Gastroesophageal reflux disease and dental erosion: the role of bile acids

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Abbreviations: gastroesophageal reflux disease (GERD), and basic erosive wear examination (BEWE).

ABSTRACT

Objectives: To identify the bile acids in the saliva of patients with and without gastroesophageal reflux disease (GERD), and to evaluate the effect of bile acids on the tooth surface. **Design:** A cross-sectional study involved 26 GERD patients and 40 controls without GERD. An expert dentist identified dental erosions. Post-prandial saliva was collected and analyzed with chromatography for bile acid identification. An *in vitro* study assessed the effect of enamel exposition to taurocholic acid in concentrations of 1µM and 10µM, and a mixture of taurocholic acid and glycocholic acid at 10µM on enamel microhardness, calcium release, and surface topography. **Results:** Salivary bile acids were analyzed from 22 GERD patients and 40 controls. All these participants presented taurocholic acid and glycocholic acid in the saliva. The salivary amount of taurocholic acid was greater than glycocholic acid in both GERD patients (area under the curve: 7946 vs. 1361; $p<0.001$) and controls (10815 vs. 1290; $p<0.001$). The salivary amount of taurocholic acid was greater in controls than in GERD patients (10815 vs. 7946; $p<0.001$). Dental erosion was more prevalent in GERD patients than in controls (27% vs. 7%; $p=0.041$). In the presence of GERD, the amount of glycocholic acid was greater in patients with dental erosion (1777 vs. 1239; $p=0.041$). Enamel exposed to taurocholic acid at 10 µM, combined or not with glycocholic acid, had their microhardness increased, accompanied by calcium release, with no changes in surface topography. **Conclusions:** Taurocholic acid was the predominant salivary bile acid, particularly in controls without GERD. This bile acid had no deleterious effect on the enamel structure.

Keywords: Bile acids; dental erosion; GERD; saliva.

INTRODUCTION

Gastroesophageal reflux disease (GERD) affects approximately 13% of the world population (Eusebi et al. 2018) and results from the frequent return of gastric contents towards the oropharynx. GERD may present several symptoms and lesions, including dental erosions (Farahmand et al. 2013; Vakil et al. 2006).

Dental erosion is the dissolution of hard dental tissues due to oral acidification, without bacterial involvement (Carvalho et al. 2015). Such chemical process can have an extrinsic origin, mainly from the ingestion of acidified food, and intrinsic origin related to acid regurgitation in patients with GERD or bulimia (Mahoney and Kilpatrick 2003). In the presence of GERD, it is believed that the hydrochloric acid present in the reflux content is the main cause of dental erosions (Bartlett and Dugmore 2008; Ranjitkar et al. 2012; Vakil et al. 2006). In these patients, the palatal surface is firstly affected (Vakil et al. 2006), which may compromise other tooth surfaces when the problem persists (Bartlett 2006). In fact, long-term treatment with acid-suppressive medications such as esomeprazole appears to stop the progress of dental erosion (Wilder-Smith et al. 2017).

In GERD patients, apart from gastric acid and pepsin, gastroesophageal reflux may contain bile acids, represented by a complex mix of conjugated and unconjugated acids (Silva et al. 2001). It is known that bile acids can damage the esophageal mucosa and may participate in the genesis of Barrett's metaplasia (Menezes and Herbella 2017; Tack 2006). The mechanism by which these changes arise is via DNA damage stimulated directly by components of duodenogastroesophageal reflux (Jolly et al. 2004; Pardon et al. 2016). Studies have found a greater amount of total bile acids in the saliva of patients with laryngopharyngeal reflux when compared to healthy individuals (Sereg-Bahar et al. 2015b). However, no study has addressed the effect of bile acids on tooth integrity.

In view of studies that demonstrate the mucosal damage caused by bile acids in patients with GERD (McQuaid et al. 2011) and laryngo-pharyngeal reflux (De Corso et al. 2021), and taking into account studies that confirm the presence of total bile acids in saliva (De Corso et al. 2021; Sereg-Bahar et al. 2015b), this study aimed to identify the bile acids present in the saliva of patients with and without GERD, and to test the *in vitro* effect of bile acids on tooth enamel. The study hypotheses were that: (1) the type and concentration of bile acids present in the saliva of patients with and without GERD are similar; (2) bile acids affect the surface topography, microhardness and calcium release of tooth enamel.

MATERIALS AND METHODS

Study design and setting

The study had two phases: i. A clinical, cross-sectional study involving patients with GERD examined at the gastroenterology department (Endopasso Clinic) and volunteers without GERD from a dental clinic (Dental School, University of Passo Fundo); ii. An *in vitro* study involving extracted human teeth. Saliva samples were analyzed at the Catholic University of Leuven, Belgium.

Clinical study

Participants

Adult patients with troublesome typical GERD symptoms were invited to participate after a confirmatory endoscopy for reflux esophagitis (Los Angeles B, C or D), according to the Lyon consensus. Twenty-six GERD patients were included between December 2017 and September 2018. Forty adult volunteers who sought consultation at a university dental clinic, who denied any gastrointestinal symptoms, participated as controls. Inclusion criteria were at least one healthy natural tooth per sextant. Exclusion criteria were xerostomia, use of proton pump inhibitors, antidepressants or any corticosteroids in the last 30 days, and bulimia. A study that involved 28 patients with laryngopharyngeal reflux and 48 healthy controls (Sereg-Bahar et al. 2015b) indicated the sample size.

The Research Ethics Committee approved the study (number 2.404.510), which followed the Helsinki Declaration and the STROBE guidelines. All participants signed an informed consent form before entering the study.

Clinical data and collection of saliva

The participants filled a clinical form and answered the following questionnaires: i. GERD symptoms, for identification of troublesome heartburn and/or acid regurgitation (Fornari et al. 2004); ii. Food questionnaire, assessing the intake of acidified food (pH < 6.0), with a score ranging between 0 (no intake) and 70 (maximal intake) (Milani et al. 2016); and iii. OHIP-14, for assessment of the oral health-related quality of life, with a score ranging between 0 (best quality) and 56 (worst quality) (Slade 1997). Weight and height were assessed to calculate body mass index.

Oral examination was performed by a calibrated dentist (first author), who is expert in dental erosion (Milani et al. 2016), using an artificial light source and clinical mirror n° 5. GERD patients had their oral cavity examined at the gastroenterology department in a common chair, whereas controls were examined at the dental clinic. The Basic Erosive Wear Examination (BEWE) (Bartlett et al. 2008) was used to characterize dental erosions, which considers all tooth surfaces. Dental erosion was classified in scores 0 (no erosion), 1 (initial loss of surface texture), 2 (hard tissue loss < 50% of the surface area), and 3 (hard tissue loss ≥ 50% of the surface area).

After that, the participants consumed a meal composed of rice, steak, fries, salad, and water. Saliva collection was done 40 min after the meal, using Salivette tubes (Sarstedt, Numbrecht, Germany). The cotton stayed at the mouth for 10 min, during which patients avoided swallowing. Saliva samples were stored in a box with carbon dioxide (approximate temperature -80°C) before freezer storage.

Saliva analysis

The saliva samples underwent centrifugation (3500 RPM for 10 minutes) (Centribio, Indianapolis, USA) and pH measurement (pH meter Digimed, São Paulo, SP), and were stored in a freezer at -80°C.

Bile salts were analyzed using a Thermo Fisher Scientific Liquid Chromatography with tandem mass spectrometry as previously described (Riethorst et al. International Journal of Pharmaceutics 2002). Briefly, this system contained a TSQ Quantum Access^{TM} triple quadrupole mass analyzer equipped with an electrospray ionization source, and attached to an Accela^{TM} U-HPLC system (San Jose, CA). Data acquisition was performed with the Xcalibur® 2.0.7 software Package. An injection volume of 25 µL with a flow rate of 450 µL/min were used. In a pilot study, the following bile acids were detected in saliva samples: taurocholic, taurochenodeoxycholic, taurodeoxycholic, glycocholic, glycochenodeoxycholic, glycodeoxycholic, tauroursodeoxycholic, glycoursodeoxycholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and cholic. Nevertheless, only the bile acids taurocholic and glycocholic were above the limit of detection. Concentrations of taurocholic and glycocholic acids were expressed as the area under curve of the spectral peak and should be considered as estimated concentrations as they were below the limit of quantification.

In vitro **study**

Specimen preparation

Sixteen permanent central and lateral incisor human teeth were obtained from a Biobank (Research Ethics Committee approval n° 2.946.129). The dental crown was separated from the root using a carburundum disc and a low-speed motor. Each dental crown generated two specimens, by sectioning the crown in the middle, separating the mesial and distal surfaces. Each fragment was included individually in acrylic resin, leaving the enamel surface exposed. The enamel was polished to obtain a flat surface using a polishing machine (Struers Abramin, Copenhagen, Denmark; silicon carbide paper

#1200). The opposite surface of the fragment was flattened (silicon carbide papers #200 and #600) to obtain two parallel opposing surfaces for the microhardness test.

Afterward, the fragments were removed from the acrylic resin and cleaned with 95% alcohol in an ultrasonic bath (Codyson, Shenzhen, China) for 5 minutes. Specimens were stored in distilled water at 37°C. Before the laboratory tests, the fragments were sealed with varnish, leaving a standard exposition area of 2.5 mm x 2.5 mm.

Exposure to bile acids

Specimens were randomly divided into three groups, according to the type of bile acid used in the erosive challenge (n = 8). The bile acids were those found in the clinical study. Taurocholic and glycocholic acids (Sigma Aldrich, Saint-Louis, USA) were used to prepare three solutions in the pharmacy laboratory in UPF; Taurocholic 1µM (pH = 5); Taurocholic 10µM (pH = 5); and taurocholic + glycocholic 10µM (pH = 6).

The erosive challenge consisted of immersing each specimen individually in 10 mL of the bile acid solution for a total time of 6 minutes, to simulate an episode of reflux (Derceli Jdos et al. 2016). At intervals of 2 minutes of immersion, the specimen was removed from the solution and washed with distilled water for 15 seconds.

Surface microhardness measurement

The Knoop microhardness test (Shimadzu, Kyoto, Japan) was performed with a 50g load and 10 seconds dwell time. Three indentations were performed in each specimen before and after 2 minutes of erosive challenge.

Analysis of calcium release

After the erosive challenge, the acidic solutions were stored and analyzed for calcium release from the enamel surfaces using atomic absorption spectrometry (Perkin Elmer, Connecticut, USA). As an initial reference, the "white solution" was used, which is a pure acidic solution that was not in contact with the teeth.

Scanning electron microscopy analysis

Images of the enamel surface were obtained with a Scanning Electron Microscope (Vega LM3/Tescan Oxford EDS Instrument), using a low vacuum. It allowed the examination of the enamel without the need for coating or any surface preparation.

Statistical analysis

Quantitative data are presented as mean ± standard deviation or when otherwise stated, whereas qualitative data are described as absolute and relative frequencies. Student *t*-test (or Mann Whitney test for skewed data) were applied for comparisons of quantitative data, whereas Fischer exact test was applied for categorical data. The analysis was performed using the software Graph Pad Prism. A p-value < 0.05 was indicative of statistical significance.

The Knoop microhardness data passed the Shapiro-Wilk normality test ($p \geq 0.05$) and were analyzed using the *t*-test for paired data ($\alpha = 0.05$). The calcium loss data (mg/L) presented non normal distribution (Shapiro-Wilk test; $p < 0.05$) and were statistically analyzed using the Kruskal-Wallis non-parametric test ($\alpha = 0.05$).

RESULTS

Participants

Twenty-six patients with GERD and 40 controls without GERD were recruited to participate in the study (Table 1). The participants were adults (≈ 40 years old). There was a predominance of males in GERD patients and females in controls ($p<0.05$). The mean body mass index was overweight but did not differ between groups. On the endoscopic evaluation, the majority of GERD patients presented esophagitis grade B (n = 19 patients) of Los Angeles, followed by grade C (n = 5) and grade D (n = 2).

The dentist observed more difficulty in collecting saliva from GERD patients compared to controls, but with borderline significance (successful collection in these groups: 88% vs. 100%; p = 0.057). Oral pH did not differ between GERD patients and controls. Acidified food intake was similar between GERD patients and controls. However, the OHIP-14 score was significantly worst in controls (dental patients without GERD) compared to GERD patients.

Dental erosion in GERD patients and controls

The prevalence of dental erosion was higher in GERD patients than in controls (27% vs. 7%; P = 0.041). In GERD patients, dental erosion was mild (BEWE score 1) in one patient and moderate to severe in six (BEWE scores 2 and 3), whereas the controls presented only moderate erosion (BEWE score 2). Regarding the tooth surfaces, GERD patients presented erosion in palatal (6 patients), and occlusal (1 patient). Controls presented dental erosion in vestibular, occlusal and palatal surfaces (one patient each).

Bile acids in saliva

Among 26 patients with GERD, four were excluded for bile acids analysis (three due to insufficient saliva and one due to spurious results in the analysis of bile acids). All 40 controls had the salivary bile acids analyzed. All saliva samples were below the limit of detection (<100 nM) for all the bile acids assessed. Nevertheless, taurocholic acid and glycocholic acid showed a higher abundance when looking at the area under the curve. The amount of these bile acids was qualitatively expressed by using the area under the curve.

In patients with GERD, the salivary amount of taurocholic acid was higher than glycocholic acid (7946 ± 1654 vs. 1361 ± 526; $p < 0.001$; statistical power = 1.0). The same was found in controls (10815 ± 2106 vs. 1290 ± 427; $p < 0.001$; statistical power = 1.0) (Figure 1).

In the comparison between patients with GERD and controls, the salivary amount of taurocholic acid was higher in controls (7946 ± 1654 vs. 10815 ± 2106; $p < 0.001$; statistical power = 1.0), while the amount of glycocholic acid did not differ between patients with GERD and controls (1361 ± 526 vs. 1290 ± 427; $p = 0.564$; statistical power = 0.84).

Among 22 patients with GERD, 5 (23%) had dental erosion. Salivary taurocholic acid did not differ between patients with and without dental erosion (7403 ± 1350 vs. 8105 ± 1736; $p = 0.417$; statistical power < 0.80), while the glycocholic acid concentration was higher in patients with erosions (1777 ± 705 vs. 1239 ± 411; $p = 0.041$; statistical power < 0.80). Among 40 healthy volunteers, only 3 (7%) had dental erosion, precluding comparisons regarding bile acids.

In vitro **study**

Bile acids and dental microhardness

Enamel exposed to taurocholic acid 1 µM (pH 5) showed similar microhardness before and after the erosive challenge (Table 2). In contrast, enamel exposed to 10 µM (pH 5), combined or not with glycocholic acid 10 µM, had their microhardness significantly increased.

Calcium release analysis

There were significant differences in calcium release between the experimental groups ($p = 0.009$) (Table 3). The enamel exposed to taurocholic acid 10 µM, combined or not with glycocholic acid 10 µM, showed significant release of calcium, while the teeth exposed to taurocholic acid 1 µM did not show calcium release.

Scanning electron microscopy analysis

Two specimens of each experimental condition were analyzed and compared with a control (polished enamel) using scanning electron microscopy. The enamel surface pattern was similar between treated and non-treated specimens. As shown in Figure 2, the dental enamel maintained its characteristics, preserving both prismatic and interprismatic integrity.

DISCUSSION

Dental erosion can develop after frequent oral acidification in patients with GERD (Bartlett 2006; Bartlett 2005). Bile acids present in gastroesophageal reflux participate in the pathogenesis of erosive esophagitis and Barrett's esophagus (Souza 2016), and might play a role in dental erosion. Therefore, this study investigated the presence of bile acids in the saliva of patients with GERD and controls without GERD and their effect on the tooth surface.

The principal study findings were: a) The main bile acids found in saliva were taurocholic and glycocholic; b) In both groups of participants, taurocholic acid was found in greater amount than glycocholic acid; c) Taurocholic acid concentration was higher in controls than in GERD patients; d) GERD patients had a higher prevalence of dental erosion, accompanied by a higher concentration of glycocholic acid in the saliva of these patients; e) Taurocholic acid at 10 µM, combined or not with glycocholic acid at the same concentration, increased enamel microhardness after *in vitro* experimentation; and f) Taurocholic acid at 10 µM, combined or not with glycocholic acid in the same concentration, caused enamel loss of calcium.

Saliva samples were analyzed by chromatography technique, and the main bile acids found in saliva were taurocholic and glycocholic acids. As far we know, this is the first study that identifies specific bile acids in the saliva, here described as area under curve because bile acids were below the limit of detection. Other studies have assessed bile acids in saliva but were limited to quantifying total bile acids rather than specific ones (De Corso et al. 2007; De Corso et al. 2021; Sereg-Bahar et al. 2015a; Sereg-Bahar et al. 2015b).

In both GERD patients and controls, the salivary amount of taurocholic acid was higher than glycocholic acid. These findings are in agreement with studies on duodenojejunal

aspirates, which found a predominance of taurocholic followed by glycocholic acid in healthy volunteers (Perez de la Cruz Moreno et al. 2006). Moreover, a greater amount of taurocholic acid was found in the saliva of controls without GERD when compared to GERD patients, rejecting the first study hypothesis, and contrary to some authors who measured the bile acids from esophageal aspirates (Nehra et al. 1999). Estimated statistical power for these analysis was higher than 80%. We believe that differences between studies are likely to occur since this technique was performed for the first time to measure bile acids in the saliva of GERD patients and compared to healthy subjects (Stachniuk et al. 2016).

The selection of GERD patients was performed with endoscopic confirmation of moderate to severe reflux esophagitis in patients off proton pump inhibitors terapy. The focus in studying the role of bile acids on dental erosion is due to their deleterious effect on the esophageal mucosa (Farre 2013). Reflux components such as bile acids, pepsin, and hydrochloric acid are believed to act synergistically, changing the DNA of esophageal cells, having a role in the development of Barrett's esophagus and esophageal adenocarcinoma (Di Ciaula et al. 2017; Souza 2016). Furthermore, laryngopharyngeal reflux containing bile acids can be a potential etiological factor for laryngeal cancer (Sereg-Bahar et al. 2015a). Other proteolytic enzimes present in saliva and upper gastrointestinal secretions (Paszynska 2017; Paszynska 2015; Schlueter 2012) may also play a role on dental erosion.

Dental erosion affected 27% of GERD patients, in agreement with other studies (Milani et al. 2016; Rauber et al. 2020). Most GERD patients (6 out 7) presented moderate to severe dental erosion, particularly on the palatal surface. In contrast, dental erosion of moderate degree was found in only 7% of controls without GERD (3 out of 40). Studies have demonstrated that dental erosion is more severe in the presence of GERD (Alves et

al. 2015; Wang et al. 2010). Due to its anatomical position, the palatal surface of the maxillary anterior teeth is the first to be affected in patients with GERD (Bartlett 2006; Moazzez et al. 2004).

Through the OHIP-14 questionnaire (Campos et al. 2021; Papagiannopoulou et al. 2012), worse quality of life related to oral health was verified in the controls. This finding is understandable since this group was composed of individuals who sought dental treatment, and these are usually dissatisfied with their oral health, whether due to pain, discomfort, or aesthetics.

The oral cavity is a complex environment, it is believed that oral fluids contribute to the formation and propagation of dental erosion, by the degradation of demineralized organic structures, and also by weakening the protective effects of the dentin pellicle (Schlueter et al. 2012). Studies in patients with anorexia have indicated that the disease affects the quantity and quality of saliva (Paszynska et al. 2015; Paszynska et al. 2017). In patients with gastroesophageal reflux disease, dental erosion is related to hydrochloric acid, which during reflux episodes can reach the oral cavity, causing demineralization of dental hard tissues (de Oliveira et al. 2015). More studies in this area are needed in order to assess the biological functions of the oral microbiome since we know that taurocholic and glycocholic acids were found in saliva.

In GERD patients, the salivary amount of taurocholic acid did not differ between those with and without dental erosion, while the amount of glycocholic acid was higher in patients with dental erosion. The reason why the amount of glycocholic acid is higher in GERD patients with dental erosion is unclear and whether it plays a role in the pathogenesis of dental erosion needs further investigation.

Some studies have reported reduced salivary flow in patients with reflux esophagitis (Campisi et al. 2008; Kao et al. 1999). Although subjective, it was more difficult to

collect saliva from GERD patients than from controls. We collected saliva in the postprandial period, in which the number of reflux episodes is more likely to occur, expecting the exposition of the teeth to regurgitate contents for at least a few seconds (Derceli Jdos et al. 2016).

Moreover, the salivary pH was similar between these groups. Sujatha and colleagues have found differences in oral pH with lower levels in GERD patients than in healthy subjects (Sujatha et al. 2016). Although saliva and esophageal protection have been extensively studied (Kongara and Soffer 1999), potential interactions between bile acids and saliva and their role on tooth structure deserve further investigation.

After salivary bile acids assessment, the *in vitro* effect on human enamel was investigated. The hypothesis that bile acids affect the surface topography, microhardness and calcium release of tooth enamel was partially rejected. Neither microhardness nor calcium release was affected by taurocholic acid 1 µM. In contrast, the enamel exposed to taurocholic acid 10 µM, combined or not with glycocholic acid 10 µM, had its microhardness increased and showed calcium release. The clinical study showed that salivary amounts of taurocholic acid were higher in controls without GERD, suggesting that this bile acid might not be associated with dental erosions. Moreover, the combination with glycocholic acid, found in greater amount in the saliva of GERD patients with dental erosion, had no deleterious effect of the enamel surface as well.

The results of the microhardness test suggest an increase in the hardness of the dental enamel, an effect contrary to dental erosion, which is characterized in this test by a softening of the enamel surface, followed by loss of volume and resulting in an underlying softened layer verified by decreasing the Knoop microhardness (Mylonas et al. 2018), due to loss of calcium and phosphate from dental enamel (Baumann et al.

2015). The microhardness test is sensitive in detecting early changes in the enamel microstructure caused by acid erosion. Yet this methodology requires a flat and polished surface (Mylonas et al. 2018). Polishing the enamel surface can produce a smear layer from dental debris (Mistry et al. 2015). The increase in microhardness observed for the two experimental groups that used taurocholic acid 10 µM resulted in increased calcium release. Therefore, these solutions could have removed the disorganized superficial layer and exposed a sound enamel layer, leading to a "false" increase in the microhardness. No changes occurred in the enamel after treatment with different solutions of bile acids, as observed by scanning electron microscopy. Previous studies confirm that polishing the enamel surface could introduce artifacts to the laboratory analysis (Schlueter et al. 2011).

What makes a solution potentially erosive is its concentration of calcium and phosphate ions and buffering capacity (Bartlett and Coward 2001; Lussi et al. 2011). The first option was excluded because there was no calcium and phosphate in the chemical composition of the evaluated bile acids, but suggesting an effect of buffering capacity of the solution, which is measured through the H+ ions available in the composition of such acids to maintain a reaction, an important factor in initial enamel demineralization (Shellis et al. 2010). Yet, during exposure of enamel to acids at constant pH for short periods of time, the erosive capacity can still be determined by the pH and type of acid (Hannig et al. 2005). This fact may have been the main cause of the differences found in our study since the pH of our solutions is not as acidic as solutions commonly evaluated in dental erosion studies. Due to the lack of previous knowledge on the erosive potential of bile acids, the effect on the enamel surface was evaluated in a more controlled setup as to avoid confunding variables. Therefore, results interpretation should consider that the oral cavity biochemistry and the biological functions of the oral microbiome were

not considered in the in vitro study. Further studies in this area are suggested in order to analyze the chemical properties of taurocholic acid and glycocholic acid, once they have been surprising in the present study.

CONCLUSION

Dental erosion was more prevalent in patients with GERD than controls without GERD. In these populations, taurocholic acid and glycocholic acid were the predominant salivary bile acids, with a higher amount of taurocholic acid in participants without GERD. These bile acids had no deleterious effect on the structure of tooth enamel. Further studies are needed to elucidate the effect of bile acids on the tooth surface.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

Daiane Cristina Milani declares that she has no conflict of interest. Márcia Borba declares that she has no conflict of interest. Ricard Farré declares that he has no conflict of interest. Luciana Grazziotin Rossatto Grando declares that she has no conflict of interest. Charise Bertol declares that she has no conflict of interest. Fernando Fornari declares that he has no conflict of interest.

Author contributions

Daiane Cristina Milani, Márcia Borba and Fernando Fornari contributed to study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Daiane Cristina Milani and Fernando Fornari, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (ethical committee of the University of Passo Fundo, number 2.404.510) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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TABLES

Table 1. Characteristics of the participants (26 GERD patients and 40 controls).

	GERD N = 26	Controls N = 40	p
Age in years, mean ± SD	40.9 ± 17	35.3 ± 11.7	0.118
Men, n (%)	18 (69)	15 (38)	0.022
Body mass index in Kg/m^2, mean ± SD	27.5 ± 4.8	25.8 ± 4.4	0.183
Participants with dental erosion, n (%)	5 (23)	3 (7)	0.041
Oral pH, mean ± SD	5.8 ± 0.6	6.1 ± 0.6	0.191
Intake of acidified food*, mean ± SD	43.1 ± 6.9	44.7 ± 5.3	0.269
OHIP-14 score§, median (interquartile range)	3 (0.7-6.3)	8 (2.3-15.7)	0.014

*Intake score, ranging from 0 (no intake) to 70 (maximal intake); §The higher the score, the worst is the oral-health related quality of life

Table 2. Enamel Knoop microhardness values before and after exposure to bile acids.

Bile acids	Microhardness (HK), mean ± SD*		
	Before	After exposure	p
TC** 1µM, pH 5.0	219 ± 89a	216 ± 92a	0.932
TC 10µM, pH 5.0	169 ± 71b	224 ± 65a	0.012
TC 10µM + GC** 10µM, pH 6.0	192 ± 43b	244 ± 63a	0.031

*Mean values followed by different letters in the same line are statistically different;

**Taurocholic acid; ***Glycocholic acid

Table 3. Median (interquartile range) of calcium release (mg/L) for the experimental groups

Bile acids	Calcium loss*
TC** 1µM, pH 5.0	0.0 (0.0 – 0.0)b
TC 10µM, pH 5.0	0.6 (0.4 – 1.8)a
TC 10µM + GC*** 10µM, pH 6.0	0.6 (0.2 – 2.1)a

*Median values followed by different letters are statistically different ($p \leq 0.05$),

**Taurocholic acid; ***Glycocholic acid

FIGURE LEGENDS

Figure 1. Qualitative amount (area under the curve; line at mean) of taurocholic acid (TC) and glycocholic acid (GC) in the saliva of patients with GERD (n = 22) and controls without GERD (n = 40).

Figure 2. Dental enamel SEM images of two experimental groups. The control group (A) was not exposed to bile acids (only polished); and the experimental group (B) was exposed to taurocholic acid (TC) and glycocholic acid (GC) 10 µM.