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Comparison of high fructose-induced cardiometabolic impairments in two different rat hypertensive models

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ABSTRACT

Over the last half century fructose has become an important component of the human diet in the industrialized countries, mainly in the form of added sugars which are added to food during its manufacture and processing. Recently it was shown that high fructose intake might evoke some cardiometabolic aberrations in predisposed individuals. In this study we assessed the effects of fructose administration to rat strains with different levels of cardiovascular impairment. High doses of fructose (as 10% solution replacing drinking water) were administered for eight weeks to 12-week-old male rats that include normotensive Wistar rats, Wistar rats receiving 40 mg/kg/day of N^G-nitro-L-arginine methyl ester to evoke nitric oxide (NO) deficiency, and spontaneously hypertensive rats (SHR). We found that fructose administration induced an increase in plasma glucose and triglycerides, elevation of blood pressure, and impairment of arterial endothelium-dependent relaxation in normotensive Wistar rats and in SHR but not in hypertensive NO-deficient rats, when compared to their respective control (non-fructose drinking) groups. Treatment with fructose led to the reduction of arterial contractile force in response to noradrenergic stimulation in SHR and in hypertensive NO-deficient rats but not in normotensive Wistar rats. Our results show that fructose consumed in excess evokes some features of metabolic syndrome in normotensive rats and SHR at similar degrees and that deficiency of NO in rats prevents the development of most of the abnormalities induced by fructose administration.

KEYWORDS: fructose, hypertension, conduit artery, nitric oxide, sympathetic nervous system

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INTRODUCTION

In recent years clinical and experimental studies have confirmed an important contribution of several environmental (lifestyle-related) factors to the development of hypertensive state or to its more intensive manifestation in genetically predisposed individuals. The nutritional factors represent one particular category and many recommendations exist with regard to foods and nutrients that are suitable or unsuitable for patients exhibiting various levels of cardiovascular impairment [1, 2]. Fructose, a monosaccharide occurring naturally in fruits and vegetables but consumed by humans mainly in the form of added sugars, has been proved to have many adverse effect on health during its excessive intake. This sugar is much more lipogenic than glucose when consumed in high doses, and it also elevates the concentration of uric acid in blood. Such adverse properties arise from the specific metabolism of fructose which takes place predominantly in the liver. Many studies demonstrated that this might lead to the development of the metabolic syndrome features including insulin resistance and cardiovascular impairment [3-7]. Long-term high fructose intake causes dysfunction of vascular endothelium associated with lower bioavailability of nitric oxide (NO) and with overproduction of vasoconstrictoric substances [8-10], increases production of reactive oxygen

species [11, 12], and also potentiates the activity of sympathetic nervous system and renin-angiotensin system [13, 14]. These disturbances are often connected with elevation of blood pressure, which was confirmed in humans as well as in experimental animals consuming fructose in excess [15, 16]. However, there are some investigators who showed that the increase in blood pressure in animals after fructose feeding is not significant [17, 18]. Such discrepancies may be a consequence of using different methodological approaches and animal strains in experiments, and also of the diverse susceptibility of individuals to cardiovascular impairment due to high fructose intake [19].

The goal of this study was to assess and compare the extent of cardiometabolic impairment due to high fructose feeding in normotensive and genetically hypertensive rats. We also aimed to evaluate the necessity of functional NO system in these effects, by studying the changes of selected cardiovascular and metabolic parameters after fructose administration in NO-deficient rats.

MATERIALS AND METHODS

The animal protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and were approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic. Twelve-week-old male normotensive Wistar and spontaneously hypertensive rats (SHR) were randomly divided into the following groups: individuals receiving fructose (as 10% solution replacing drinking water) for the period of eight weeks (up to 20th week of age), and the age-matched control rats receiving just tap water. In another group of Wistar rats, N^{G}-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day, administered for eight weeks in drinking water or in fructose solution) was used to induce NO deficiency. All rats were housed at 22-24 °C on a 12:12-h dark-light cycle (06.00-18.00 h lights on) and maintained on a standard laboratory rat chow *ad libitum*.

During the experimental period (*i.e.* from 12th to 20th week of life of rats) systolic blood pressure was measured in conscious animals by the non-invasive tail-cuff method. At the end of the treatment, rats were sacrificed under CO_2 anesthesia, and the relative left heart ventricle weights and liver weights (as the ratio of left heart ventricle or liver weight to body weight) were determined and samples of their blood were collected and used for measurement of plasma glucose and triglyceride concentration.

The selected conduit arteries (thoracic aorta and superior mesenteric artery) were removed and prepared for isometric tension recording. The arteries were cut into rings (3.0-3.5 mm in width) and suspended in 20 ml organ baths with oxygenated (95% O_2 + 5% CO_2) modified Krebs solution maintained at 37 °C. The Krebs solution had the following composition (in mmol/l): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, and $CaNa_2$.EDTA 0.03. The arterial rings were set up for isometric tension recording using a force-displacement transducer Sanborn FT 10 (Sanborn, Baltimore, USA). The preparations were equilibrated under a resting tension of 10 mN for 60-90 min, and the solution was changed every 15 min.

To examine the endothelium-dependent vasorelaxation, the preparations of aorta were first precontracted by phenylephrine (10^{-6} mol/l). When the contraction reached a plateau, increasing concentrations of acetylcholine (10^{-9}-10^{-5} mol/l) were applied in a cumulative manner.

Adrenergic contractions in endothelium-intact mesenteric arteries were determined as the responses to cumulatively applied exogenous noradrenaline (10^{-10}-10^{-5} mol/l) or as the neurogenic responses elicited by electrical stimulation of periarterial sympathetic nerves. The arterial rings were stimulated by two parallel platinum plate electrodes placed on either side of the preparation and connected to an electrostimulator ST-3 (Hungary). Frequency-response curves to electrical stimuli were obtained using square pulses of 0.5 ms in duration, at supramaximal voltage (> 30 V), applied at 1-32 Hz, for a period of 20 s. The contractions of rat mesenteric arteries elicited by electrical stimulation (using the described parameters of stimulation) are blocked by phentolamine or tetrodotoxin, indicating that they are induced mainly by nerve-released (endogenous) noradrenaline.

In mesenteric arteries, contractions to 100 mmol/l KCl were also determined.

The results are presented as means ± S.E.M. Arterial isometric responses to respective doses of

pharmacological substances or to particular frequencies of transmural electrical stimulation were expressed as absolute value in mN and they were normalized to the cross section area of the respective ring preparation. Area under curve (AUC, in arbitrary units) was calculated from concentration (frequency)-response curves in each experimental group using the rectangular rule for numerical integration, according to Pruessner *et al.* [20].

Statistical evaluation was carried out by using one-way analysis of variance (ANOVA). The results were considered to be significant when $p < 0.05$.

RESULTS

Eight-week-lasting fructose administration caused body weight increase in SHR but not in normotensive Wistar rats and NO-deficient (hypertensive) Wistar rats (Fig. 1A). High-fructose-induced elevation in relative liver weight (Fig. 1B) and in plasma concentrations of glucose (Fig. 1E) and triglycerides (Fig. 1F) was observed in SHR as well as in normotensive Wistar rats; however, none of these parameters was changed in NO-deficient rats when compared to their respective control groups.

High fructose feeding led to increase in blood pressure (Fig. 1C) and impairment of acetylcholine-induced endothelium-dependent relaxation of thoracic aorta (Fig. 2A) in SHR and in normotensive Wistar rats but not in NO-deficient rats. Relative weight of left heart ventricle was not influenced in any experimental group (Fig. 1D).

Diminution in adrenergic contractions (in response to endo- or exogenous noradrenaline) due to high fructose intake was observed in mesenteric artery of SHR. In NO-deficient rats, only the responses of mesenteric artery to exogenous (but not endogenous) noradrenaline were found to be reduced by fructose administration. The adrenergic contractions of arteries from normotensive Wistar rats were not significantly changed (Figs. 2B, D). Contractile responses of mesenteric arteries to excitation by high K^+ concentration in bath solution (100 mmol/l KCl) were not influenced after fructose administration in any experimental group (Fig. 2C).

DISCUSSION

In this study we confirmed that high fructose consumption leads to important metabolic changes that may evoke some features of metabolic syndrome in adult Wistar rats. The observed impairments included enlargement of liver together with increase in plasma concentration of glucose and triglycerides, diminution in arterial endothelium-dependent relaxation, and elevation of systolic blood pressure. Similarly, we detected the manifestation of these alterations in genetically/spontaneously hypertensive rats. In SHR, moreover, weight gain and decreased arterial contractile responses to noradrenaline were observed after fructose feeding.

The mild but significant blood pressure increase in normotensive as well as in hypertensive rats (SHR) after eight weeks of fructose administration was not accompanied by increase in relative left heart ventricle weight. However, weaker relaxant responses to acetylcholine were detected in isolated thoracic aortas from Wistar rats and SHR treated with fructose. We assume that the fructose-induced impairment of endothelium-dependent relaxation might lead to the rise in vascular resistance and contribute to the observed elevation of blood pressure in these rats. Similarly, other studies showed that vascular relaxation is reduced after feeding normal rats with excess of fructose and this is associated with decreased endothelial production of NO and elevated blood pressure [8, 9]. It is supposed that the high plasma concentration of uric acid produced in the liver during fructose over-consumption plays an important role in decreasing the NO bioavailability and this mechanism might also contribute to the development of type 2 diabetes and hypertension in subjects with metabolic syndrome [16, 21, 22]. Large amount of uric acid eliminates NO by several ways including its direct scavenging [23], increasing of oxidative stress [11, 24, 25], or by stimulation of arginase [26]. Furthermore, Corry *et al*. [25] demonstrated that uric acid stimulates the proliferation and production of angiotensin II in vascular smooth muscle cells. The described alterations are responsible for endothelial dysfunction and prevalence of vasoconstriction, leading to increase in vascular resistance and in blood pressure. Decreased amount of endothelial NO and reduced blood flow to skeletal muscle and peripheral tissues deteriorate insulin action and glucose uptake by the cells and may lead to insulin resistance. Compensatory hyperinsulinemia can stimulate sympathetic nervous

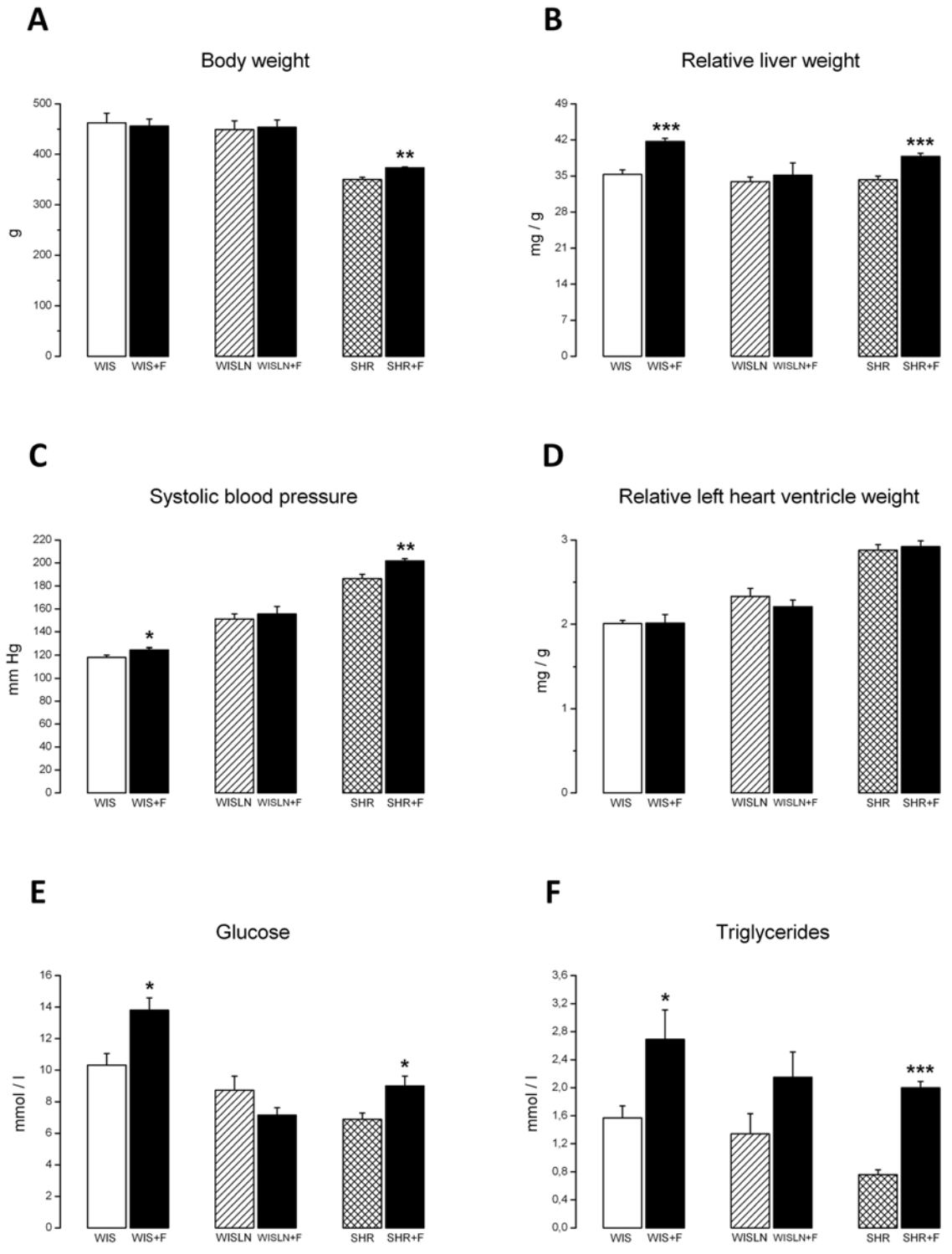

Fig. 1. Effect of eight-week-lasting high fructose intake (*+F*) on selected cardiovascular parameters in normotensive Wistar rats (WIS), NO-deficient (L-NAME-treated) Wistar rats (WISLN), and spontaneously hypertensive rats (SHR). Data are presented as means ± SEM obtained from six to ten different animals in each experimental group. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to respective control (non-fructose drinking) groups of rats.

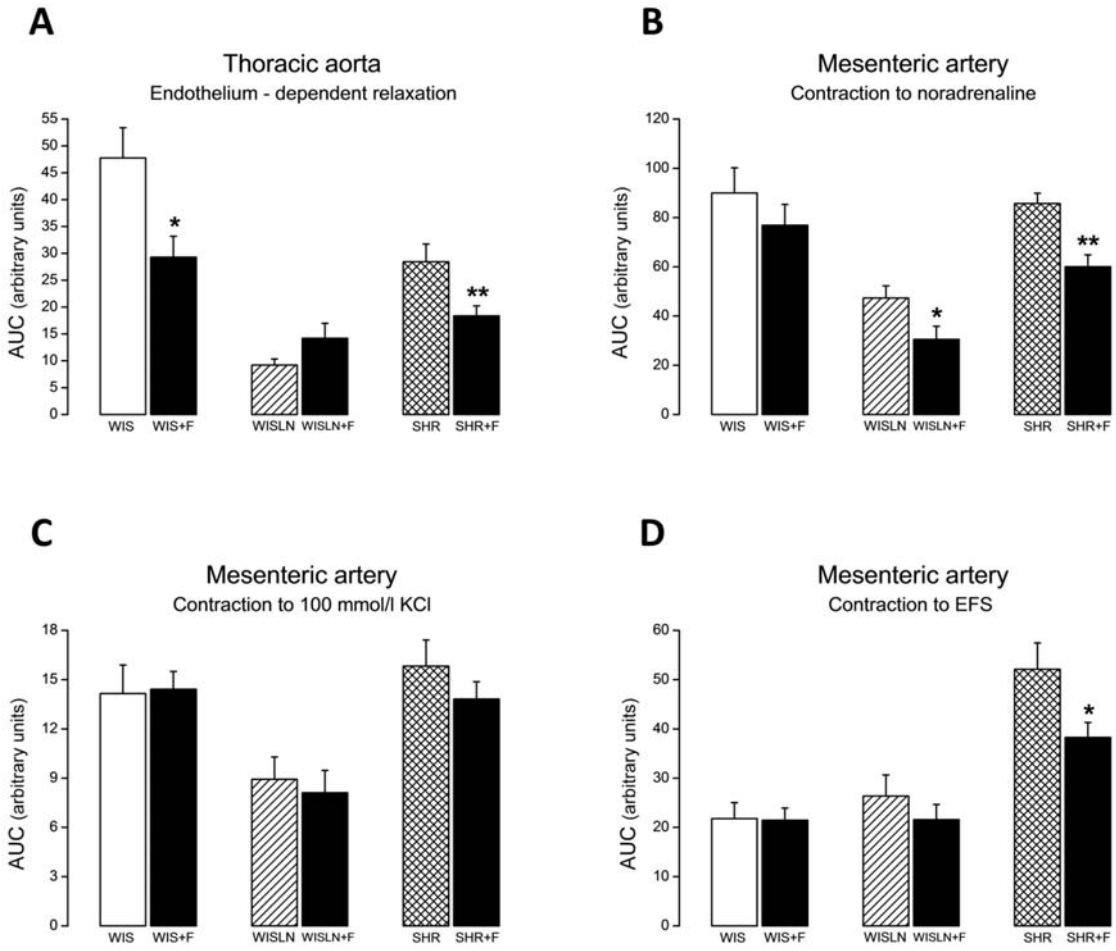

Fig. 2. Effect of eight-week-lasting high fructose intake (+*F*) on arterial reactivity in normotensive Wistar rats (WIS), NO-deficient (L-NAME-treated) Wistar rats (WISLN), and spontaneously hypertensive rats (SHR). Data are presented as means ± SEM obtained from six to ten different animals in each experimental group. *$p < 0.05$ and **$p < 0.01$ compared to respective control (non-fructose drinking) groups of rats.

system activity and sodium reabsorption in the kidney, changes which also promote elevation of blood pressure [27].

Nitric oxide deficiency in Wistar rats caused severe hypertension and multiple impairments in arterial functions; however, when L-NAME-administered rats were simultaneously fed with high amounts of fructose, no further aberrations were seen (in contrast to fructose-induced alterations detected in L-NAME-untreated Wistar rats or in SHR). This may indicate that when there is no basal level or reserve in NO production, most of the changes due to high fructose consumption do not manifest. On the contrary, in adult SHR, in spite of their high blood pressure and diminished endothelium-dependent relaxations in conduit arteries, the NO synthase activity is elevated and the resting vasorelaxation is sufficiently NO-dependent [28]. This may be the reason why we could detect further impairment in acetylcholine-induced response and elevation of blood pressure after high fructose feeding in SHR similar to that in normotensive Wistar rats.

Eight-week-lasting fructose administration to normotensive Wistar rats had no effect on contractile responses of their mesenteric arteries induced by exogenous or endogenous noradrenaline. On the other hand, in fructose-fed SHR, we unexpectedly detected the reduction of both exogenous noradrenaline-induced contractions as well as neurogenic contractions. Kamata *et al*. [29] also observed the

decrease in arterial noradrenaline contractions in fructose-fed mice and they proposed that this effect could be mediated through elevation of NO production induced by increased activity of the GTP-binding protein coupled to endothelial alpha-2 adrenoreceptors. However, our results showed that despite the conditions of strong inhibition of NO generation in L-NAME-treated rats, there is a significant decrease in contractile force of mesenteric artery in response to noradrenaline after long-term fructose consumption.

Bunnag *et al*. [30], presenting similar observations of reduced arterial contractions to adrenergic stimulation in fructose-fed rats, explained this finding as the reactive response to enhanced sympathetic activity. Such idea could elucidate our observations with SHR; it is well known that these rats have genetically determined sympathetic hyperinnervation which significantly contributes to the elevation of their blood pressure even during their juvenile period [31]. After high fructose feeding which leads to further increase in sympathetic efferentation [13, 32], the adaptive reaction of specific physiological systems may cause the decline in adrenergic responses of certain cells and tissues. This could explain the reduction of adrenergic responses in mesenteric arteries from fructose-fed SHR detected in our experiments. It is also in accordance with the finding of fructose-induced decrease in the development of contractile force in mesenteric arteries in response to noradrenaline in NO-deficient rats. As L-NAME treatment is associated with augmentation of sympatho-adrenergic system [33], after its further potentiation with high fructose intake we could again expect the negative adaptation of adrenergic receptor system to these excessive stimuli. However, while in NO-deficient rats this adaptation seems to involve only postsynaptic processes (seen as a reduction in response to exogenously applied noradrenaline), in SHR, it might affect the adrenergic nervous system at the presynaptic level as well because the neurogenic contractions were also decreased after fructose treatment in these rats.

Since the contractile responses of mesenteric arteries to nonspecific depolarisation by high potassium in all fructose-fed rat groups were not changed, we can suppose that the mechanical contractility of arterial smooth muscle is not impaired and that the sympatho-adrenergic system could be selectively influenced due to fructose treatment.

In our study we demonstrated strong lipogenic and pro-diabetic consequences of high fructose consumption in normotensive rats as well as in SHR. Our finding of increased liver mass supports the idea of the link between fructose intake and development of non-alcoholic fatty liver disease which has been indicated recently [34]. Moreover, we have found that inhibition of NO production by treating rats with L-NAME eliminated the high fructose-evoked increase in liver weight and in plasma concentration of glucose and lipids. It is known that NO is implicated in many pathways regulating liver metabolisms and its higher amount may have potential for both protection of the liver as well as exacerbation of its injury [35]. According to the results of some authors, inducible NO synthase (iNOS) seems to be the key factor in this process [36]. In mice receiving high amounts of fructose [36] or in rats with cholestasis [37], various parameters indicating the status of inflammation and fibrosis in the liver were ameliorated after treatment with unspecific NO synthase inhibitor like L-NAME, and they were not detected in iNOS knockout mice. This may partially elucidate our observations of the failure of fructose administration in inducing the abnormalities in liver and in plasma parameters of NO-deficient rats.

CONCLUSION

In conclusion, we demonstrated that eight-week-lasting excessive fructose consumption in normotensive Wistar rats and in SHR leads to similar cardiometabolic alterations; however, weight gain and decreased adrenergic contractile responses in mesenteric arteries were detected only in SHR. Nitric oxide deficiency prevented the occurrence of abnormalities observed during fructose administration in normal Wistar rats. Therefore, our results show that deterioration of NO system could contribute to most of the adverse effects induced by high fructose intake.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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