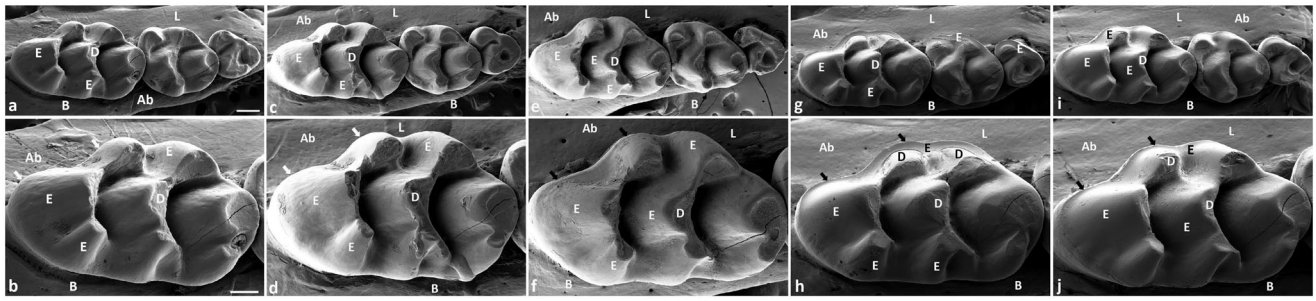

Figure 3. SEM images of maxillary molars from wild type (a, b) and NOD control (c, d) mice, and sports drink (e, f), cola light drink (g, h), and cola drink (i, j) NOD mice. (a, c, e, g, i) Occlusal view of all three maxillary molars from wild type and NOD control mice, and sports drink, cola light drink, and cola drink NOD mice, respectively. (b, d, f, h, j) Higher magnification of occlusal view of maxillary first molar in panels a, c, e, g and i, respectively. The white arrows in panels b and c indicate the unaffected and intact lingual surface of the first maxillary wild type and NOD control molar, respectively. The black arrows in panels f, h and j indicate the affected enamel on the lingual surface of the sports drink, cola light drink, and cola drink first maxillary NOD molars. The step indicates the border between the unaffected cervical part and the affected occlusal part of the tooth. The bar represents 300 μm in panels a, c, e, g and i, and 200 μm in panels b, d, f, h and j. E: enamel; D: dentine; Ab: alveolar bone; B: buccal side; L: lingual side.

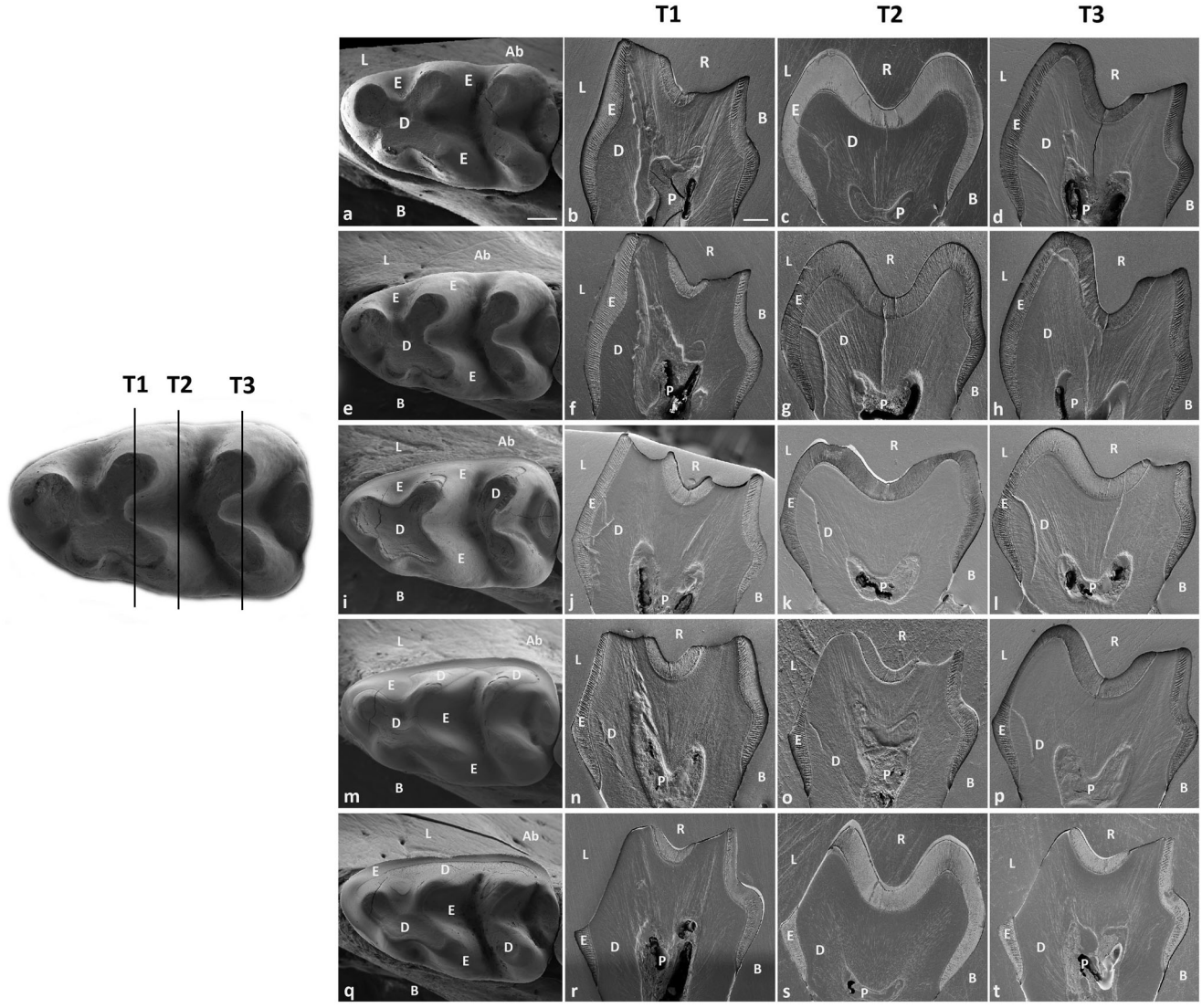

Figure 4. SEM images of transversely ground planes of mandibular first molars from wild type (a–d) and NOD control (e–h) mice, and sports drink (i–l), cola light drink (m–p), and cola drink (q–t) NOD mice. The image on the left shows a representation of a mandibular first molar, indicating the position of the transversely ground planes T1, T2, and T3. In both wild type and NOD control molar, the lingual enamel exhibits full thickness (b–d and f–h). In the sports drink (j–l), cola light drink (n–p), and cola drink (r–t) molars, the lingual enamel exhibits a distinct step with varying degrees of erosion in an occlusal direction. The bar represents 200 μm in panels a, e, i, m and q, and 100 μm in panels b–d, f–h, j–l, n–p and r–t. E: enamel; D: dentine; P: pulp; Ab: alveolar bone; R: resin; B: buccal side; L: lingual side.

In both groups, all three mandibular molars exhibited a typical rounded cuspal appearance with considerable erosion also on the occlusal aspects of the cusps (Figure 2(o,p,s,t)), more pronounced in the cola drink molars (Figure 2(s,t)). Accordingly, on the occlusal aspect of the molars, erosion increased the size of normally obliging enamel-free areas, which were continuous with the lingual dentine in the cola drink molars and partly continuous in the cola light molars (Figure 2(n,r)). In the cola drink maxillary molars, a marked erosion step on the lingual side was observed on all three molars (Figure 3(i,j)), whereas in the cola light maxillary molars the erosion step, with large areas of exposed dentine, was even more distinct (Figure 3(g,h)). However, in both cola light and cola group, the erosive effect was more variant in the maxillary molars. Furthermore, the incisors in both groups were unaffected (results not shown).

The transversely ground planes of the cola light and cola drink first mandibular molars showed an erosion pattern that reflected the observations of the sports drink molars; however, there was greater erosive destruction of the lingual

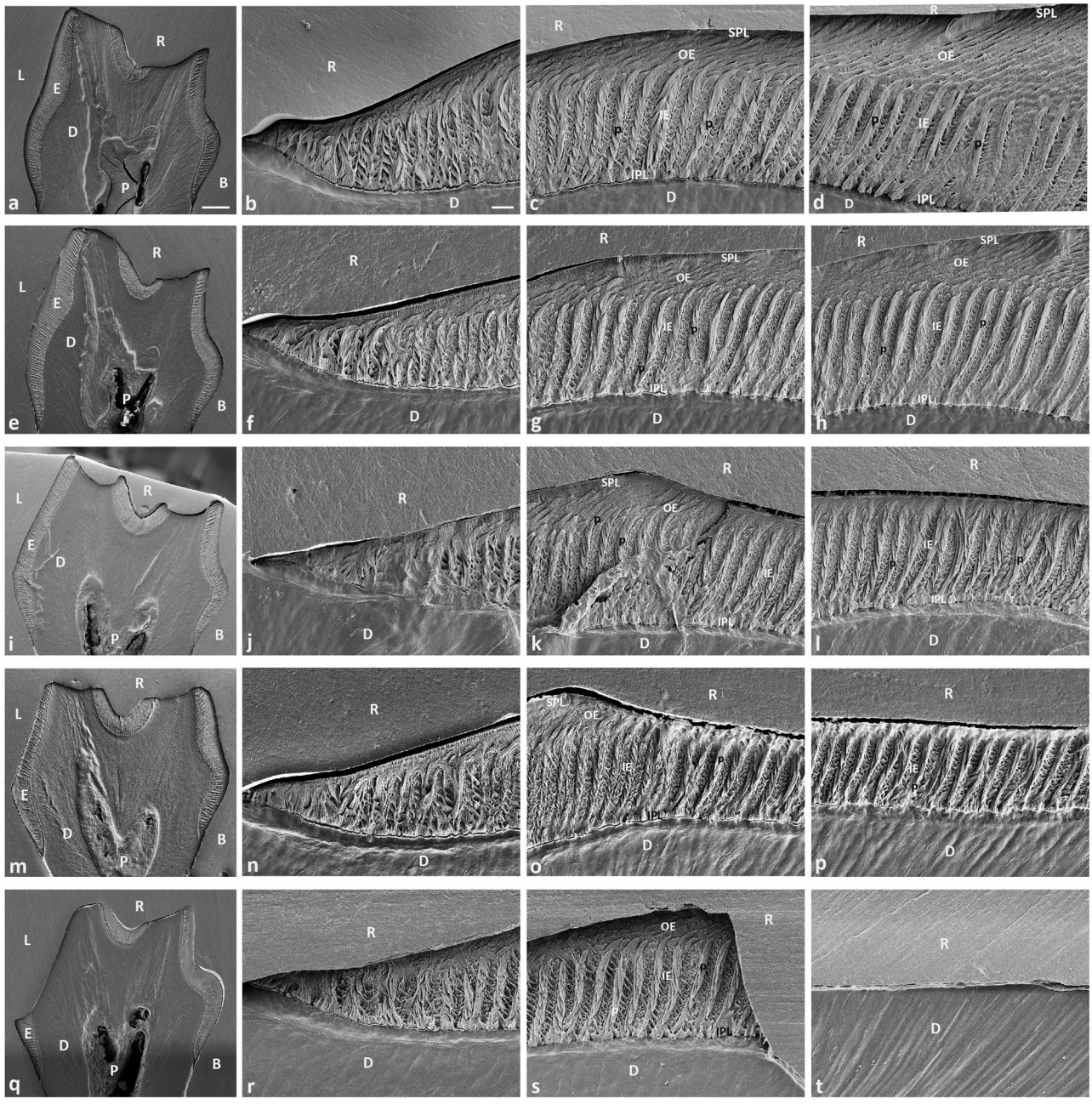

Figure 5. SEM images of lingual mandibular first molar enamel from wild type (a–d) and NOD control (e–h) mice, and sports drink (i–l), cola light drink (m–p), and cola drink (q–t) NOD mice. (b–d, f–h, j–l, n–p, r–t) Higher magnification of lingual enamel from panel a, e, i, m and q, respectively. In both wild type and NOD control molar, the lingual enamel exhibits full thickness and normal basic enamel structure with four layers (b–d and f–h). In the sports drink molar, the lingual enamel exhibits a distinct step indicating the border between the eroded and unaffected enamel (k). An artefact is presented in the middle of panel k due to remnants of organic materials, often appearing in SEM after etching of enamel. The superficial and outer enamel is completely eroded (l). In the cola light drink molar, the enamel is even more eroded, including the partial erosion of inner enamel (p). In the cola drink molar, the lingual enamel shows a distinct step with total erosion of enamel in an occlusal direction (s, t). The bar represents 100 μm in panels a, e, i, m and q, and 10 μm in panels b–d, f–h, j–l, n–p and r–t. E: enamel; D: dentine; P: pulp; R: resin; B: buccal side; L: lingual side; IPL: inner prism-free layer; IE: inner enamel; OE: outer enamel; SPL: superficial prism-free layer; p: prism.

enamel (Figures 4 and 5), including significant erosion of the dentine in cola drink molars (Figures 4(r–t) and 5(q)). In both cola light and cola drink mandibular first molars, the lingual and buccal cusps were eroded and reduced in height (Figure 6(c,d)). The lingual tooth height was 26% lower (589 μm vs. 792 μm) in the cola light drink molars and 37% lower (501 μm vs. 792 μm) in cola drink molars compared to the control (Table 1). The enamel on the buccal aspect of the molars was unaffected (Figures 4 and 5); however, the buccal tooth height in the cola light and cola drink molars was reduced by 9% (523 μm vs. 572 μm) and 12% (503 μm vs. 572 μm), respectively (Table 1).

The erosive effects on the cola drink molars were more severe compared to that in the cola light molars, and consequently the erosion of the enamel was more extended in the cervical direction as judged by the presence of the erosive step (Figures 2 and 6). At the transversely ground plane T1, approximately 211 μm, as measured from the enamel–cementum junction, an erosive step was evident in the cola light molars (Figure 6(c); Table 1). In cola drink molars, this step was present at approximately 162 μm from the enamel–cementum junction (Figure 6(d); Table 1). In cola light molars, proceeding from this step in an occlusal direction, the enamel layers were gradually lost, with complete loss of superficial and outer enamel (Figure 5(o,p)). The lingual enamel in cola light molars, based on the measurements on the transversely ground plane T1, was approximately 62% thinner (25 μm vs. 66 μm) compared to the control molars (Table 1). However, in the cola drink molars, the enamel with all its layers was completely eroded occlusally for the erosive step (Figure 4(r–t)). As judged by the lingual outline of the dentine surface at this level, dentine erosion was evident (Figures 4(r–t) and 6(d)).

The pattern of acid-induced dental hard tissue destruction

SEM observations of the morphology (Figure 2) and transversal sections (Figures 4 and 5) of mandibular first molars from both control and all three experimental groups, gave the possibility to study the pattern and consecutive sequence of acid induced dental hard tissue loss (Figure 6). The control molars were not affected (Figure 6(a)), and erosive lesions were evident in increased succession from sports drink (Figure 6(b)), cola light (Figure 6(c)) to cola drink (Figure 6(d)) molars. Accordingly, loss of enamel was initiated at the occlusal half of the tooth, including the tip of the cusps, resulting in reduced enamel thickness and dentine exposure at the tip of the cusps L1 and L2 (Figure 6(b)). The erosive step, marking the border between the affected and unaffected cervical enamel, was evident (Figure 6(b)). The persistent erosion resulted in further loss of dental enamel on cusps L1 and L2 in both the cervical and mesio-distal directions, and simultaneously with the evident initiation of enamel erosion at cusp L3 (Figure 6(c)). At this point, synchronous loss of enamel towards the underlying dentine together with further progression of the erosive step in cervical direction, was observed. Collectively, the enamel became thinner and the total eroded area above the erosive step was increased (Figure 6(c)). At this stage, coincidental expanding exposure of dentine together with initial erosion of dentine at the top of the cusps was evident; the molars appeared with typical rounded cuspal morphology (Figure 6(c)). At the final stage, almost all enamel above the erosive step, except for some remnants in the pits between the cusps, was destroyed, and the exposed dentine emerged as a continuous layer with progressive erosion in the cervical, mesio-distal, and even pulpal direction (Figure 6(d). The extensive erosion of dentine in the pulpal direction resulted in loss of the curved outline of the lingual tooth surface (Figure 6(d)).

Discussion

A similar erosion pattern, as described here in NOD mice, has been observed in rats [7–9] and has recently also been described in our study using mice with normal salivary flow [13]. However, the NOD mice showed significantly more erosion. In this study, we aimed to design the experiments and the evaluation method with SEM, similar to the study on mice with normal salivary flow [13], in order to enable a comparison of the results and thereby explore the role of salivary flow rate on development of erosive lesions. However, due to the complexity of NOD mice with regard to when the salivary flow rate decreases, it was not possible to use animals of the same age, i.e. the study was initiated at 7 weeks in normal mice vs. 16 weeks in NOD mice. Therefore, from the time the NOD mice arrived at the animal facility until the beginning of the experiments, and during the 6-week erosive induction (Figure 1), we aimed to minimize attrition by softening the standard laboratory fodder with water. All animals in this study, in both the control and experimental groups, were maintained on the same fodder both before and during the experiment. Importantly, as judged by the tooth morphology at the end of the experiment, the mandibular molars in the control NOD mice (Figure 2(e–h)) did not show increased tooth wear compared neither to that in the wild-type control (Figure 2(a–d)) nor compared to the mice with normal salivary flow in our previous study [13]. Accordingly, we assert that the tooth wear observed in the experimental NOD molars was predominantly due to erosion, and that the comprehensive registration of erosive lesions and their depths in this study are comparable with the findings in mice with normal salivary flow [13].

Different types of acids, amount, lifestyle, and drinking habits and technique have a collected impact on the risk for development of erosive lesions in humans [4,6,32–34]. In this study, the NOD mice were given sports drink (citric acid, pH 3.39), cola light drink (phosphoric acid, pH 3.10), or cola drink (phosphoric acid, pH 2.27) as the only drink for 6 weeks. Therefore, due to these exaggerated conditions, the results have to be carefully extrapolated to humans, in whom the consumption of acidic drinks is more varied and is often combined with other drinks, such as water. In accordance with previous animal studies, the results showed some small individual variations in the erosive effects

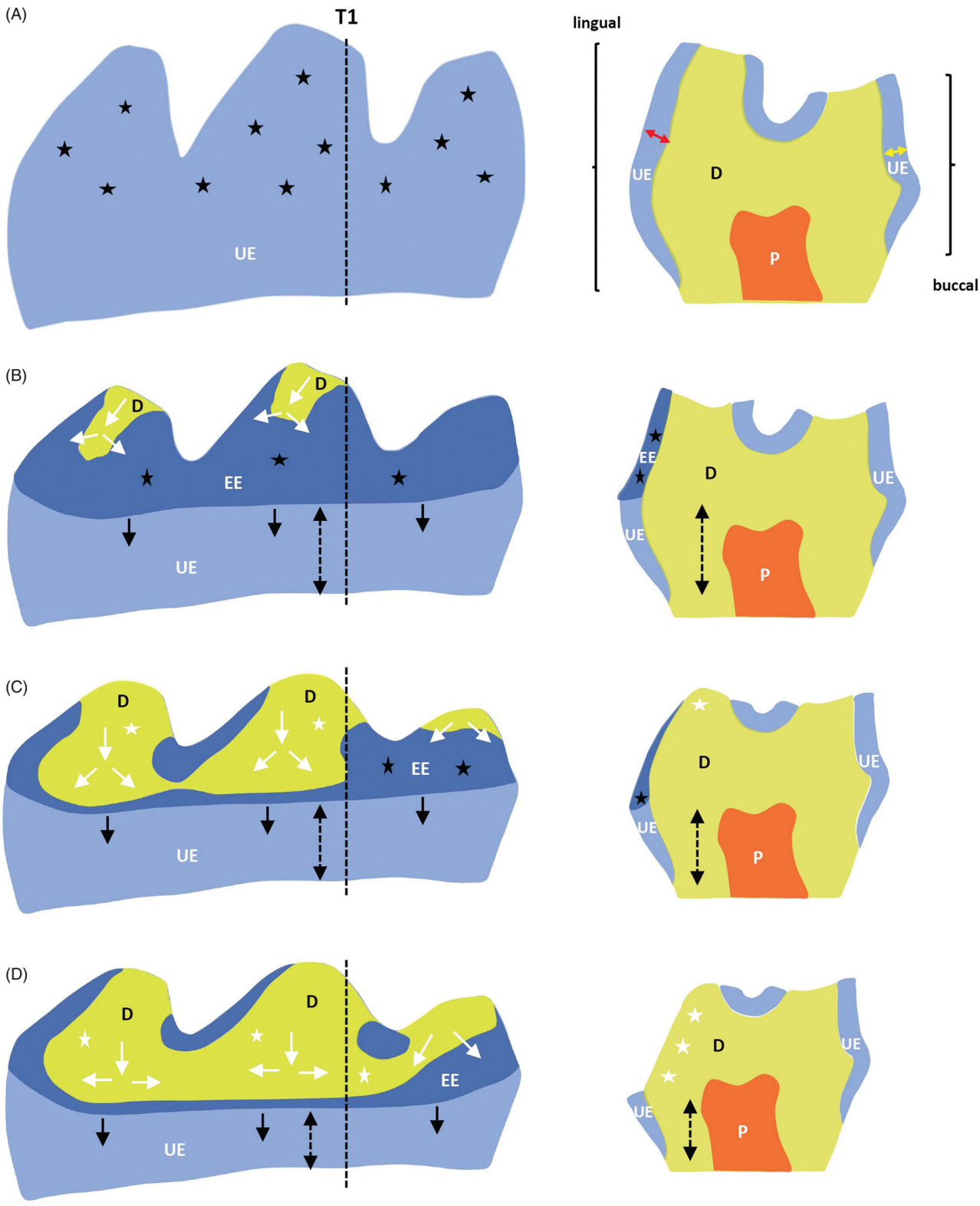

Figure 6. The pattern and consecutive sequence of acid-induced dental hard tissue loss. Schematic representation of lingual view and transversely ground planes (T1) of mandibular first molar from control (A), sports drink (B), cola light drink (C), and cola drink (D) NOD mouse. The stippled vertical line marked T1 indicates the position of the transversal sections presented on right. The black stars in A indicate the part of the tooth at which the enamel erosion will start. The black stars in B and C indicate progression of enamel erosion towards the underlying dentine. The white stars in C and D indicate progression of erosion into dentine and towards pulpal direction; white arrows represent the direction of cervical and mesio-distal erosion. The black arrows show the progression of erosion and movement of the erosive step in the cervical direction; the stippled black arrows indicate the distance between the step and enamel–cementum junction. UE: unaffected enamel; EE: eroded enamel; D: dentine; P: pulp.

between mice within the same experimental group, which may be due to the differing frequency and amount of drink consumed, which was not possible to control during the experiment. However, the overall consumption of drinks was not significantly different between the groups. In general, the findings show that all three acidic drinks resulted in higher erosive tooth destruction of NOD molars compared with that in mice with normal salivary flow [13]. Despite this, no erosive influence on the incisor teeth was observed in the groups. It appears that the protective property of yellow-brown iron pigmentation on the labial enamel of mouse incisors, which is more resistant to acid [35], was decisive in this outcome. As previously reported in clinical [4,36] and animal [13] studies, NOD mouse maxillary molars are considerably less affected compared to mandibular molars (Figures 2 and 3), probably 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 evident that the reduced salivary flow rate has an impact on the development of erosive lesions in the maxillary molars. Compared to the findings in mice with normal salivary flow rates [13], NOD mouse maxillary molars had a marked erosion step on the lingual side of all three molars in the cola and cola light drink groups (Figure 3). However, the reason that maxillary molars in cola light group were more affected compared to that in the cola group, which in general exhibited more severe lesions, is uncertain.

The progression of dental erosion is influenced by both physical factors (temperature, salivary flow rate), and chemical factors (degree of saturation, presence of inhibitors, type of acid, buffering, pH, fluoride) [33]. The association between low salivary flow rate and the occurrence of dental erosive lesions has been investigated in a few studies only [11,18–21]. Regarding the acidic drinks, this study and the previous study [13] confirms that several factors are involved in the complicated chemistry of dental erosion, such as type of acid, low acid dissociation constant (pKa), and low pH. These factors influence the development and severity of dental erosion in mouse molars. Erosive lesions were evident in increasing succession from sports drink (Figure 6(b)), cola light (Figure 6(c)) to cola drink (Figure 6(d)) molars. In all experimental NOD mice groups, acidic drinks exhibited higher erosive potential compared to corresponding groups in the mice with normal salivary flow [13]. It was also evident that the erosive step in the experimental NOD molars was extended significantly further in the cervical direction compared with that in the mice with the normal salivary function. However, the most cervical part of the experimental NOD mouse lingual enamel was not affected. The same erosive pattern has been shown in rat [8] and mouse [13] molars. Since the enamel is not gradually eroded along the whole lingual surface that is exposed to acid, it may be assumed that the gingiva covering the cervical part serves as protection.

In this study, the transversal ground planes of mouse molars enabled detailed measurements of tooth erosion. As previously discussed [13], achieving an ideal transversal ground plane of mouse molars through the correct cusps is technically difficult due to their small size, which may have affected the accuracy of the measurements. However, it was considered unlikely that this slight variation would mask the significant differences in enamel thickness after erosion between the groups. This is reflected by the enamel thickness measurements in the wild-type mice and NOD control mice in this study, and NOD control mice from previous study [13] using the same method, which showed accurate and similar values in both (Table 1).

NOD mice were selected in this study, since they represent a well-known model of Sjögren's syndrome with documented progressive loss of salivary gland function and significantly reduced salivary flow rate between week 17 and 24 [28,29]. In order to investigate the impact of decreased salivary flow rate on development of erosive lesions, mice at about this age were used, with significantly reduced salivary flow compared to wild type, measured at both 17 and 22 weeks of age. However, challenges during the study were experienced; 15 NOD mice died during the experiment (Figure 1). According to the animal supplier Taconic Biosciences, the present mouse model exhibits destructive autoimmune pancreatic insulitis as early as 4 weeks of age, and insulin-dependent diabetes is found in some females beginning at 12 weeks of age and in approximately 50% of females by 24 weeks of age. Other studies have also shown that NOD mice spontaneously develop diabetes, the progression of which is similar to that of human type 1 diabetes [37,38]. Interestingly, type 1 diabetes incidence in NOD mice and the rate of disease progression are affected by the gut microflora and the drinking water pH, which also affects the composition and diversity of commensal bacteria in the gut [39]. It has been shown that female NOD mice that were maintained on acidic water developed more frequently insulitis and hyperglycaemia compared with those on neutral pH water [39]. Accordingly, it is likely that some of the NOD mice that were exposed to acidic drinks in our study developed diabetes with lethal outcome. However, it was also experienced that two mice from the control group, maintained only on distilled water, died (Figure 1). Even though, in NOD mice, the common clinical symptoms of diabetes are present as in humans, the mice have larger resistance to development of ketoacidosis. In consequence, they can remain alive approximately 2–4 weeks after the onset of disease without treatment with insulin. Finally, if not treated, death results from dehydration, rather than ketoacidosis [40,41]. Since the onset of salivary gland infiltration and salivary flow reduction in NOD mice has been shown to be independent of blood glucose status [42], we assume that the salivary flow in this study was not influenced by poorly regulated diabetes. Further, the salivary flow rate, as measured in this study, decreased as previously shown [28] with age, and was not influenced by diet since it was similarly reduced in all NOD mice. Collectively, it was assumed that sick animals that were excluded from the study developed diabetes either spontaneously or due to consumption of acidic and sugar containing drinks. However, although treatment of overtly diabetic NOD mice is possible [43], in this study those animals were terminated.

There are several studies looking at a possible association between the presence of dental erosions and saliva. However, most of those have been *in vitro* and *in situ*. Considering guiding principles underpinning the humane use of animals in scientific research, the three Rs, we did not find it reasonable to include more than approximately 10 NOD mice, prone to develop diabetes, in each group. This study is the first *in vivo* experiment that highlights the role of saliva in development of dental erosion. Use of animal models, and especially mice, when studying oral pathology in humans is widely accepted. In this study, a standardized *in vivo* model was used, which is widely described in our previous paper [13] and has been shown to be suitable for studying even initial erosive lesions. In addition, our SEM images as shown previously [13] and in this study show the same pattern of erosion on mouse molars as in the humans, with the typical cuppings/pits on the occlusal surface of the molars [36].

In conclusion, animal models are highly advantageous due to the salivary influence and soft tissue interactions that resemble the human oral environment. NOD mice, exhibiting low salivary flow rate at a certain age, may serve as a beneficial model for studying reduced saliva as a risk factor for dental erosive wear, however, the frequent development of diabetes may be challenging. Erosive lesions appearing in increased succession from sports drink, cola light to cola drink molars indicated that chemical factors such as the low pH and type of acid are important factors for development of dental erosive lesions. It may be concluded that the severity of the erosive lesions increases when an important protective factor, such as saliva, is reduced or missing. In addition to salivary flow rate, the composition of saliva may be important factor having impact on its protective properties. Different fluoride treatments should also be explored as part of prophylactic treatment for individuals at risk for dental erosion. These aspects may be important to address in future studies using similar animal model and experimental method.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was funded by the Department of Oral Biology, University of Oslo and Department of Medical Biochemistry, Oslo University Hospital.

References

[1] Lussi A, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2014;25:1–15.
[2] Mulic A, Vidnes-Kopperud S, Skaare A, et al. Opinions on dental erosive lesions, knowledge of diagnosis, and treatment strategies among Norwegian Dentists: a questionnaire survey. Int J Dent. 2012;2012:716396.
[3] Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2014;25:55–73.
[4] Sovik JB, Skudutyte-Rysstad R, Tveit AB, et al. Sour sweets and acidic beverage consumption are risk indicators for dental erosion. Caries Res. 2015;49(3):243–250.
[5] Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res. 2004;38(1):34–44.
[6] Barbour ME, Lussi A. Erosion in relation to nutrition and the environment. Monogr Oral Sci. 2014;25:143–154.
[7] Sorvari R, Pelttari A, Meurman JH. Surface ultrastructure of rat molar teeth after experimentally induced erosion and attrition. Caries Res. 1996;30(2):163–168.
[8] Sorvari R, Kiviranta I, Luoma H. Erosive effect of a sport drink mixture with and without addition of fluoride and magnesium on the molar teeth of rats. Eur J Oral Sci. 1988;96(3):226–231.
[9] Sorvari R, Kiviranta I. A semiquantitative method of recording experimental tooth erosion and estimating occlusal wear in the rat. Arch Oral Biol. 1988;33(4):217–220.
[10] Sorvari R. Effects of various sport drink modifications on dental caries and erosion in rats with controlled eating and drinking pattern. Proc Finn Dent Soc. 1989;85(1):13–20.
[11] Aldosari MA, Scaramucci T, Liu SY, et al. Susceptibility of partially desalivated rats to erosive tooth wear by calcium-supplemented beverages. Oral Dis. 2018;24(3):355–362.
[12] Higo T, Mukaisho K, Ling Z-Q, et al. An animal model of intrinsic dental erosion caused by gastro-oesophageal reflux disease. Oral Dis. 2009;15(5):360–365.
[13] Tulek A, Saeed M, Mulic A, et al. New animal model of extrinsic dental erosion-Erosive effect on the mouse molar teeth. Arch Oral Biol. 2018;96:137–145.
[14] Zwier N, Huysmans MC, Jager DH, et al. Saliva parameters and erosive wear in adolescents. Caries Res. 2013;47(6):548–552.
[15] Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. Monogr Oral Sci. 2014;25:197–205.
[16] Buzalaf MA, Hannas AR, Kato MT. Saliva and dental erosion. J Appl Oral Sci. 2012;20(5):493–502.
[17] Tenovuo J. Salivary parameters of relevance for assessing caries activity in individuals and populations. Commun Dent Oral Epidemiol. 1997;25(1):82–86.
[18] Mulic A, Tveit AB, Songe D, et al. Dental erosive wear and salivary flow rate in physically active young adults. BMC Oral Health. 2012;12:8.
[19] Johansson AK, Norring C, Unell L, et al. Eating disorders and oral health: a matched case-control study. Eur J Oral Sci. 2012;120(1):61–68.
[20] Jensdottir T, Buchwald C, Nauntofte B, et al. Saliva in relation to dental erosion before and after radiotherapy. Acta Odontol Scand. 2013;71(3–4):1008–1013.
[21] Jarvinen VK, Rytomaa II, Heinonen OP. Risk factors in dental erosion. J Dent Res. 1991;70(6):942–947.
[22] Humphreys-Beher MG, Peck AB. New concepts for the development of autoimmune exocrinopathy derived from studies with the NOD mouse model. Arch Oral Biol. 1999;44 (Suppl 1):S21–S25.
[23] Cha S, Peck AB, Humphreys-Beher MG. Progress in understanding autoimmune exocrinopathy using the non-obese diabetic mouse: an update. Crit Rev Oral Biol Med. 2002;13(1):5–16.
[24] Faveeuw C, Gagnerault MC, Lepault F. Expression of homing and adhesion molecules in infiltrated islets of Langerhans and salivary glands of nonobese diabetic mice. J Immunol. 1994;152(12):5969–5978.
[25] Yamano S, Atkinson JC, Baum BJ, et al. Salivary gland cytokine expression in NOD and normal BALB/c mice. Clin Immunol. 1999;92(3):265–275.
[26] Humphreys-Beher MG, Brayer J, Cha S, et al. Immunogenetics of autoimmune exocrinopathy in the nod mouse: more than meets the eye. Adv Exp Med Biol. 2002;506(Pt B):999–1007.
[27] Rosignoli F, Roca V, Meiss R, et al. Defective signalling in salivary glands precedes the autoimmune response in the non-obese diabetic mouse model of sialadenitis. Clin Exp Immunol. 2005;142(3):411–418.

[28] Jonsson MV, Delaleu N, Brokstad KA, et al. Impaired salivary gland function in NOD mice: association with changes in cytokine profile but not with histopathologic changes in the salivary gland. Arthritis Rheum. 2006;54(7):2300–2305.
[29] Roescher N, Lodde BM, Vosters JL, et al. Temporal changes in salivary glands of non-obese diabetic mice as a model for Sjogren's syndrome. Oral Dis. 2012;18(1):96–106.
[30] Risnes S. Multiangular viewing of dental enamel in the SEM: an apparatus for controlled mechanical specimen preparation. Eur J Oral Sci. 1985;93(2):135–138.
[31] Lyngstadaas SP, Moinichen CB, Risnes S. Crown morphology, enamel distribution, and enamel structure in mouse molars. Anat Rec. 1998;250(3):268–280.
[32] Zero DT, Lussi A. Erosion–chemical and biological factors of importance to the dental practitioner. Int Dent J. 2005;55(S4): 285–290.
[33] Shellis RP, Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci. 2014;25:163–179.
[34] Johansson AK, Lingstrom P, Imfeld T, et al. Influence of drinking method on tooth-surface pH in relation to dental erosion. Eur J Oral Sci. 2004;112(6):484–489.
[35] Moinichen CB, Lyngstadaas SP, Risnes S. Morphological characteristics of mouse incisor enamel. J Anat. 1996;189 (Pt 2):325–333.
[36] Mulic A, Tveit AB, Skaare AB. Prevalence and severity of dental erosive wear among a group of Norwegian 18-year-olds. Acta Odontol Scand. 2013;71(3–4):475–481.
[37] Makino S, Kunimoto K, Muraoka Y, et al. Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu. 1980;29(1):1–13.
[38] Gorsuch AN, Spencer KM, Lister J, et al. Evidence for a long prediabetic period in type I (insulin-dependent) diabetes mellitus. Lancet. 1981;2(8260–61):1363–1365.
[39] Sofi MH, Gudi R, Karumuthil-Melethil S, et al. pH of drinking water influences the composition of gut microbiome and type 1 diabetes incidence. Diabetes. 2014;63(2):632–644.
[40] Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med. 1999;5(6):601–604.
[41] Mathews CE. Utility of murine models for the study of spontaneous autoimmune type 1 diabetes. Pediatr Diabetes. 2005;6(3): 165–177.
[42] Hu Y, Nakagawa Y, Purushotham KR, et al. Functional changes in salivary glands of autoimmune disease-prone NOD mice. Am J Physiol. 1992;263(4 Pt 1):E607–E614.
[43] Ogawa N, List JF, Habener JF, et al. Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. Diabetes. 2004;53(7):1700–1705.