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Evaluation of salivary parameters and remineralizing effects of yogurt in counteracting the cariogenic impact of candy consumption: An in vivo study

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ABSTRACT

Background: The consumption of sugary snacks like candies alters the oral environment, increasing the risk of dental caries. This study evaluates changes in salivary pH, calcium, phosphorus, alkaline phosphatase, and total antioxidant capacity (TAC) following the consumption of candies and yogurt.

Aim: To compare the impact of candies on the oral environment and assess the effectiveness of rinsing with water versus consuming yogurt in reversing cariogenic changes in saliva.

Materials and methods: Baseline saliva samples (5 mL) were collected from 60 children. Participants consumed candies, and saliva was collected 10 min post-consumption. They were divided into two groups: Group 1 rinsed with water, and Group 2 consumed yogurt. Saliva samples were collected at 10, 20 and 30 min post-intervention and analyzed for pH, calcium, phosphorus, alkaline phosphatase, and TAC. Data were analyzed using paired t-tests and post hoc Tukey tests.

Results: Candy consumption significantly decreased pH, calcium, phosphorus, and alkaline phosphatase levels (p < 0.001) and increased TAC levels (p < 0.001). Both water rinsing and yogurt consumption reversed these changes. Yogurt showed superior remineralization, with pH, calcium, phosphorus, and alkaline phosphatase levels returning to baseline more effectively than water rinsing (p < 0.001 for all comparisons). TAC levels were also restored more quickly with yogurt (p < 0.05).

Conclusion: Candy consumption induces a cariogenic environment. Both water rinsing and yogurt consumption effectively reversed these effects, with yogurt showing enhanced remineralization potential.

1. Introduction

Dental caries is a prevalent chronic disease among children, often initiated by the consumption of fermentable carbohydrates. These carbohydrates are metabolized by acidogenic bacteria in dental plaque, producing organic acids like lactic acid, which lower salivary pH and lead to enamel demineralization through the loss of calcium and phosphate ions from the subsurface of enamel. ^{1,2} An estimated 514 million children have been affected by dental caries in their primary teeth, while around 2 billion people have experienced dental caries in their permanent teeth. ^{3,4}

In the 20th century, dietary habits, particularly the increased consumption of chocolates and candies between meals, have significantly contributed to the rise of dental caries at an early age. The frequent consumption of sugar-rich products and their prolonged presence in the oral cavity leads to enhanced cariogenicity. Studies have shown that candies with high sucrose content and delayed oral clearance are more cariogenic than other foods. Despite this, there is limited data on how the consumption of candies impacts various salivary parameters such as calcium, phosphorus, alkaline phosphatase, and total antioxidant capacity (TAC) in vivo.

The cariogenic potential of foods is influenced by factors such as the

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retention time of acidic carbohydrates in the mouth, the role of saliva in oral clearance, and the presence of remineralizing ions like calcium and phosphate, which can buffer the acidic environment in the mouth. 5,6 Previous research has shown that reduced levels of calcium, phosphorus, and pH in saliva are associated with higher caries prevalence. 7,8 , Additionally, the imbalance in free radicals and reactive oxygen species, which play a role in dental caries development, highlights the significance of antioxidants in saliva. 9,10

At the early stages of demineralization, salivary calcium and phosphorus ions help reverse the damage by diffusing into the subsurface enamel. ^{7,11} However, in high caries-risk children, the salivary defence mechanisms may not be sufficient, requiring additional sources of these ions. Diet counselling, which aims to limit sugar intake, is a common preventive approach. However, due to poor patient compliance, alternative solutions are needed. Traditionally, rinsing with water after sugar intake has been considered beneficial for oral clearance, but its effects on salivary parameters remain unclear.

Milk and milk-based products, such as yogurt, have gained attention for their potential in preventing dental caries and promoting remineralization. These products contain beneficial components such as casein phosphopeptides, proteins, fats, vitamins, calcium, and phosphate, which contribute to their anticariogenic properties. ¹² Additionally, the presence of lactoferrin and casein in milk inhibits bacterial adherence to teeth. ¹³ Yogurt, being an accessible and cost-effective product, stimulates saliva production, enhancing its buffering capacity and promoting faster carbohydrate clearance. ¹⁴ Despite these known benefits, there is limited research on the antioxidant properties of yogurt and its ability to counteract the cariogenic effects of sweetened candies. This study aims to evaluate the effectiveness of yogurt in mitigating the cariogenic challenge induced by candy consumption.

2. Materials and Methods

The study was conducted at A. B. Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangaluru, India. Sixty children aged 5–12 years reporting to the Department of Pediatric and Preventive Dentistry were recruited. The sample size of 60 was calculated using the formula $n=2(Z\alpha+Z1-\beta)^2\sigma^2/\Delta^2$, where $Z\alpha=1.96$ for a 5 % α -error (two-sided) and $Z1-\beta=0.8416$ for 80 % power. The standard deviation (σ) and effect size (Δ) were derived from the study by Tayab et al. 15

Children with DMFT/deft scores of 3–5, as per WHO criteria, were included. Exclusion criteria were caries-free children, those with enamel defects, systemic or metabolic diseases, children on medication, and children with special healthcare needs. Ethical clearance was obtained from the Institutional Ethics Committee (ABSM/EC/75/2013), and informed consent was provided by parents/guardians, along with verbal assent from the children.

Screening was conducted using a mouth mirror and probe, and oral prophylaxis was performed 24 h prior to the study to standardize oral conditions. On the day of saliva collection, participants were instructed to refrain from brushing and to avoid eating or drinking for 2 h before the study.

Baseline unstimulated saliva (5 mL) was collected from all participants in the Coachman's position, using passive drooling into sterilized plastic containers. Each child was then given one sweetened candy (Pim Pom candy, Jakarta Raya, Indonesia) to consume, taking approximately 5 min. Saliva samples (5 mL) were collected 10 min after candy consumption.

Participants were randomly assigned to two groups using a lottery method to ensure an unbiased distribution. Each participant's name was written on identical slips of paper, which were then placed into a sealed container and thoroughly mixed. A neutral third party drew the slips, allocating participants to one of two groups, each comprising 30 individuals.

Group 1: Rinsed with water.

Group 2: Swished 20 g of yogurt (Amul Premium Dahi, India) in their mouth and swallowed.

Subsequent saliva samples (5 mL) were collected 10, 20, and 30 min after intervention. Participants were asked to brush their teeth thoroughly at the end of the study to remove any residual candy particles.

The saliva samples were stored at $-20\ ^{\circ}\text{C}$ and centrifuged at 3000 rpm for 10 min. The supernatant was analyzed for pH, calcium, phosphorus, alkaline phosphatase, and total antioxidant capacity (TAC) using standard biochemical methods.

The pH was measured using a digital pH meter based on the principle that hydrogen ions in the solution migrate toward the glass electrode, displacing metal ions on the electrode coating. This creates a voltage difference that is amplified and converted into a pH value by the voltmeter. 16 Calcium concentration was analyzed using the modified OCPC method. In this method, calcium reacts with ortho-cresolphthalein to form a violet-colored complex, and the absorption of this complex, which is proportional to calcium concentration, is measured colorimetrically at a wavelength of 578 nm after a 5-min incubation. 17 Phosphorus was measured using the phosphomolybdate method with an inorganic phosphorus kit (Agappe Diagnostics Ltd., Kerala, India). This method involves the reaction of ammonium molybdate with phosphorus in the presence of sulfuric acid to form a phosphomolybdic complex. The absorbance was measured at a wavelength of 340 nm after 1 min. 18 Alkaline phosphatase was analyzed using the DGKC-SCE method with an alkaline phosphatase reagent kit (Agappe Diagnostics Ltd., Kerala, India). In this method, para-nitrophenyl phosphate reacts with alkaline phosphatase, resulting in the formation of p-nitrophenol and inorganic phosphate. The yellow color of p-nitrophenol was measured at a wavelength of 405 nm after 1 min of incubation. ¹⁹ The total antioxidant capacity (TAC) was determined using the phosphomolybdenum method with a spectrophotometer. This assay is based on the reduction of molybdenum (Mo VI) to molybdenum (Mo V) by antioxidants, which then forms a green-colored complex with phosphate under acidic conditions. The intensity of the green complex was measured spectrophotometrically at a wavelength of 695 nm.²⁰

The interventions were performed simultaneously within the same time frame for all participants in both groups, ensuring consistency in sample processing and analysis.

Paired t-tests were used to analyze changes in salivary parameters, including pH, calcium, phosphorus, alkaline phosphatase, and total antioxidant capacity (TAC), at baseline, 10 min after candy consumption, and at 10, 20, and 30 min after the consumption of water and yogurt. To evaluate intergroup differences (yogurt vs. water), post hoc Tukey's test was applied. The level of significance was set at p < 0.05.

3. Results

The salivary parameters analyzed included pH, calcium, phosphorus, alkaline phosphatase, and TAC. A comparison of the changes in these parameters at baseline, 10 min after candy consumption, and at 10, 20, and 30 min post-intervention (water or yogurt) was conducted.

3.1. pH

The mean salivary pH decreased significantly after candy consumption (p < 0.001). Post-intervention, pH levels increased progressively over time in both groups. In the yogurt group, pH exceeded baseline levels at 30 min (p < 0.001), whereas the water group saw partial recovery, with pH remaining slightly below baseline. Intergroup analysis revealed that the yogurt group had a significantly higher pH recovery than the water group at all time intervals (p < 0.001) (Table 1).

Table 1Changes in Salivary pH at Different Time Points After Consumption of Candies, Water, and Yogurt.

Parameter	Mean (±SD)	Paired Differences	t	p-value
Baseline (Candies and	7.04	_	_	<0.001***
Water Group)	(± 0.31)			
10 min after Candies	6.16	$-0.88~(\pm 0.30)$	-16.34	< 0.001 ***
(Group 1)	(± 0.34)			
10 min after Water	6.48	$-0.21~(\pm 0.23)$	-5.05	< 0.001***
(Group 1)	(± 0.38)			
20 min after Water	6.69	$-0.21~(\pm 0.22)$	-5.27	< 0.001***
(Group 1)	(± 0.38)			
30 min after Water	6.89	$-0.15~(\pm 0.17)$	-4.79	< 0.001***
(Group 1)	(± 0.30)			
Baseline (Candies and	6.87	-	-	< 0.001***
Yogurt Group)	(± 0.42)			
10 min after Candies	6.21	$-0.35~(\pm 0.41)$	-4.58	< 0.001 ***
(Group 2)	(± 0.46)			
10 min after Yogurt	6.55	$-0.53~(\pm 0.38)$	-7.67	< 0.001 ***
(Group 2)	(± 0.38)			
20 min after Yogurt	7.08	$-0.07~(\pm 0.33)$	-1.15	0.26 ^{ns}
(Group 2)	(± 0.37)			
30 min after Yogurt	7.15	$-0.28~(\pm 0.36)$	-4.30	< 0.001 ***
(Group 2)	(± 0.29)			
Intergroup	Yogurt >	-	-	<0.001***
Comparison (after	Water			
30 min)				

p < 0.001: Very Highly Significant; ns = Not Significant.

3.2. Calcium

A significant decline in salivary calcium levels was observed after candy consumption (p < 0.001). Recovery was noted in both groups post-intervention, with the yogurt group showing a sustained and significant increase beyond baseline levels at 30 min (p < 0.001). Calcium levels in the water group returned to baseline but did not exceed it. The intergroup comparison confirmed the superior effect of yogurt in restoring calcium levels (p < 0.001) (Table 2).

Table 2Changes in salivary calcium levels at different time points after consumption of candies, water, and yogurt.

Parameter	Mean (±SD) (mg/dl)	Paired Differences	t	p-value
Baseline (Candies and	9.81	_	-	<0.001***
Water Group)	(± 1.13)			
10 min after Candies	7.60	$-2.21~(\pm 0.98)$	-12.36	<0.001***
(Group 1)	(± 1.19)			
10 min after Water	8.85	$-0.49~(\pm 0.92)$	-2.94	*0.006
(Group 1)	(± 1.31)			
20 min after Water	9.35	$-0.65~(\pm 0.68)$	-5.21	<0.001***
(Group 1)	(± 1.07)			
30 min after Water	9.99	$-0.19~(\pm 0.67)$	-1.53	0.137 ns
(Group 1)	(± 1.12)			
Baseline (Candies and	7.63	-	-	<0.001***
Yogurt Group)	(± 1.48)			
10 min after Candies	6.17	$-0.92~(\pm 1.23)$	-4.08	<0.001***
(Group 2)	(± 1.57)			
10 min after Yogurt	7.08	$-0.85~(\pm 1.09)$	-4.28	<0.001***
(Group 2)	(± 1.61)			
20 min after Yogurt	7.94	$-0.69~(\pm 1.32)$	-2.85	*0.008
(Group 2)	(± 1.42)			
30 min after Yogurt	8.62	$-0.99~(\pm 0.98)$	-5.58	<0.001***
(Group 2)	(± 1.85)			
Intergroup	Yogurt >	-	-	<0.001***
Comparison (after	Water			
30 min)				

p < 0.001: Very Highly Significant; ns = Not Significant.

3.3. Phosphorus

Salivary phosphorus levels decreased significantly after candy consumption (p < 0.001). In the water group, phosphorus levels peaked at 20 min but remained below baseline at 30 min (p = 0.043). Conversely, in the yogurt group, phosphorus levels increased significantly, peaking at 20 min and remaining higher than baseline at 30 min (p < 0.001). The intergroup comparison indicated that yogurt was significantly more effective than water in restoring phosphorus levels at 30 min (p < 0.001) (Table 3).

3.4. Alkaline phosphatase

Candy consumption caused a significant reduction in alkaline phosphatase activity (p < 0.001). Post-intervention, both groups exhibited recovery, with the yogurt group achieving higher levels at 20 min (p < 0.05). However, the levels returned closer to baseline by 30 min in both groups, with no statistically significant differences observed between the groups (p > 0.05) (Table 4).

3.5. Total antioxidant capacity (TAC)

TAC levels increased significantly after candy consumption ($\mathbf{p} < \mathbf{0.001}$). Following intervention, TAC decreased in both groups over time, with the water group returning to baseline at 30 min. In the yogurt group, a transient increase was noted at 20 min, but final TAC levels at 30 min were similar to baseline ($\mathbf{p} > \mathbf{0.05}$). While the yogurt group demonstrated marginally higher TAC levels at all intervals, the differences were not statistically significant at 30 min ($\mathbf{p} > \mathbf{0.05}$) (Table 5).

4. Discussion

Salivary calcium and phosphorus are crucial in maintaining enamel's mineral balance during demineralization and remineralization processes. Demineralization begins when organic acids produced by cariogenic bacteria lower the pH of dental plaque, initiating the dissolution of hydroxyapatite (HA) crystals and releasing calcium (Ca²⁺) and phosphate ions (PO₄³⁻) from enamel into the surrounding environment. ²¹ Saliva acts as a reservoir, replenishing these ions and enabling

Table 3Changes in salivary phosphorus levels at different time points after consumption of candies, water, and yogurt.

Parameter	Mean (±SD) (mM/L)	Paired Differences	t	p-value
Baseline (Candies and Water Group)	3.02 (±1.19)	-	-	<0.001***
10 min after Candies (Group 1)	1.38 (±0.90)	$-1.34~(\pm 1.17)$	-6.27	<0.001***
10 min after Water (Group 1)	2.72 (±1.60)	$-0.25~(\pm 0.93)$	-1.47	0.153 ns
20 min after Water (Group 1)	2.97 (±1.22)	0.11 (±0.82)	0.71	0.482 ns
30 min after Water (Group 1)	2.86 (±1.12)	$-0.16~(\pm 0.42)$	-2.12	*0.043
Baseline (Candies and Yogurt Group)	2.07 (±0.38)	-	-	<0.001***
10 min after Candies (Group 2)	1.23 (±0.64)	$-0.74~(\pm 0.83)$	-4.83	<0.001***
10 min after Yogurt (Group 2)	1.97 (±0.59)	$-2.04~(\pm 1.27)$	-8.80	<0.001***
20 min after Yogurt (Group 2)	4.00 (±1.40)	$-0.69~(\pm 1.32)$	-2.85	0.173 ns
30 min after Yogurt (Group 2)	3.55 (±1.18)	$-1.48~(\pm 1.06)$	-7.69	<0.001***
Intergroup Comparison (after 30 min)	Yogurt > Water	-	-	<0.001***

p < 0.001: Very Highly Significant; p < 0.05: Significant; ns = Not Significant.

Table 4Changes in salivary alkaline phosphatase levels at different time points after consumption of candies, water, and yogurt.

Parameter	Mean (±SD) (U/L)	Paired Differences	t	p-value
Baseline (Candies	48.00	_	_	<0.001***
and Water Group)	(± 35.30)			
10 min after Candies	26.28	$-18.37\ (\pm 27.55)$	-3.65	0.001
(Group 1)	(± 16.94)			
10 min after Water	44.65	$0.70~(\pm 23.50)$	0.16	0.871 ^{ns}
(Group 1)	(± 32.87)			
20 min after Water	43.95	$-4.82~(\pm 22.49)$	-1.17	0.25 ^{ns}
(Group 1)	(± 24.10)			
30 min after Water	48.77	$-0.77~(\pm 28.09)$	-0.15	0.882 ^{ns}
(Group 1)	(± 25.61)			
Baseline (Candies	49.98	-	-	< 0.001 ***
and Yogurt Group)	(± 15.38)			
10 min after Candies	30.39	$-5.80~(\pm 21.25)$	-1.49	0.146 ^{ns}
(Group 2)	(± 11.21)			
10 min after Yogurt	36.19	$-22.09\ (\pm 51.35)$	-2.36	0.025*
(Group 2)	(± 21.59)			
20 min after Yogurt	58.27	$2.84~(\pm 53.35)$	0.29	0.772 ^{ns}
(Group 2)	(± 51.95)			
30 min after Yogurt	55.43	$-5.45~(\pm 23.09)$	-1.29	0.206 ^{ns}
(Group 2)	(± 22.23)			
Intergroup	Yogurt >	-	-	< 0.001 ***
Comparison (after	Water			
30 min)				

p < 0.001: Very Highly Significant; p < 0.05: Significant; p < 0.01: Highly Significant; ns = Not Significant.

Table 5Changes in salivary TAC levels at different time points after consumption of candies, water, and yogurt.

Parameter	Mean (±SD) (mM/L)	Paired Differences	t	p-value
Baseline (Candies and Water Group)	0.98 (±0.49)	-	-	<0.001*
10 min after Candies (Group 1)	1.95 (±1.15)	0.66 (±1.12)	3.21	0.003*
10 min after Water (Group 1)	1.30 (±0.49)	$0.38~(\pm 0.68)$	3.05	0.005*
20 min after Water (Group 1)	0.92 (±0.44)	0.06 (±0.60)	0.55	0.587 ^{ns}
30 min after Water (Group 1)	0.86 (±0.36)	0.13 (±0.64)	1.08	0.29 ^{ns}
Baseline (Candies and Yogurt Group)	1.00 (±0.34)	-	-	0.176 ^{ns}
10 min after Candies (Group 2)	1.17 (±0.51)	0.16 (±0.44)	1.98	0.057 ^{ns}
10 min after Yogurt (Group 2)	1.01 (±0.29)	$-0.22~(\pm 0.46)$	-2.59	0.015*
20 min after Yogurt (Group 2)	$1.23~(\pm 0.33)$	$0.21~(\pm 0.68)$	1.66	0.109 ns
30 min after Yogurt (Group 2)	1.02 (±0.56)	$-0.01~(\pm 0.72)$	-0.10	0.923 ^{ns}

p < 0.001: Very Highly Significant; p < 0.05: Significant; p < 0.01: Highly Significant; ns = Not Significant.

remineralization once the pH stabilizes. The buffering capacity of saliva is instrumental in neutralizing acids and restoring pH to levels favorable for remineralization. At neutral or slightly basic pH, calcium and phosphate ions redeposit onto demineralized enamel, forming acid-resistant hydroxyapatite crystals. 22

However, this dynamic balance is significantly influenced by salivary flow and composition. ^{6,8} Conditions like xerostomia or medication-induced reductions in salivary flow compromise the availability of these ions, leaving enamel vulnerable to prolonged demineralization. Fluctuations in salivary pH and ion concentration also modulate mineral loss or recovery, emphasizing the importance of maintaining salivary health. Routine assessments, dietary adjustments,

and fluoride applications can support remineralization and reduce caries risk.

The cariogenic potential of foods depends on factors such as carbohydrate content, frequency of consumption, oral retention time, and their ability to stimulate salivary flow. ^{1,2} Sweetened candies, chosen for this study, are well-documented for their cariogenic properties, aligning with research on the detrimental effects of fermentable sugars. ⁵

In contrast, milk and dairy products, essential for a balanced diet, exhibit anticariogenic properties. Dairy products, rich in proteins, calcium, phosphorus, and bioactive components, combat dental caries through various mechanisms. ^{12,13} Casein phosphopeptides (CPP), found in dairy, create a protective barrier on enamel, inhibiting demineralization and bacterial adhesion. ²³ Additionally, lactoperoxidase and lysozyme in dairy suppress *Streptococcus mutans* metabolism, reducing acid production. ²⁴

Our study found a significant drop in salivary pH 10 min after candy consumption, consistent with reports by Shah TJ et al., 5 Verakaki et al., 25 and Hegde et al. 26 This aligns with the Stephan curve, where plaque bacteria metabolize fermentable carbohydrates, producing organic acids like lactic and acetic acids. However, a water rinse quickly elevated the pH, highlighting the role of oral hygiene in neutralizing acids and enhancing salivary buffering. 5

Conversely, yogurt consumption resulted in a sustained increase in salivary pH, surpassing baseline levels within 30 min. These findings corroborate previous studies by Tayab et al., ¹⁵ Rugg-Gunn et al., ²⁷ Higham et al., ²⁸ and Sonmez et al., ²⁹ which observed similar effects with cheese. Interestingly, unlike Ravishankar et al., ³⁰ our study did not record an initial pH drop post-yogurt consumption, suggesting yogurt's buffering capacity and its ability to stimulate saliva mitigate acidogenic effects.

Yogurt also significantly increased salivary calcium and phosphorus levels within 10 min, consistent with Hegde et al. 28 This highlights yogurt's remineralization potential, supported by Ferrazano et al. 31 and Varghese et al. 32 Yogurt consistently outperformed water in enhancing salivary ion levels, reaffirming its efficacy as an anticariogenic dietary option.

Interestingly, prior literature, such as Jensdottir et al.,³³ suggests certain candies transiently increase salivary calcium and phosphorus levels due to calcium fortification. This emphasizes the influence of candy composition on salivary ion dynamics. However, our study did not observe significant increases after candy consumption, likely due to the absence of calcium fortification or timing variations in sample collection.

In terms of remineralization, yogurt's high mineral content and CPP stabilized calcium and phosphorus, facilitating their deposition onto enamel. Alkaline phosphatase (ALP), a mineralization marker, decreased after candy consumption but significantly increased with yogurt intake. This contrasts with studies by Pandey et al. ³⁴ and Gandhy et al., ³⁵ which linked higher ALP with caries activity.

The study also noted a rise in salivary total antioxidant capacity (TAC) after candy consumption, reflecting bacterial activity in response to sugar. ⁵ Yogurt, however, reduced TAC levels, suggesting a potential to alleviate oxidative stress. Despite this, yogurt and water showed no significant differences in intergroup TAC comparisons, warranting further exploration of dairy's antioxidant effects.

To strengthen these findings, future research should address limitations such as sample size, observation duration, and confounding variables like dietary habits and oral hygiene. Additionally, the low pH of yogurt (mean 4.7 in this study) may transiently influence salivary dynamics. Testing the pH of products before study initiation and comparing yogurt with other dairy products like milk or cheese would yield deeper insights.

In conclusion, yogurt demonstrates the ability to counteract the cariogenic effects of candy by elevating salivary calcium and phosphorus levels, promoting remineralization, and mitigating acid exposure. These findings support yogurt as a dietary recommendation,

particularly for children at risk of dental caries. Future studies should explore the complex interplay of food composition, salivary ions, and oral health to refine dietary strategies for caries prevention.

5. Conclusion

Yogurt demonstrated superior anticariogenic properties compared to water, as evidenced by its ability to restore and maintain salivary pH, calcium, and phosphorus levels after a cariogenic challenge. The findings support the inclusion of yogurt in the diet as a natural, accessible, and effective means to promote oral health and prevent dental caries.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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