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High-fat diet effect on periapical lesions and hepatic enzymatic antioxidant in rats

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ABSTRACT

Aims: To evaluate the effects of a high-fat diet (HFD) on the progression of apical periodontitis (AP), local inflammation, systemic antioxidant status, and blood lipid profile in rats.
Main methods: Sixteen male *Wistar* rats were fed a standard diet (SD) or a HFD. At the sixth experimental week, the pulp chambers of the mandibular first molars were exposed to develop AP. A glucose tolerance test was performed the week before euthanasia. At the tenth experimental week, the animals were euthanized and the livers were collected to estimate catalase (CAT) and reduced glutathione (GSH) levels. Blood was acquired for biochemical analysis. The size of AP was estimated from radiographs and described as AP size-to-body weight ratio; inflammatory grade of AP was determined by histological analysis.
Key findings: At the end of the experimental period, the rats fed the HFD had 30% less weight ($P < 0.0001$) and higher blood glucose levels after 30 min of sucrose intake ($P < 0.05$) than those fed the SD. Animals from the HFD group had lower levels of CAT ($P < 0.01$), but the same was not observed in the GSH levels. Plasma insulin and total cholesterol were not affected by the diet. The rats fed the HFD presented greater AP than those fed the SD ($P < 0.05$). However, the local inflammatory infiltrate was similar in both groups.
Significance: The alterations promoted by the consumption of a HFD were not only observed systemically, but also locally, producing greater AP in rats than a SD.

1. Introduction

Apical periodontitis (AP) is an oral disease characterized by the destruction of the bone perirradicular tissues along with the development of an inflammatory process [1]. Microorganisms from the oral cavity can colonize the root canal system, mainly due to untreated caries, and the infection may culminate in the AP development [1]. AP is also associated with inadequate root canal-treated teeth and with insufficient coronal restorations [2]. As AP is related to both untreated and treated teeth, the disease is highly prevalent, affecting up to 52% of the worldwide population [3].

The course of some oral diseases, such as AP and periodontitis, can be modified by systemic alterations and individuals' behavioral attitudes [4,5]. Diabetes, osteoporosis, chronic alcohol consumption, among others, accentuate body inflammation, contributing to the bone loss in the oral cavity [6–8]. Chronic intake of a high-fat diet (HFD) has also increased periodontal loss in induced experimental periodontitis model in rats [9].

Eating habits have been severely modified in most of the countries lately, with an increase in the consumption of hypercaloric diets, preferably rich in fat and with poor nutritional value [10]. Chronic consumption of these types of diets can generate many health problems,

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especially given that they are risk factors for a series of non-communicable diseases, including type 2 diabetes and cardiovascular diseases [11]. Moreover, HFD consumption can compromise bone healing [12], with a decrease in bone mineral content and density [13–15]. A recent study has shown that an HFD affects bone by inducing osteoclastogenesis and inflammatory gene expression [16].

Furthermore, dietary pattern can determine systemic alterations through oxidative stress caused by the excessive production of reactive oxygen species (ROS) and/or by reduced activity of the endogenous antioxidant defense system [17]. The endogenous antioxidant defense system, composed of enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione reductase and glutathione peroxidase] and non-enzymatic antioxidants [uric acid, vitamin C, vitamin E, reduced glutathione (GSH)], acts to combat the harmful effects of oxidative stress [18,19]. Recently, the involvement of oxidative stress has been demonstrated in the pathophysiology of the apical periodontitis [20–22]. Oxidative stress also jeopardizes bone turnover [23] and contributes to chronic inflammation of tissues [24].

Since AP is a highly prevalent oral disease, and a high-fat dietary pattern is present worldwide, it is important to know whether these diets, which affect general health, also modify the progression of the periapical disease. In this context, the aim of this study was to evaluate the influence of an HFD on the AP progression, local inflammation, systemic antioxidant status, and blood lipid profile in rats.

2. Materials and methods

2.1. Animals

Experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals from National Institutes of Health. The study was approved by the local animal ethics committee (CEUA – protocol number 13/00360) of the Pontifícia Universidade Católica do Rio Grande do Sul (CEUA, PUC, Rio Grande do Sul, Brazil). Sixteen male *Wistar* rats (30 days old) were housed under controlled conditions (temperature 22 °C ± 1 °C, 70% humidity, and a 12 h light-dark cycle) and free access to food and filtered water. Animals' weight was verified weekly from the third week until the end of the experimental period, using a digital scale.

2.2. Diets

The animals were randomly divided into two groups according to their diets, of which they were fed throughout the 10-week experimental period: a standard diet (SD) (Nuvilab Produtos Agropecuários Ltda, Curitiba, Paraná, Brazil), or a high-fat diet (HFD), based on a protocol used previously [15]. The SD pellets had 3.5% of fat, yielding 331 kcal/100 g, whereas HFD pellets had 13.4% of fat with 389 kcal/100 g. The HFD group also received 20% sucrose in the drinking water during the first four weeks. The chow intake was measured at three different moments.

2.3. Apical periodontitis induction

The protocol to develop apical periodontitis was performed as previously described [25,26], with minor adaptations. Briefly, the rats were anesthetized by an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg). The opening of the left mandibular first molar was performed at the sixth experimental week with a round, long shank bur number 1011 (KG Sorensen®, São Paulo, SP, Brazil) and using high-speed rotation under constant irrigation. The pulp chambers were left exposed to the oral cavity for four weeks to permit the development of the AP [1].

2.4. Glucose tolerance test (GTT)

One week before euthanasia, GTT was measured at four moments: after 16 h of overnight food deprivation and after 30 min, 60 min, and 120 min of sucrose solution (2 g/kg body weight) intake [27]. The animals were not fed until the end of the test. The blood glucose was determined by using a glucometer (OneTouch Select Simple®, Johnson & Johnson Medical Devices & Diagnostics Group, Latin America, L.L.C.) with the blood sample collected through a small puncture at the tip of the rat's tail [28].

2.5. Euthanasia and biochemical analyses

At the tenth experimental week, the animals were euthanized by a deep anesthesia with isoflurane. The blood was rapidly acquired by a heart puncture with a vacutainer disposal needle (BD Vacutainer Eclipse® Blood Collection Needle). Extracted blood was centrifuged at 3640*g* for 15 min. Serum was used for the analyses of insulin and total cholesterol.

Insulin levels were measured by radioimmunoassay (Immunotech®, Beckman Coulter Company, Marseille, France), and total cholesterol levels were determined spectrophotometrically by enzymatic colorimetric tests (Bioclin®, Belo Horizonte, Minas Gerais, Brazil) by following the manufacturer's recommendations.

Liver samples were homogenized in saline and centrifuged at 3500*g* for 10 min for analysis of endogenous antioxidant status. CAT activity and GSH levels were determined according to the methodology described previously [29].

2.6. Radiographic analysis

After euthanasia, the left side of the mandibles were removed, dissected, and placed in neutral-buffered formalin (10%) for five days. Digital periapical radiographs were conducted in a standardized manner by using a Soredex® digital sensor (Tuusula, Finland) and an x-ray unit (Gnattus®, Ribeirão Preto, SP, Brazil), with an exposure time of 0.2 s. The x-ray beam was perpendicularly positioned to be 30 cm from the mandible-sensor set. The apical periodontitis size (in pixels) was determined by a trained endodontist, blinded to the experimental groups, using the Adobe Photoshop CS6 software (Adobe Systems Incorporated®, San Jose, CA) [25,26]. Measurements were carried out by the same examiner twice and intraclass correlation coefficient (ICC) was estimated to determine the intra-examiner reliability. ICC values and their confidence intervals were calculated using single measurement, absolute agreement, and two-way mixed-effects model [30]. The apical periodontitis size results were described as AP size-to-body weight ratio.

2.7. Histopathology analysis

After fixation in neutral-buffered formalin, the mandibles were decalcified in 17% EDTA (pH 7.0) for 45 days. EDTA solution was substituted every other day. The samples were included in paraffin blocks and were sectioned at a thickness of 3 μm in the mesiodistal plane. The slices that showed both the apical foramen and the periapical tissues were stained with hematoxylin and eosin. Pictures of the slices were taken with a light microscope (Zeiss Axio Imager M2 Light Microscope, Carl Zeiss®, Gottingen, Germany) using ×100 and ×400 of magnification. They were assessed by an experienced pathologist blinded to the experimental groups. The qualitative analysis of the inflammatory infiltrate was undertaken according to their intensity: (0) absent, (1) discrete, (2) moderate, or (3) severe [25,26].

2.8. Statistical analysis

The sample size was established by 8 animals per group and was

based on previous studies [25,26]. Statistical analyses were performed using the statistical software GraphPad Prism 5.0 (GraphPad® Software Inc., San Diego, CA). The Shapiro-Wilk test was applied to test the normality of the data, and the means of the variables of body weight, GSH, AP size-to-body weight ratio, and total cholesterol were compared using the *t*-test. The two-way ANOVA was applied to analyze the influence of the diet and the time on the results of GTT, whereas the insulin and catalase levels, and inflammatory infiltrate results were compared using Mann Whitney test.

Data were expressed as mean and standard error of the mean (SEM), and α was established at 5% to determine the statistical significance level.

3. Results

3.1. Body weight and chow intake

At the beginning of the experiment, the animals' weight in both groups was not statistically different ($P > 0.05$). As seen from the third week of experiment on, the HFD fed animals gained less weight then the SD fed animals (Supplementary Fig. 1A). At the end of the experimental period, the diet showed to have a significant impact on the body weight of the animals ($P < 0.0001$), and the rats fed the HFD had 30% less weight than the animals fed the SD (Table 1).

Measurements of the chow intake showed that the animals from the HFD group ate less amount of chow than the animals from the SD group (Supplementary Fig. 1B).

3.2. GTT

The results of the glycemic curve showed that the time and the diet had a significant impact on the results. The animals fed the HFD showed higher glucose concentrations compared to the SD group after 30 min of sucrose administration ($P < 0.05$); however, after 60 min of sucrose intake, both groups presented similar glycemic levels (Fig. 1).

3.3. Plasma insulin and total cholesterol levels

There were no statistically significant differences in total cholesterol and plasma insulin levels between the groups, although the insulin concentration was higher in the HFD group (Table 1).

3.4. Catalase activity and GSH levels

The HFD group showed reduced CAT activity compared to the SD group ($P < 0.01$), whereas GSH levels were similar between the groups (Table 1).

3.5. Radiographic analysis

ICC value was 0.92 (95% CI = 0.79–0.97), indicating that the intra-examiner reliability was "good" to "excellent" [30].

The mean of AP size-to-body weight ratio in the animals fed the HFD was significantly higher than in the animals fed the SD ($P < 0.05$) (Fig. 2A). Fig. 2B and C shows the AP in the first mandibular molar of the rats.

Table 1
Final body weight, plasma insulin, total cholesterol, catalase, and GSH levels in rats fed a Standard diet (SD) and a High-fat diet (HFD). Values are expressed as mean and standard error of mean (of 7 to 8 animals).

	Standard diet	High-fat diet	P value
Final body weight (g)	372.3 ± 11.28	258.6 ± 3.65	**P < 0.0001**
Plasma insulin (ng/ml)	0.26 ± 0.04	0.38 ± 0.08	P > 0.05
Total cholesterol (mg/dl)	63.48 ± 5.044	62.87 ± 3.075	P > 0.05
Catalase (mmol/min/mg protein)	8.55 ± 1.38	2.83 ± 0.94	**P < 0.01**
GSH (mmol/min/mg protein)	1.48 ± 0.28	1.31 ± 0.20	P > 0.05

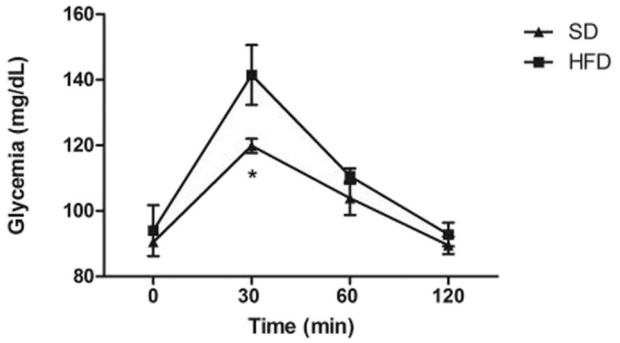

Fig. 1. Glucose tolerance test showing a significant higher blood sugar after 30 min of sucrose intake in the animals fed the HFD than those fed the SD ($P < 0.05$).

3.6. Histopathology analysis

Images in the high-power view (×400) showed the predominance of two cellular types, neutrophils and lymphocytes, and those cells were distributed similarly in the two groups (Fig. 2D and E) ($P > 0.05$).

4. Discussion

Highly fatty diets constitute a known environmental factor that may alter the susceptibility to oral [8,31] and systemic [32,33] diseases. In this study, we investigated the progression of AP in rats submitted to a high-fat diet. We also examined the diet influence on local inflammation, systemic antioxidant status, and blood lipid profile. To the best of our knowledge, this is the first time that a study has evaluated the influence of a dietary composition in the endodontic context.

When considering the AP size-to-body weight ratio, animals fed the HFD showed greater AP size comparing to those fed the SD. Even though the HFD induced the same intensity of local inflammation in the histological analysis, the AP extension in the rats fed the high-fat diet was greater than in those fed the SD, as seen by radiographic images. It could be inferred that the HFD caused a more severe manifestation of the AP by extending the inflammatory process, even though it promoted a similar degree of inflammation than the SD.

The endodontic infection caused by the harmful event of the opening of the tooth is combated through the organism's inflammatory response [1]. In the classic study of Kakehashi et al. [1], they described the evolution of bacterial invasion into the root canal and its progression into the apical tissues. Both non-specific and specific immune responses are stimulated in the periapical bone tissues by microbial antigens, culminating in destruction of the bone area surrounding the root apex and formation of inflammatory tissue [34,35]. The endodontic infection by itself causes the inflammatory reaction in the bone tissue [1], and HFD consumption is associated with an increase in the inflammatory state [36]. However, in the present study, histologic evaluations showed similar patterns of inflammation intensity in both groups. These findings are comparable with a previous one, using induction of AP in a type 2 diabetes model by sucrose intake in rats, in which the level of inflammation was mainly moderate, without difference between diabetic and control animals [26].

Contradictory to expectation, at the end of the experimental time, the HFD group had 30% less body weight compared to animals fed SD. Totsch et al. [37] showed that an HFD influenced body weight gain in female rats, but not in male ones, demonstrating the difference between both genders in the metabolism of a diet [37]. Furthermore, studies have shown high levels of the appetite-suppressing hormone leptin in animals fed an HFD [38], mainly in male ones [37]. The amount of chow intake

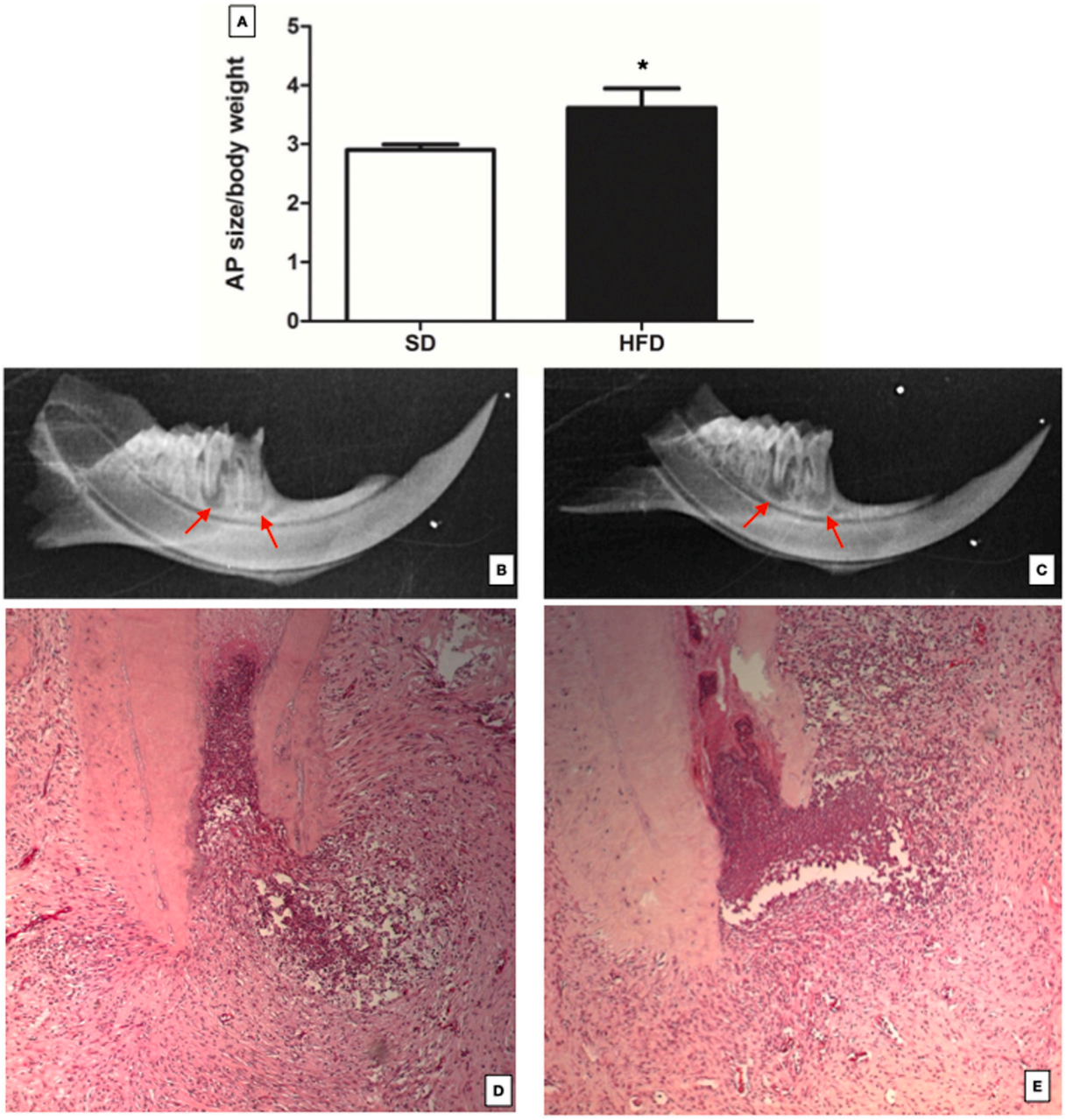

Fig. 2. A) AP size-to-body weight ratio was significantly greater in the animals from the HFD group ($P < 0.05$); B, C) representative images of the AP associated with the first mandibular molars in the rats fed an SD and an HFD, respectively; D, E) inflammatory infiltrate showing a severe grade of inflammation in the SD and the HFD groups, respectively (×100).

for the HFD fed animals was significantly smaller than for the animals fed the SD in the days that the food consumption was weighed (29th, 30th, and 37th day of experiment), which possibly explain these findings.

Our results showed a significantly higher concentration of blood glucose after 30 min of sucrose intake in the HFD group; however, the GTT result and insulin levels do not represent a type 2 diabetes model. Therefore, this pathway does not seem to support the difference in the AP size between the groups. HFD intake is related to the onset and progression of type 2 diabetes [39], and high blood glucose, also called hyperglycemia, is the defining characteristic of the disease. Hyperglycemia inhibits osteoclastogenesis, impairs osteoblast growth and promotes osteoblast apoptosis [40–42], but little is known about its effect on bone metabolism [43]. Regarding oral conditions, it is well evidenced in the literature that type 2 diabetes is highly associated with periodontal disease [4]. In animals with diabetes, visible changes in the progression of AP were only noticed in those with high blood glucose levels, consistent with type 1 diabetes or uncontrolled type 2 diabetes [8,44].

A poor nutritional diet, based on fat and sugar, is associated with an increase of oxidative stress and metabolic dysfunctions [45]. An imbalance between the production of ROS and antioxidants causes a state of oxidative stress, which can damage proteins, lipids, and nucleic acids [46]. Here, we also observed a higher reduction in the CAT activity in the liver of the HFD group compared to the SD group, which indicates the consumption of this endogenous enzymatic antioxidant. The impairment in the endogenous antioxidant system is a direct indicator of increased ROS [47]. Although ROS is needed in normal cellular functions, their overproduction can aggravate oxidative stress and initiate multiple deleterious events, eventually developing cellular dysfunction

and death [48]. Alterations in the GSH level, a non-enzymatic antioxidant, were not observed, which may indicate partial preservation of cellular protection. However, changes in markers of oxidative stress must be interpreted carefully, considering that some pathways might be altered as a compensatory mechanism in disease states. This might likely explain the changes of CAT as a compensatory mechanism, without any changes of GSH. It is also possible to infer that the time adopted for the HFD and AP induction might differently modulate these pathways. In mammalian cells, CAT is found at high concentrations in peroxisomes, along with a variety of oxidases and peroxidases [49]. This enzyme protects cells through the removal of the hydrogen peroxide, thereby preventing the toxic accumulation of ROS [50]. Moreover, the Km (Michaelis constant; a measure of the stability of the enzyme-substrate complex) for catalytic activity of the CAT is >10 mM, indicating the substrate amount needed to saturate/inhibit 50% of that enzyme. Then, at low intracellular concentrations of hydrogen peroxide, this reaction is kinetically unfavorable [51], and can be assumed that to reduce the CAT activity, the damage (apical periodontitis and HFD) would necessarily have to be high. These considerations corroborate our findings of the CAT activity reduction and, consequently, of the potential damage induced by HFD and apical periodontitis. Previous studies showed that both trans fatty acids (TFA) supplementation *per se* [52] or associated with ultraviolet radiation [53] reduced the CAT activity in the skin. Other study showed increased oxidative damages and lower antioxidant defenses, as observed by increased levels of protein carbonyl and reduced CAT activity in both cortex and hippocampus of trans fatty acids-supplemented rats [54]. In fact, the incorporation of TFA in neurons of these brain areas reduced the membrane fluidity, thus affecting membrane bound enzymes [55], which can be related to the modified antioxidant activity of the CAT, as observed in the current study.

Lastly, oxidative stress triggered in the aging process and inflammation has been associated with bone loss [56–58]. Even though the real mechanism of bone resorption through oxidative stress is not totally understood, pieces of evidence state that ROS can indirectly activate pro-resorption pathways, besides being found in contact with activated osteoclasts [59]. Thus, this mechanism possibly may help to explain the larger AP size in the HFD group.

5. Conclusion

Within the limits of this *in vivo* study, the alterations promoted by the consumption of a high-fat diet were not only observed systemically, but also locally, producing greater periapical lesions in rats than a standard diet. The present findings may imply in more extended periapical resorptions in compromised endodontic teeth in patients under an imbalanced diet. Metabolic disorders, such as type 2 diabetes, might influence the healing of such lesions as well. Endodontists, physicians, and patients must be aware of this association, which can alter the treatment plan and follow-up length of the cases. Alternatively, oral infections can also have negative impacts on comorbidities related to western diets enriched with ultra processed foods, including cardiovascular diseases. Future molecular and clinical studies are expected to pave the way regarding the relationship between inadequate diets and oral infection burden.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2020.118637.

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The authors declare that there is no conflict of interest regarding the publication of this paper.

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