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Oral supplementation with a prebiotic, mannan oligosaccharide, mitigates intestinal dysbiosis in young rats chronically exposed to passive smoking

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Efeito da Suplementação Oral de Mananoligossacarídeo na Mitigação da Disbiose Intestinal em Ratos Jovens Expostos ao Tabagismo Passivo

RESUMO

Em humanos, foi demonstrado que o tabagismo crônico passivo altera a microbiota e aumenta o risco de infecções por patógenos e/ou bactérias oportunistas. O objetivo deste estudo foi avaliar o efeito da suplementação de mananoligossacarideo (MOS) na concentração de *Escherichia coli* nas fezes de ratos fumantes passivos. Sessenta ratos jovens (23 dias) foram alocados aleatoriamente em quatro grupos (n=15): dois grupos suplementados com MOS e expostos ou não cronicamente à fumaça do cigarro e não suplementados com MOS e expostos ou não cronicamente à fumaça do cigarro. A exposição à fumaça de cigarro foi realizada duas vezes ao dia durante 180 dias e amostras fecais foram coletadas quatro vezes (dias 0, 60, 120 e 180). Após a coleta de fezes, as populações bacterianas foram amplificadas por PCR em tempo real. As médias dos dados foram analisadas por meio de ANOVA unidirecional seguida de análise post-hoc de Student-Newman-Keuls, considerando 5% como nível de significância. Os resultados revelaram que a exposição crônica a fumaça de cigarro aumentou (P<0,05) de maneira tempo-dependente a população de *E. coli*. No grupo de ratos suplementados com MOS a concentração de *E. coli* não aumentou durante o período experimental de 180 dias. A média da concentração de *E. coli* dos grupos de ratos somente suplementados com MOS e controle não diferiram (P>0,05). Os resultados permitem-nos concluir que a suplementação de MOS atenua o efeito crônico da fumaça do cigarro na concentração da bactéria *E. coli* em ratos jovens, como modelo pré-clínico.

PALAVRAS-CHAVE: MOS. Tabagismo. Alimento Funcional. Microbiota Intestinal.

Oral supplementation with a prebiotic, mannan oligosaccharide, mitigates intestinal dysbiosis in young rats chronically exposed to passive smoking

ABSTRACT

In humans, passive smoking has been shown to alter the microbiota and increase the risk of infections by pathogens and/or opportunistic bacteria. The objective of this study was to evaluate the effect of MOS supplementation on the concentration of *E. coli* in the feces of passive smoking rats. Sixty young rats (23 days) were randomly allocated into four groups (n=15): supplemented or not with MOS in the diet; and chronically exposed or not to cigarette smoke. Exposure to cigarette smoke was performed twice a day for 180 days and fecal samples were collected four times (days 0, 60, 120, and 180). After fecal collection, bacterial populations were amplified by real-time PCR. Data means were analyzed using one-way ANOVA followed by Student-Newman-Keuls post-hoc analysis, considering 5% as the level of significance. The results revealed that MOS supplementation significantly (P<0.05) reduced the *E. coli* population resulting from chronic exposure to cigarette smoke. The mean results of the group of rats supplemented with MOS and exposed to cigarette smoke did not differ (P>0.05) from the groups not exposed to cigarette smoke and supplemented or not with MOS. The results allow us to conclude that MOS supplementation mitigates the chronic effect of cigarette smoke on the concentration of *E. coli* bacteria in young rats, as a preclinical model.

KEY-WORDS: MOS; Smoking; Functional Food, Intestinal Microbiota.

Efecto de la Suplementación Oral de Mananoligosacárido en la Mitigación de la Disbiosis Intestinal en Ratas Jóvenes Expuestas al Tabaquismo Pasivo

RESUMEN

En humanos, se ha demostrado que el tabaquismo pasivo crónico altera la microbiota y aumenta el riesgo de infecciones por patógenos y/o bacterias oportunistas. El objetivo de este estudio fue evaluar el efecto de la suplementación con mananoligosacárido (MOS) en la concentración de *Escherichia coli* en las heces de ratas expuestas al tabaquismo pasivo. Sesenta ratas jóvenes (23 días) fueron asignadas aleatoriamente a cuatro grupos (n=15): dos grupos suplementados con MOS y expuestos o no crónicamente al humo de cigarrillo, y dos grupos no suplementados con MOS y expuestos o no crónicamente al humo de cigarrillo. La exposición al humo de cigarrillo se realizó dos veces al día durante 180 días, y se recolectaron muestras fecales en cuatro momentos (días 0, 60, 120 y 180). Tras la recolección de las heces, las poblaciones bacterianas fueron amplificadas mediante PCR en tiempo real. Los datos promedio fueron analizados mediante ANOVA unidireccional, seguido de un análisis post-hoc de Student-Newman-Keuls, considerando un nivel de significancia del 5%. Los resultados revelaron que la exposición crónica al

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humo de cigarrillo aumentó (P<0,05) de manera dependiente del tiempo la población de *E. coli*. En el grupo de ratas suplementadas con MOS, la concentración de *E. coli* no aumentó durante el período experimental de 180 días. El promedio de la concentración de *E. coli* en los grupos de ratas suplementadas únicamente con MOS y el grupo control no presentó diferencias significativas (P>0,05). Los resultados permiten concluir que la suplementación con MOS atenúa el efecto crónico del humo de cigarrillo en la concentración de la bacteria *E. coli* en ratas jóvenes, utilizándose como modelo preclínico.
PALABRAS CLAVE: MOS. Tabaquismo. Alimento Funcional. Microbiota Intestinal.

1 INTRODUCTION

Current studies warn of the economic costs and deaths caused by passive smoking. Every year, 603,000 people die worldwide as a result of passive smoking, of which 160,840 are children (INCA, 2024)

In humans, tobacco smoke, through passive smoking, has been shown to alter the microbiota and increase the risk of infections by pathogens and/or opportunistic bacteria (HUANG; SHI, 2019). Tobacco smoke contains many extremely toxic products, including cyanide and nicotine, and tests with animal models, including mice, hamsters, rats, and chick embryos, have been performed to identify the effects of smoke-derived components on intestinal microbiota and other pathologies, and can be used as sentinels for environmental and public health (BAGAITKAR; DEMUTH; SCOTT, 2008; REIF, 2011).

Escherichia coli colonize and reside in the gastrointestinal (GI) tract of animals and humans along with thousands of different microorganisms. Some strains have beneficial properties, such as the antibacterial secretion of colicin, and can provide antioxidant, anti-inflammatory, and antitumor molecules. Additionally, some strains can secrete enzymes to convert sucrose and fructose into insulin and mannitol, respectively, and have been shown to be effective in preventing the onset of metabolic and multifactorial diseases, including cancer (GATTUPALLI; GATTUPALLI, 2023; HASEBE et al., 2022; MOREIRA DE GOUVEIA; BERNALIER-DONADILLE; JUBELIN, 2024). However, *Escherichia coli* can also be responsible for serious infections, being identified as an opportunistic pathogen (GUILLAUME DALMASSO, 2015; MARTINSON; WALK, 2020; MARTINSON et al., 2019).

To remedy the effects of altered microbiota, functional foods, such as prebiotics [mannan oligosaccharide (MOS) – extracted from sugar cane yeast], have demonstrated a role in maintaining the epithelial barrier integrity and modulating innate immunity through secretion of pro- and anti-inflammatory cytokines, switches in macrophage polarization and function, neutrophil recruitment and migration, and dendritic cell and regulatory T-cell differentiation (GUIMARÃES et al., 2020; PUJARI; BANERJEE, 2021). Furthermore, it is known that the mode of action of prebiotics is based on their ability to bind to the fimbriae of pathogenic bacteria, making them unavailable for adherence to the epithelium and preventing them from colonizing the gastrointestinal tract (C. TAM; M. LAND; W. CHENG, 2020).

Therefore, it is in the interest of the scientific community to seek viable alternatives that can reduce the impacts caused by passive smoking on humans, especially children. Therefore, since literature data are scarce regarding this factor, the present study aimed to quantify the effect of supplementation with the oral prebiotic mannan-oligosaccharide, on the population mass of *Escherichia coli* bacteria in young rats chronically exposed to tobacco

cigarette smoke. In this way, mannan oligosaccharides could be used to mitigate the effects of cigarette smoke on one of the components of the intestinal microbiota, such as the population of *Escherichia coli*.

2 MATERIAL AND METHODS

2.1 Animal ethics

The experimental study was conducted according to the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation and was approved by the Institutional Ethics Committee for Animal Research (No. 2656), of the Faculty of Medicine, Western São Paulo University (UNOESTE), Presidente Prudente, São Paulo, Brazil.

2.2 Experimental design

Sixty young male rats (*Rattus novergicus*), twenty-three days old, with a mean body weight of 45 g, were kept in collective cages, under the same lighting conditions (12/12 hour light/dark cycle), with a controlled temperature (22±1.5ºC), and water and diet were provided ad libitum during the experimental period of 187 days: seven days of adaptation to the management and basal diet; and 180 days of treatment. The animals were randomly distributed to the four experimental groups (n=15) by means of a sequence table generated by the R program (HARRELL, 2015), and fed with the following diets: Control (C), standard diet (Supra Lab – Alisul ind. Alimentos Ltda, São Leopoldo, RS, Brazil); Prebiotic (Pre), standard diet supplemented with 10 $g.Kg^{-1}$ of prebiotic [Mannan oligosaccharide (MOS) - Immunowall® - ICC USA, Inc., Louisville, Kentucky – USA). The Control Smoking (CS) and Prebiotic Smoking (PreS) groups were fed the C and Pre diets, and submitted to the protocol of exposure to cigarette smoke.

To expose the animals to cigarette smoke, an inhalation chamber was used, built based on Cendon's experiments (CAMARGO FILHO et al., 2011). During the experimental period, commercial cigarettes were lit daily, burned, and the smoke was pumped for two half-hour periods, at 8:00 am and 6:00 pm, into a chamber where the rats from the CS and PreS groups were housed. The mean concentrations of tar, nicotine, and CO were analyzed every 15 days during the experimental period (RENNE et al., 2006). The concentrations of tar (11.3±0.1 mg/cigarette), nicotine (0.9±0.1 mg/cigarette), and CO (10.7±0.2 mg/cigarette) were analyzed monthly during the 180-day trial.

During the 180 days of the experimental period, adverse effects, such as diarrhea and death, were monitored, and feces was collected from all animals at four sampling periods (days 0, 60, 120, and 180). Feces were collected from each rat in a laminar flow hood, after previous asepsis of the perianal region with 70% alcohol. To induce defecation, the rats were lifted by the tail. At least one fresh pellet was collected with sterile forceps from each rat and immediately placed in an Eppendorf-type microtube, previously sterilized for 20 minutes under UV in the laminar flow hood.

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Pools of feces were collected in the tubes in order to better represent the diversity of each group, with 5 microtubes being used for each group. Extractions were carried out after collection using the QIAmp DNA Stool Mini Kit from QIAGEN, specific for fecal DNA extraction. The quantification of DNA was performed on a NanoDrop ND-1000 spectrophotometer, NanoDrop Technologies. The evaluation of the intestinal microbiota was performed through real-time PCR of the 16S RNA region corresponding to the Escherichia coli gene. The Real-time PCR was performed using the Microbial DNA qPCR Assays Kit (QIAGEN) according to the manufacturer's recommendations. The reaction was used to measure the number of single copies of the 16S rRNA gene.

2.3 Statistical analyses

When comparing the mean of two groups, the P value was computed with the Mann-Whitney Rank Sum test for nonparametric data. When comparing the mean from three or more groups, the P value was computed using one-way ANOVA followed by Student-Newman-Keuls post-hoc analysis. The analyses were conducted using Biostat 5.3 software, considering 5% as the level of significance (AYRES, 2007).

3 RESULTS

In the group of rats only exposed to cigarette smoke, 15 days after the beginning of the experimental period, the feces became pasty and had a bad odor. This aspect continued for remainder of the experimental period, but there were no animal deaths or any other clinical signs. In the groups of animals supplemented with MOS, the feces continued to have a normal consistency and we did not observe clinical alterations in the animals.

The results of the statistical analyses are summarized in (Table 1), together with the cycle threshold (CT) values. The CT value corresponds to the point at which the threshold crosses the amplification line, enabling identification of the minimum number of cycles necessary for amplification of the sequence of interest, being inversely proportional to the amount of target DNA present in the sample. The results showed that at the beginning of the study (day 0) there were no significant differences between the animal groups (P=0.0636) for the *E. coli* population in feces. Sixty days after the beginning of the study, the animals fed a control diet or supplemented with prebiotic and exposed or not to cigarette smoke did not differ from each other (P=04498) in relation to the intestinal population of *E. coli.* After 120 and 180 days, the group of rats not supplemented with prebiotic and exposed to cigarette smoke (CS) differed statistically (P=0.0068 and P=0.0034, respectively) from the other experimental groups (C, Pre, and PreS).

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Table 1. Influence of oral prebiotic supplementation, mannan oligosaccharide (MOS), on the *E. coli* population in rats chronically exposed to cigarette smoke (values expressed as mean ± standard deviation).

Groups	***E. coli* spp. population**				
	Study period, day				***P value**,2**
	Day 0	**Day 60**	**Day 120**	**Day 180**	
Control(C)	32.56 ± 1.23^{Aa}	33.72 ± 1.69^{Aa}	33.10 ± 1.73^{Aa}	34.86 ± 2.06^{Aa}	*P=0.3545*
Control Smoking (CS)	31.52 ± 0.73^{Aa}	36.08 ± 3.15^{Aa}	42.41 ± 2.09^{Bb}	46.52 ± 2.39^{Bb}	*P=0.0027*
Prebiotics (Pre)	31.52 ± 1.47^{Aa}	32.81 ± 2.18^{Aa}	32.94± 1.35^{Aa}	32.72 ± 1.92^{Aa}	*P=0.5230*
Prebiotics Smoking (PreS)	32.56 ± 1.24^{Aa}	34.72 ± 1.85^{Aa}	34.90 ± 1.34^{Ab}	35.81 ± 1.41^{Ab}	*P=0.0436*
P value,1*	*P=0.4861*	*P=0.4498*	*P=0.0068*	*P=0.0034*	

**P value* was computed using one-way ANOVA followed by Student-Newman-Keuls post-hoc analysis. [1]Different superscripts (^{A,B}) denote significant differences between treatments in the same period of time (days) (*P<0.05*). [2]Different superscripts (^{a,b}) denote significant differences between collection times (days) of the same treatment (*P<0.05*). Source: Authors,2024

The results revealed no significant differences (P=0.3545 and P=0.5230, respectively) between the mean fecal *E. coli* concentrations during the 180-day experimental period in the control and prebiotic-treated groups. In the smoking control group, the results revealed a significant increase in the *E. coli* population throughout the experimental period (P=0.0027). The group of rats treated with prebiotic and chronically exposed to cigarette smoke showed a significant increase (P=0.0436) in the *E. coli* population 120 days after the beginning of the experimental period.

The statistically significant decrease in the *E. coli* population in the intestinal microbiota of rats that received prebiotics, and especially in the rats exposed to cigarette smoke, reflects the beneficial effect of prebiotics, including in the microbiota after cigarette interference, suggesting that the damage caused by cigarettes can be modulated through functional nutrition (Figure 1).

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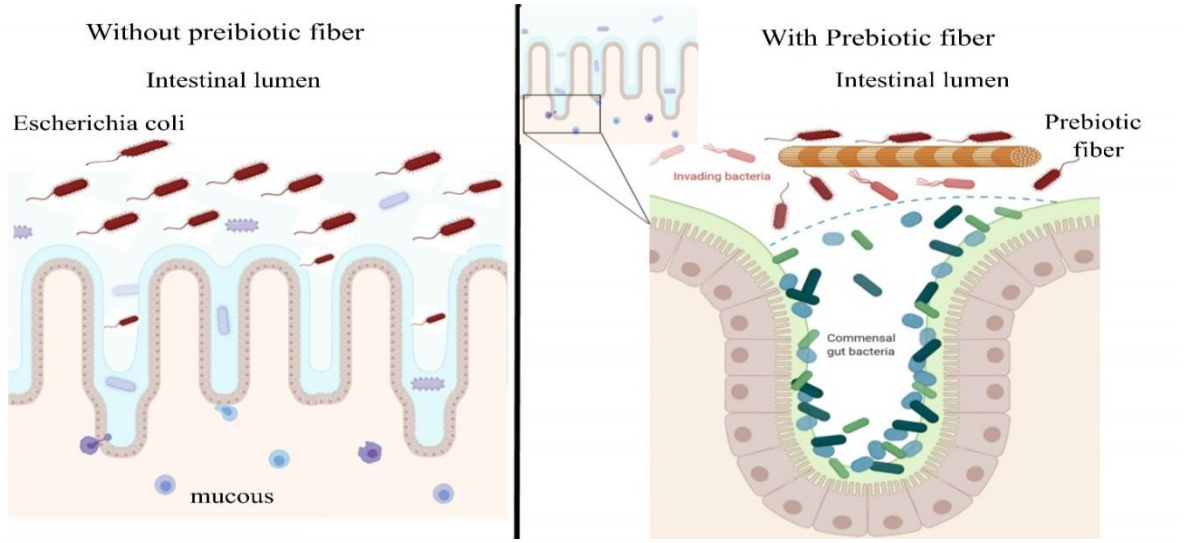
Figure 1. Prebiotic, mannan oligosaccharide, fiber action. Pathogenic microorganisms recognize and adhere to prebiotic fiber; therefore, these oligosaccharides act by adsorbing pathogenic microorganisms to the intestinal epithelium and eliminating them in the feces.



Source: Authors, 2024

4 DISCUSSION

Many chronic conditions, including smoking, have been associated with modifications in the gut microbiota (OHUE-KITANO et al., 2024). In an attempt to remedy the effects of these alterations, functional foods such as prebiotics have shown beneficial effects (KAZEMI et al., 2019; PAIVA et al., 2024; ROMBI *et al.*, 2021; RUFINO et al., 2018). The current work evaluated the effect of oral prebiotic supplementation, mannan oligosaccharide, on the *E. coli* bacteria population in rats chronically exposed to smoke from burning tobacco.

The results revealed in this study confirm that the balance of the intestinal microbiota is highly dynamic, and can be disturbed by many factors, such as dietary habits (prebiotic supplementation, mannan oligosaccharide) and environmental factors (such as cigarette smoke) (FOSTER; MCVEY NEUFELD, 2013; GUI; YANG; LI, 2021; HUANG; SHI, 2019).

Smoke from burning tobacco is a complex chemical mixture, including nicotine, aldehydes, polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and heavy metals, etc., which can be passively inhaled into the lungs as aerosol particles or free in a gaseous state, mainly by children (GHIO et al., 2008; GUI; YANG; LI, 2021; LEE et al., 2018). In the group of rats exposed to cigarette smoke during a period of chronic exposure for 180 days, the results revealed a significant increase in the concentration of *E. coli* bacteria. The toxic elements of cigarette smoke ingested in the gastrointestinal tract induce dysbiosis of the gastrointestinal microbiota, in humans and animals, through different mechanisms, such as antimicrobial activity and regulation of the intestinal microenvironment, leading to changes in the composition of the intestinal microbiota (BERKOWITZ et al., 2019; KOBAYASHI; FUJIWARA, 2013). The main mechanisms by which smoking affects the gut microbiota include the following: raising the pH of the intestinal environment, inducing chronic low-grade

inflammation or inflammation-related diseases by inducing an increased abundance of proinflammatory bacteria, and promoting oxidative stress (MCLEAN; JUN; KOZYRSKYJ, 2019).

More surprisingly, this dysregulation of the microbiota affects not only organs that are in direct contact with smoke, such as the oral cavity, contiguous cavities, and the upper and lower respiratory tract, but, in fact, can also affect more distant organs, such as the intestine, heart, vessels, and genitourinary tract. These observations provide deeper insights into the mechanisms involved in the pathogenesis of smoking-related diseases, suggesting a role for dysbiosis, with a recurring pattern of depletion of beneficial bacterial species and a proliferation of pathogens.

MOS supplementation in the diet of the group of rats not exposed to smoke did not result in any difference in results compared to the control group, fed a control diet, during the experimental period of 180 days. In the group of animals supplemented with MOS and chronically exposed to cigarette smoke, we observed a significant reduction in the mean concentration of *E. coli* in feces in relation to the group of animals only exposed to cigarette smoke. This beneficial effect of MOS supplementation is likely due to the presence in the prebiotic of receptors similar to those expressed in the cells of the hosts' digestive tract and which allow the bacteria to bind to the surface of the prebiotic fiber, saturating its receptors and thus not adhering to the intestinal mucosa, and being excreted (ANAND; MANDAL; TOMAR, 2019; GONZÁLEZ-ORTIZ et al., 2014; MOLIST et al., 2014; TRAN; EVERAERT; BINDELLE, 2018; WEI et al., 2020)

The existence of molecular mimicry between some sites of the prebiotic fiber and the receptors of the host's intestinal epithelial cells was previously suggested, which pathogenic microorganisms recognize and adhere to. Therefore, these oligosaccharides act by adsorbing the pathogenic microorganisms from the intestinal epithelium. This interaction is explained by the presence of numerous receptors in pathogenic *E. coli* pathotypes that, in addition to the host cells, also recognize the oligosaccharides (HUANG; SHI, 2019). This adhesion capacity of *E. coli* is mainly conferred by the specific D-mannose adhesin of type 1 fimbriae (FimH), located at the tip of type 1 fimbriae of strains of this bacterium, which specifically binds to terminal epitopes of glycans with high content of mannosylated glycans conjugated to uroplakin 1a (UP1a), a receptor specifically expressed on the surface of digestive tract or urothelial cells (KATANI et al., 2021; WHELAN; LUCEY; FINN, 2023).

The fact that the reduction in the population of *E. coli* is beneficial to the host, is based on the existence of pathogenic *E. coli* pathotypes, such as uropathogenic *E. coli*, enteropathogenic *E. coli,* and enterohemorrhagic *E. coli*, which are responsible for very serious intestinal and extra intestinal infections that express adhesins related to the pathogenesis of these bacteria and which are not expressed on the surface of commensals *E. coli*. Furthermore, type 1 fimbriae is an important virulence factor for medical device infections, and expression is associated with increased biofilm formation and catheter-associated urinary tract infections. Studies have demonstrated that the type 1 pilus adhesin, FimH, mediates not only bacterial adherence, but also invasion of human bladder epithelial cells (BLUMER et al., 2005; DONLAN, 2001; MARTINEZ, 2000; TRAN; EVERAERT; BINDELLE, 2018).

Furthermore, in animals and humans, mannan oligosaccharides stimulate the structure of the epithelial barrier and the functionality of the intestinal mucosa, increase the

surface area of microvilli and the number of goblet cells in the small intestine, and stimulate the host's immune system and the adsorbent capacity against toxins. In addition, this prebiotic is not toxic when administered orally, even in very large concentrations (FAUSTINO et al., 2023; FIRON; OFEK; SHARON, 1982; MADRIGAL-SANTILLÁN et al., 2007; TORRECILLAS et al., 2015).

One of the major limitations of this study was the lack of evaluation of other members of the intestinal microbiota. In addition, metabolome and metagenomics analyses were not performed. However, the data provided by this study showed that prebiotic fiber was able to modulate one of the main bacterial species that colonize the intestinal microbiota, and could be a potential modulator for conditions that cause alterations in the intestinal microbial population, such as passive exposure to cigarette smoke, which mainly affects the most vulnerable, children and older adults.

Our data can be applied to other studies that more specifically evaluate the role of prebiotics in modulating the composition of the intestinal microbiota.

5. CONCLUSION

The use of the prebiotic mannan oligosaccharide supplemented in the diet reduced the concentration of *E. coli* in feces and at the same time mitigated the adverse effect, diarrhea, in young rats chronically exposed to cigarette smoke. The supplemented dose of MOS prebiotic did not cause diarrhea or any other adverse clinical effects.

Even though we are far from using prebiotics as a systematic and rational therapeutic approach, we can speculate that modulating the microbiota could help in the prevention and treatment of some diseases in the future.

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7. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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