

ORIGINAL ARTICLE

Prevention of erosive/abrasive enamel wear due to orange juice modified with dietary supplements

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OBJECTIVE: The aim of this study was to evaluate the erosive/abrasive enamel wear after contact with orange juices modified with different dietary supplements.

METHODS: A total of 96 bovine enamel samples were prepared and allocated to eight groups (1–8; $n = 12$). Samples were eroded (120 s) in 200 ml of the following eight solutions: 1: water (control), 2: orange juice, 3: water + calcium effervescent tablet, 4: orange juice + calcium effervescent tablet, 5: water + 0.75 g acid/base regulating powder (Probase), 6: water + 0.375 g Probase, 7: orange juice + 0.75 g Probase and 8: orange juice + 0.375 g Probase. After erosion, the samples were brushed with 40 brushing strokes (load 2.5 N). Enamel wear was measured using surface profilometry after 20 and 40 cycles of erosion/abrasion respectively.

RESULTS: Highest mean enamel wear (\pm SD) after 20 and 40 cycles of erosion/abrasion was observed for the unmodified orange juice (group 2) ($0.605 \pm 0.240 \mu\text{m}$; $1.375 \pm 0.496 \mu\text{m}$ respectively). The enamel wear in all other groups (3–8) was significantly lower ($P < 0.0001$ respectively) with no significant difference within these groups and compared with water (control).

CONCLUSION: Erosive/abrasive enamel wear induces by orange juice and tooth brushing could be reduced significantly by modification with free available dietary supplements.

Oral Diseases (2011) 17, 508–514

Keywords: erosion; tooth wear; prevention

Introduction

The prevalence of dental erosion is rising (Nunn *et al*, 2003) over the last decades and has entered into the focus of dental research (Zero and Lussi, 2005; Jaeggli and Lussi, 2006).

To prevent erosive tooth wear or the associated dental hard tissue softening (Wiegand *et al*, 2008b), different preventive approaches have been established (Amaechi and Higham, 2005; Wegehaupt *et al*, 2010). These preventive approaches are often based on the application of different kinds of fluoride, namely amine fluoride (Wegehaupt *et al*, 2009), sodium fluoride (Ganss *et al*, 2008), monofluorophosphate (Kato *et al*, 2010) or titanium tetrafluoride (Wiegand *et al*, 2008a) on enamel or dentine. Furthermore, a preventive effect against erosive tooth wear was observed after the application of solutions containing stannous chloride (Yu *et al*, 2010) or cerium chloride (Wegehaupt *et al*, 2010). The mode of action of these preventive approaches is described as an increase of the acid resistance of the so treated dental hard tissue.

Besides increasing the acid resistance of the enamel or dentine, other approaches tried to reverse the erosion-associated tissue softening (Fowler *et al*, 2009). It is desired to reharden and stabilize the softened enamel or dentine, as the softened dental hard tissues are more susceptible because of mechanical effects and the dental hard tissue loss is increased significantly if the softened enamel or dentine is brushed direct after the erosion or even after hours (Attin *et al*, 2000; Bartlett, 2005).

In how far erosive tooth wear may be reduced or the erosively softened dental hard tissue may be rehardened by preventive approaches based on the topical application of fluorides is discussed controversially in the literature (Amaechi and Higham, 2005; Magalhaes *et al*, 2009b).

All the above-mentioned preventive approaches are applied on the effected substance, namely enamel and dentine. As extrinsic erosions are frequently caused by the contact of acidic beverages with the dental hard tissues (Lussi *et al*, 2004), prevention of erosions by modification of the erosive substrates, such as beverages, are also imaginable. The erosive potential of beverages is not only determined by the pH of the beverage but also by its amount of titratable acidity and its content of calcium, phosphate and fluoride (Lussi and Jaeggli, 2006; Lussi *et al*, 2009).

Already in 1950, Spencer and Ellis (Spencer and Ellis, 1950) demonstrated that the erosive potential of

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Received 5 October 2010; revised 3 December 2010; accepted 13 December 2010

grapefruit juice is reduced when fluoride is admixed. Due to systemic toxicological reasons, it is not possible to admix a sufficient amount of fluoride to acidic beverages required to reduce the erosive potential significantly (Larsen and Richards, 2002). Taking this limitation into consideration, other additives like monocalcium phosphate and sodium phosphate (Reussner *et al*, 1975), polyphosphates (Hooper *et al*, 2007), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Ramalingam *et al*, 2005), different combinations of ions (Ca, Fe, P and F) (Attin *et al*, 2005; Magalhaes *et al*, 2009a) and proteins like ovalbumin (Hemingway *et al*, 2008) have been tested. The modified beverages showed a lower erosive potential compared with the unmodified beverages. All these modifications are experimental and not available to the consumers. The aim of this study was to test the erosive potential of some orange juice modifications, which can be established by customers at home and with free available dietary supplements (effervescent tablet containing calcium and an acid/base regulating powder).

The null hypothesis of this study was that there would be no differences in reducing the potential for erosive tooth wear between the orange juice modified with the dietary supplements and the unmodified version.

Materials and methods

Sample preparation and allocation

For the study, 96 freshly extracted bovine lower incisors were sectioned at the cementum–enamel junction with a water-cooled diamond disc. The pulp tissue was removed from the coronal part of the tooth with endodontic files. The crowns were placed in sample moulds with the buccal surface downwards and embedded in acrylic resin (Palavit G; Kulzer, Wehrheim, Germany). After curing of the acrylic resin, the samples were removed from the moulds and the enamel on the buccal surface was ground with water-cooled carborundum discs (800, 1000, 1200, 2400 and 4000 grit, Water proof silicon carbide paper; Struers, Erkrath, Germany) until a smooth, flat area of enamel was exposed. On the exposed enamel surface, two parallel indents were placed using a scalpel blade. The space between the indents measured 1.5 mm and served as test area during the later experiments. The enamel adjacent to the marked area was used as a reference area for the surface profilometry. The samples were stratified and allocated to eight (1–8) groups ($n = 12$) according to their Knoop microhardness. For the determination of the Knoop microhardness, six microhardness indentations were performed with a digital microhardness tester (model no. 1600-6106; Buehler, Lake Bluff, IL, USA) on the enamel surface of the reference area and the average of the six indentations was calculated.

Experimental procedure

From each sample five baseline profiles were recorded with a profilometer (Perthometer S2; Mahr, Göttingen,

Germany) with a distance of 100 μm between each profile. To ensure an exact repositioning of the samples during and after the experimental procedure, the profilometer and the samples are equipped with a custom-made jig. After the recording of the baseline profiles, the reference areas adjacent to the marked test area were covered with tape (Tesa; Beiersdorf, Hamburg, Germany) to avoid any alterations of the enamel during the following erosive cycling or the toothbrush abrasion.

The samples were eroded by immersion for 120 s in 200 ml of different test solutions. The test solutions are prepared by mixing water or orange juice (M-Budget; Migros, Zürich, Switzerland) with either an effervescent tablet containing calcium (Actilife; Migros) or an acid/base regulating powder (Burgerstein Probase, Burgerstein, Rapperswil, Switzerland). The mixtures and allocation to the different groups is given in Table 1. The orange juice was a 100% orange juice drink. The effervescent tablet contained citric acid, calcium carbonate, sodium bicarbonate, sorbite, rice amyllum, flavour, artificial sweetener and vitamin D (Migros). The acid/base regulating powder is recommended for the harmonisation of the acid-base balance special for athletes to reduce the muscle hyperacidity. The ingredients of the acid/base regulating powder were: calcium carbonate, magnesium carbonate, potassium hydrocarbonate, sodium bicarbonate, silicon dioxide, zinc gluconate and manganese gluconate (Burgerstein). After the erosive attack the samples were rinsed with tap water and toothbrush abrasion was performed in an automatic brushing machine (Imfeld, 2001). Per sample 40 brushing strokes were applied with a constant brushing load of 2.5 N. During brushing, the samples were covered with toothpaste slurry prepared by mixing 300 ml artificial saliva (Wegehaupt *et al*, 2008) and 100 ml fluoridated toothpaste (Elmex; Gaba, Münchenstein, Switzerland). The used toothpaste contained 1400 ppm amine fluoride (Olafluor). After brushing, the samples were removed from the brushing machine and remnants of the toothpaste slurry were rinsed off with tap water.

After performing 20 cycles of erosion (immersion in test solution) and abrasion (tooth brushing), the tape was removed from the reference areas of the samples and five new surface profiles per samples were recorded. After this determination, the reference areas were again covered with adhesive tape and another 20 erosion/abrasion cycles were performed. Afterwards five profiles per sample were additionally recorded. By exact superimposition of the untreated reference areas, the enamel wear was calculated by a custom-made software (4D Client, custom designed software; University Zurich, Zurich, Switzerland), comparing the baseline profiles with the respective post-treatment profiles after 20 and 40 cycles. The stylus profilometer used has a lowest measurement limit of 0.105 μm , as determined in a previous study (Attin *et al*, 2009).

To test possible influences of the dietary supplements on the taste of the modified orange juice, a blinded test with two of the authors was performed.

Table 1 Mixture of the solutions and allocation to the eight test groups

	Group							
	1 (control)	2	3	4	5	6	7	8
Water	200 ml	–	200 ml	–	200 ml	200 ml	–	–
Orange juice (M-Budget; migros, Switzerland)	–	200 ml	–	200 ml	–	–	200 ml	200 ml
Calcium effervescent tablet (actilife; migros, Switzerland, LOT:CH.01430138)	–	–	1 tab.	1 tab.	–	–	–	–
Acid/base regulating powder (Burgerstein Probase; Burgerstein, Switzerland, LOT: 220607)	–	–	–	–	0.75 g	0.375 g	0.75 g	0.375 g

Calculation of degree of saturation

For all tested solutions, the degree of saturation with respect to hydroxyapatite (HA), octacalciumphosphate (OCP) and calcium fluoride (CF₂) was calculated before the samples were immersed in the solutions. The calculation was performed with the computer program IONPRODUCT (Shellis, 1988). The concentration of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), manganese (Mn) and zinc (Zn) was measured using atomic absorption spectroscopy (2380 Atomic Absorption Spectrophotometer; Perkin-Elmer, Schwerzenbach, Switzerland). For the measurement of chloride (Cl) and fluoride (F) concentration ion-selective electrodes were used (Orion fluoride ion-selective electrode Typ 94-04 and Orion chloride ion-selective electrode Typ 94-17B both Orion Research, Cambridge, USA). The concentration of phosphate (PO₄) and citrate was measured using a spectrophotometer (Spectrophotometer U-2010; Hitachi High-Technologies Corporation, Tokyo, Japan). The ion concentration and calculated degrees of saturation with respect to the different kinds of apatite are given in Table 2.

Statistical analysis

Statistical analyses were conducted with Stat View (version 5.0.1, SAS Institute Inc., Cary, NC, USA). For data presentation, the mean enamel wear of the five profiles per sample and the mean loss per group were calculated after performing 20 and 40 cycles of ero-

sion/abrasion. Statistical analysis was performed using ANOVA followed by Bonferroni/Dunn *post hoc* test for comparison of the experimental groups at 20 and 40 cycles. All testes were performed at 5% level of significance.

To compare the enamel wear after 20 and 40 cycles within the same group, ANOVA followed by one-sample *t*-tests was performed.

Results

At baseline, the mean microhardness (\pm SD) of the samples in each group was 292.6 ± 22.6 KHN– 293.3 ± 19.8 KHN.

Wear (mean \pm SD) of enamel after 20 and 40 cycles for the different groups is given in Figure 1.

After 20 cycles of erosion/abrasion, the highest wear of enamel (0.605 ± 0.240 μ m) was observed in the unmodified orange juice group 2). The enamel wear in all other groups (1, 3–8) was significantly lower compared with the wear in the unmodified orange juice group ($P < 0.0001$ respectively). No significantly higher enamel wear was observed after 20 cycles of erosion/abrasion for the modified orange juice group 4 (orange juice + Ca tablet), 7 (orange juice + 0.75 g Probase) and 8 (orange juice + 0.375 g Probase) compared with the water control group 1 ($P = 0.9236$, 0.7883 and 0.0295 respectively). Within all groups, except group 2, no significant difference in the enamel

Table 2 The ion concentration (mM) and calculated degrees of saturation with respect to the different apatite

Group	Composition	Concentration of ions (mM)										pH	Calculated saturation		
		Ca	PO ₄	F	Mg	K	Na	Cl	Mn	Zn	Citrate		HA	OCP	CF ₂
1	Water (control)	1.06	0.00	<0.01	0.31	0.02	0.24	<0.1	0.00	0.02	0.00	6.92	–	–	0.12
2	Orange juice	3.80	15.47	<0.01	2.79	43.47	0.43	<0.1	0.00	0.00	6.13	3.96	0.20	0.11	0.12
3	Water + Ca tablet	74.96	0.55	<0.01	0.49	0.44	25.89	<0.1	0.01	0.03	25.42	4.76	1.01	0.33	0.26
4	Orange juice + Ca tablet	83.31	17.55	<0.01	3.51	45.09	24.15	<0.1	0.01	0.00	76.99	4.18	0.88	0.42	0.22
5	Water + 0.75 g Probase	55.06	0.05	<0.01	10.72	6.70	6.52	<0.1	0.07	0.21	0.00	8.14	42.38	3.23	0.23
6	Water + 0.375 g Probase	47.78	0.01	<0.01	6.72	2.94	3.48	<0.1	0.03	0.06	0.00	8.22	28.34	1.93	0.25
7	Orange juice + 0.75 g Probase	16.68	16.60	<0.01	11.71	51.24	6.66	<0.1	0.06	0.47	1.65	4.69	1.46	0.61	0.17
8	Orange juice + 0.375 g Probase	4.10	15.92	<0.01	6.21	47.15	3.03	<0.1	0.04	0.09	1.71	4.28	0.35	0.18	0.12

HA, hydroxyapatite; OCP, octacalciumphosphate; CF₂, calcium fluoride.

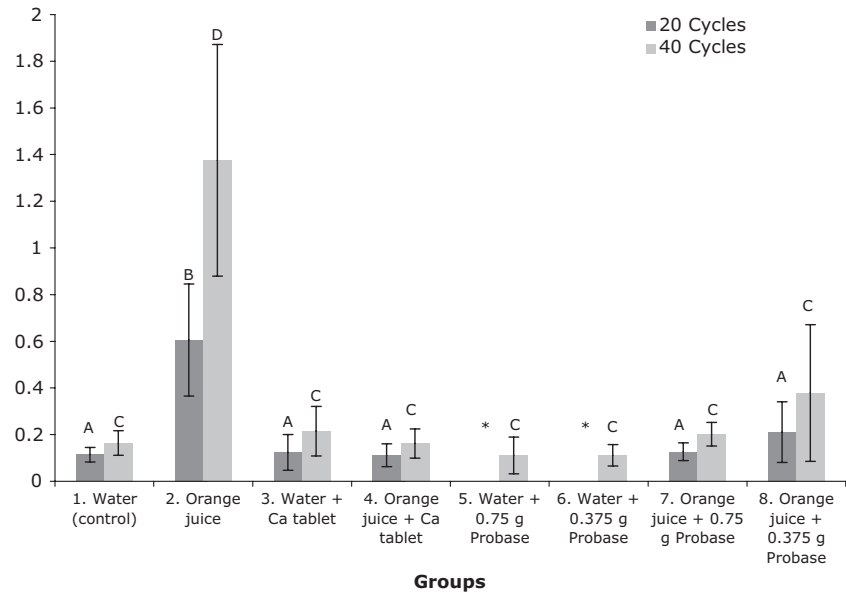


Figure 1 Mean wear and SD (μm) of enamel in the different groups (1–8) after 20 and 40 cycles of erosion/abrasion. Values that are not statistically significant different are marked with same capital letters. Values marked with asterisk are below detection limit

wear after 20 cycles of erosion/abrasion was observed ($P > 0.0018$ respectively). Wear of enamel after 20 cycles in group 5 (water + 0.75 g Probase) and 6 (water + 0.375 g Probase) was below the detection limit of 0.105 μm . Therefore, these values were set to 0 μm .

Within all groups, a significant increase of enamel wear due to erosion/abrasion was observed after 40 cycles when comparing with the respective values for 20 cycles ($P < 0.05$ respectively).

When comparing the enamel wear after 40 cycles of erosion/abrasion, again the highest wear ($1.375 \pm 0.496 \mu\text{m}$) was observed for the unmodified group 2. All other groups showed significantly lower enamel wear ($P < 0.0001$ respectively). When comparing the enamel wear of the modified orange juice group 4 (orange juice + Ca tablet), 7 (orange juice + 0.75 g Probase) and 8 (orange juice + 0.375 g Probase) with the wear of the water control group 1 after 40 cycles of erosion/abrasion, no statistically significant difference was observed ($P = 0.9771$, 0.6532 and 0.0149 respectively). Moreover, no significant difference in the enamel wear was observed within all groups, except group 2, after 40 cycles ($P > 0.0018$ respectively).

Discussion

In this study, the samples have been prepared from bovine enamel. Substitution of human enamel by bovine enamel has been performed in various other studies testing the susceptibility of enamel to erosive/abrasive tooth wear (Rios *et al*, 2006; Moretto *et al*, 2010; Wegehaupt and Attin, 2010). When comparing human and bovine enamel with regard to mineral content, mineral distribution as well as mechanical properties, no significant difference has been observed (Esser *et al*, 1998). Main advantage of using bovine enamel instead of human enamel is that it is easier to obtain a sufficient number of sound bovine teeth compared with human

teeth (Oesterle *et al*, 1998). Furthermore, bovine teeth do not suffer from caries and do not have a history of fluoridation measures that might influence erosive tooth wear in an unknown manner.

During the erosion/abrasion cycling of this study, the samples were immersed in the respective solutions. The use of commercially available orange juice simulates the common clinical situation of the patients (Lussi *et al*, 2004). Other studies (Attin *et al*, 2003; Vieira *et al*, 2006) dealing with erosive tooth wear and the prevention of the erosive tooth wear have used pure citric acid adjusted to different pH values. As the erosive potential of acidic beverages is not determined only by their kind of acid, pH value, titratable acidity and mineral content but also by their adhesiveness (Ireland *et al*, 1995), the use of pure citric acid does not seem to be appropriate to simulate the clinical situation. The exposure time of the samples in the solutions, during a single erosive/abrasive cycle of this study, has been set to 120 s. A previous study (Meurman *et al*, 1987) has shown this duration to be representative for a rapid consumption of an acidic beverage.

In contrast to other studies concerning erosive/abrasive tooth wear (Attin *et al*, 2007; Ganss *et al*, 2007b), no storage in remineralization solution such as artificial saliva was performed to simulate the clinical situation in this study. The reason for this was that the present study wanted to simulate the worst-case-scenario, like it occurs when the patient brushes immediately after the consumption of the juice for example in the morning after the breakfast (Attin *et al*, 2000). Another reason for not using remineralization media was that when the modification of the juice shows a positive effect under the here used worst-case-conditions, no other results might be expected with intermittent storage in a remineralization solution. The only difference that might be imaginable is that a longer duration of the experimental procedure, in this case a higher number of erosive/abrasive cycles, is needed until significant dif-

ferences of the tooth wear between the different groups can be observed. Moreover, it has been shown (Ganess *et al*, 2007a) that a remineralization period between erosion and tooth brushing has only a minor effect on the erosion/abrasion tooth wear.

When testing the taste of the modified orange juices, no taste change was reported for the orange juice modification with the two concentrations of Probase. When testing the orange juice modification with the calcium effervescent tablet, one test person reported a slight change in the taste. The taste of the modification was described as more tasteful.

The null hypothesis of this study has to be rejected. The erosive/abrasive tooth wear in the groups with the orange juice modifications was significantly lower compared with the unmodified orange juice group. This finding is in agreement with the findings of other studies, finding a lower erosive potential of acidic beverages like soft drinks (Hooper *et al*, 2007), sports drinks (Hooper *et al*, 2004), grapefruit juice (Spencer and Ellis, 1950) or black currant juice (West *et al*, 2003) modified by adding calcium or fluoride. It has to be kept in mind that these beverage modifications do not fully prevent the erosive tooth wear due to contact with enamel.

A recent study (Larsen and Richards, 2002) showed that fluoride admixtures to acidic solutions in a concentration excluding toxicologically side effects seem unable to arrest erosive lesions. Furthermore, besides acute poisoning due to the uptake of a toxic dose of fluorides, chronic effects of fluorides like dental fluorosis and skeletal fluorosis are observed (Krishnamachari, 1986; Fejerskov *et al*, 1994). As the admixtures of fluorides to acidic beverages, in non-toxic amounts, were not able to reduce the erosive potential significantly and under consideration of the chronic side effects, it seems not to be advisable to follow this approach. Also for the used dietary supplements, possible negative side effects due to an excessive intake, especially of the calcium, might be imaginable. For the Probase, the manufacture recommends a daily dose of 5 g for harmonization of the body acid-base balance. This amount contains 800 mg calcium. Even the highest amount of Probase used in this study (0.75 g) was only approximately one-seventh of the recommended daily dose. For the calcium tablets, the recommended dose is one tablet per day. This single tablet contains 500 mg calcium. For children over 1 year of age, the WHO (Vitamin and mineral requirements in human nutrition, World Health Organization, Food and Agriculture Organization of the United Nations) recommends a daily intake of 500 mg. For adolescents (10–18 years) a daily intake of 1300 mg is recommended. An upper limit of calcium intake of 3 g is recommended. Possible toxic side effects of calcium have only been described, when this amount of calcium is given as calcium carbonate. In this case, the side effects are related to the alkali effect of the carbonate (Burnett and Commons, 1949). Taking into consideration these findings and the recommendations of the manufactures, toxic side effects of the here used orange juice modifications seem to be unlikely.

The findings of this study demonstrate that orange juice modified with an effervescent tablet containing calcium, with low pH value showed no significantly higher erosive potential compared with water. This is in agreement with the observation that yogurt with a low pH (~ 4.0) has barely any erosive effect (Lussi *et al*, 1991). These results support the statement by Lussi *et al* (2009) that the erosive potential is not exclusively dependent on the pH value of beverages or foodstuff. It is more the degree of saturation of the erosive agent with respect to tooth minerals, determined by the pH but also the content of calcium, phosphate and fluoride which acts as a driving force for enamel dissolution (Lussi *et al*, 2009). Both, yogurt and the orange juice modification used in this study had low pH values (4.0–4.2), but showed no erosive potential, due to their high degree of saturation or even supersaturation with respect to apatite. In this study, the unmodified orange juice showed the lowest saturation concerning HA and OCP while presenting the highest enamel wear. When modified with the effervescent tablet containing calcium or Probase, the saturation increases and the wear was reduced significantly. Lowest wear was observed for the water with Probase (0.375 g and 0.75 g). These groups had the highest saturation concerning HA and OCP.

In contrast to other studies (Hooper *et al*, 2004, 2007) that attempted to modify erosive beverages by adding minerals to the beverages under laboratory conditions, the modifications employed in this present study can be performed by the individual consumer with free available dietary supplements (effervescent tablet containing calcium and a acid/base regulating powder) at home with no need of special laboratory equipment or chemical background.

Taking into consideration that there was no significant difference in the erosive/abrasive tooth wear of the modified orange juice groups and the water control group of this study and assuming that water has no erosive potential, the orange juice modifications used in this study were able to eliminate the erosive potential of the juice.

In the oral cavity, certain other factors influencing the erosive or erosive/abrasive enamel wear *in vivo*, have to be taken into consideration. One of these factors is the manner how an acidic beverage is drunk. It has been shown by Johansson *et al* (Johansson *et al*, 2004) that the pH drop is heavily influenced by the way of drinking. The highest pH drop was observed, when the beverage was held in the mouth, with a less pronounced drop due to long-sipping. The smallest drop of intra oral pH was observed, when the beverage was gulped. In the oral cavity, the erosive attack is also modified by the protective effect of the acquired pellicle. This protective effect was observed especially when less severe erosive challenges were simulated (Hara *et al*, 2006). Another factor is the diluting and buffering capacity of the saliva (Zero and Lussi, 2005). All these factors might lead to lower absolute erosive/abrasive enamel wear *in vivo*, although the relative protective effect of the orange juice modifications should be very similar to those found under *in vitro* conditions.

It can be concluded that the erosive/abrasive enamel wear due to the contact of enamel with orange juice followed by tooth brushing could be significantly reduced by the performed modifications of the orange juice with free available dietary supplements.

Author contributions

F. J. Wegehaupt was responsible for manuscript preparation. Günthart and Sener performed the study. Attin designed the study.

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