The effects of a 50-Hz magnetic field on the cardiovascular system in rats

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ABSTRACT

A 50-Hz magnetic field (MF) is a potential health-risk factor. Its effects on the cardiovascular system have not been fully investigated. This study was conducted to explore the effects of long-term exposure to a 50-Hz MF on the cardiovascular system. In the study, an exposure system was constructed, and the distribution of the 50-Hz MF was determined. Sixty-four Sprague-Dawley (SD) rats were exposed to a 50-Hz MF at 100 μT for 24 weeks, 20 h per day, while another 64 rats were sham exposed. During the exposure, blood pressure was measured every 4 weeks. After 24 weeks, echocardiography, cardiac catheterization and electrocardiography were performed. Moreover, heart and body weight were recorded, and haematoxylin–eosin staining and real-time PCR were conducted. The results showed that compared with the sham group, exposure to a 50-Hz MF did not exert any effects on blood pressure, pulse rate, heart rate or cardiac rhythm. Furthermore, echocardiography and cardiac catheterization showed that there were no significant differences in the cardiac morphology or haemodynamics. In addition, histopathological examination showed that exposure to a 50-Hz MF had no effects on the structure of the heart. Finally, expression of the cardiac hypertrophy–related genes did not show any significant differences between the 50-Hz MF exposure group and the sham group. Taken together, in SD rats, exposure to a 50-Hz/100 μT MF for 24 weeks did not show any obvious effects on the cardiovascular system.

KEYWORDS: 50-Hz magnetic field, cardiovascular system, rat

INTRODUCTION

With increasing reliance on alternating current (AC) electricity [1], the opportunity for exposure to time-varying magnetic fields (MFs) has also increased over the past decades. The frequency of MFs is mainly 50 Hz or 60 Hz, and it is widely recognized that MFs are commonly produced by power transmission lines and various AC-powered appliances [2]. Further, a 50-Hz MF is a physical field and is a part of the electromagnetic field (EMF). Once a human’s or animal’s body gets close to this field, a weak electric current will be induced in the body [3]. Although there are currently no known biophysical mechanisms that could potentially lead to health effects from exposure to 50-Hz MFs, research in this area continues to be of interest to the scientific community [4].

In 1979, Wertheimer and Leeper first suggested an association between residential 50-Hz MF exposure and childhood leukaemia, but inadequate justification was provided [5]. As a result, a large number of studies were initiated, focusing on the biological effects of a 50-Hz MF with respect to cancer [6–8] and other adverse health effects (e.g. on reproductive [9–11], central nervous system [12, 13], and cardiovascular system health [14, 15]). The cardiovascular system consists of macrocirculation, microcirculation, the lymphatic system and the heart, which is the most important component. The cardiovascular system is very important to human health because it is in direct contact with body tissues and cells, and is responsible for supplying nutrients and oxygen, while removing metabolites and engaging in thermoregulatory functions [16].
Potential association of exposure to 50-Hz MFs with cardiovascular diseases has previously been studied in humans [17]. These studies have mainly concentrated on two aspects: the effects of 50-Hz MFs on blood pressure and on heart rate [14, 18]. For example, in 1996, Korpinen and Partanen first reported the effects of 50-Hz EMFs on human blood pressure. During the measurements, male volunteers spent 1 h inside the fields, and the results showed that 1 h exposure to 1.4–6.6 μT power-frequency EMFs did not alter their blood pressure [19]. In addition, Graham et al. exposed 24 men to a much higher MF (127.3 μT), and heart rate variability (HRV) measurements from the same individual were compared across three relevant test conditions: intermittent field exposure, continuous field exposure, and control ‘no exposure’. HRV was not altered by either field exposure condition compared with the control condition [20]. Further, there were also several epidemiology studies that reported no effects on the cardiovascular system in workers at high-voltage railway substations [21].

As indicated in the World Health Organization Environmental Health Criteria Monograph No. 238, the evidence did not support an association between MF exposure and cardiovascular diseases [17]. However, most of the studies on the effects of 50-Hz MFs on the cardiovascular system have focused on short-term rather than long-term effects. Actually, investigating the effects of long-term exposure to 50-Hz MF is more important, because the current or the energy induced by the 50-Hz MF cannot break the structure of DNA or exert direct biological effects [4].

Few studies focused on the long-term effects of MFs on the cardiovascular system have been conducted. In 1985, studies carried out by Checcucci et al. found no effects on the cardiovascular system in 1200 persons employed at high-voltage railway substations (4–15 μT) [22]. Moreover, in another health survey of 627 railway high-voltage substation workers, no differences were found in the electrocardiograms (ECGs) between exposed and control groups [23]. The existing research appears to conclude that long-term exposure to 50-Hz MFs exerts no effects on the human cardiovascular system. However, this conclusion needs further investigation, because the majority of these studies are cohort studies, which may have a variety of confounding factors, such as age, gender, other exposure factors and the flux density of the fields.

Therefore, we carried out the present study to explore the long-term effects of 50-Hz MFs on the cardiovascular system. To investigate the potential health effects, we employed rats as subjects. The flux density of 50-Hz MF was not consistent in previous human and rodent studies. Thus, in the present study, the flux density of 50-Hz MF at 100 μT was used according to the guideline proposed by the International Commission on Non-Ionizing Radiation and Protection (ICNIRP) [3, 24] and the State Environmental Protection Administration (SEPA) of China [25]. Moreover, the long-term effects on the cardiovascular system of exposure to 50-Hz MFs was evaluated by many more parameters, including blood pressure, pulse rate, heart rate, cardiac function and cardiac morphology.

MATERIALS AND METHODS

Ethics, consent and permissions

All of the animals used in the study were housed in the Animal Centre of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST, Wuhan, China). The experimental protocols were approved by the Animal Care and Use Committee of HUST, and were performed according to the Principles of Laboratory Animal Care [26], the Office for Protection from Research Risks (OPRR) Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised 2015) [27], and the U.S. Animal Welfare Act 1966 (as amended in 2013) were followed, as well as the specific national laws (i.e. the current version of the Japanese Animal Welfare Law 2013).

Animals

Sixty-four male Sprague-Dawley (SD) rats at the age of 8 weeks were exposed to a 50-Hz/100 μT MF, and another 64 rats were sham exposed. Both groups were exposed/sham exposed for 24 weeks, and for the 50-Hz MF exposure group, the animals were exposed for 20 h per day (12 p.m.–8 a.m.). During the exposure, all of the animals were in good health, and no animal died. The rats were raised under standard laboratory conditions (12 h light/12 h dark cycle, light cycle starting at 7 am with light on; the room temperature remained at 22–25°C, and the relative humidity was 45–55%). All animals were housed in polycarbonate ventilated cages (45 cm × 20 cm × 35 cm, L × W × H) and received irradiated corncob bedding. The cages were changed every two weeks, and the bedding was changed weekly. The food was sterilized with high pressure and the drinking water was sterilized using filtration and ozone. All efforts were taken to minimize the suffering of the animals. Anaesthetization of animals was done with intraperitoneal injections of a xylazine (5 mg/kg) and ketamine (80 mg/kg) mixture, and the rats were placed in a supine position before they were sacrificed. At the end, the animals were euthanized by CO2 inhalation in a professional and compassionate manner by skilled personnel. The rats were sacrificed after 24 weeks of exposure, and cardiac tissue samples were snap frozen in liquid nitrogen for real-time PCR or fixed with 4% neutral formalin for paraffin embedding.

Exposure system

The exposure system consisted of an exposure device and a plastic rack, both of which were made with acrylonitrile butadiene styrene plastic, which is firm and insulated. There were five layers in the exposure device (200 cm x 100 cm x 200 cm, L x W x H). Inside the exposure device, a plastic rack (140 cm x 70 cm x 200 cm, L x W x H) with four floors was set. Bronze wires were used to conduct the current and generate the 50-Hz MF. In order to generate the uniform magnetic field, the wiring ratio of the five coils was 2:1:1:1:2, and the 3% uniformity area for each floor was 150 cm x 50 cm (L x W). The wires were fixed tightly to the framework in order to decrease the vibration and the noise. When the system was powered on, the sound from the exposure system and the temperatures of the living conditions were close to those in the environment, and the conditions for the animals living inside it were considered normal. In the present study, we constructed two systems, one used for 50-Hz MF exposure and the other one for sham exposure. For each system, there were two sets of bronze wires inside the framework. However, the combinations of the wires differed between the systems. For the 50-Hz MF exposure group, the two sets of wires had current flowing in the same direction, whereas the currents were in opposite directions.
Table 1. Primers for the cardiac hypertrophic markers

| Primer  | Forward                  | Reverse                  |
|---------|--------------------------|--------------------------|
| ANP     | 5'-GCTTCGGGGGTAGGATTGAC  | 3'-TCTCAGTGCAATGGCCAGA   |
| BNP     | 5'-TCCAAGATGCGACATGTTCAAG| 3'-CAACCTCAGCGTCACAG     |
| α-MHC   | 5'-CAGAAAATGCAGATGGAGA   | 3'-GCATTGATTTATGGG       |
| β-MHC   | 5'-CCCTTCCTGGCAAATCTGA   | 3'-ACAGTCACCGTCTGCCATTC  |
| GAPDH   | 5'-GGCAAGTTCACGGCAGAG    | 3'-CGCCAGTAGACTCCACGACAT|

ANP = atrial natriuretic peptide, BNP = brain natriuretic peptide, α-MHC = alpha myosin heavy chain, β-MHC = beta myosin heavy chain, GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Fig. 1. Distribution of the 50-Hz MF. (A, B) Perspective views of the exposure device. The size of the exposure device was 2000 mm × 1000 mm × 2000 mm (L × W × H), and the layer-to-layer distance was 350 mm. (C) The distribution of MF was measured with Narda efa-300 as the plotting map. For each point, three measurements were obtained, and the mean value was used to draw the distribution map. (D) The distributions of the 50-Hz MF in the rack. Inside the exposure device, a plastic rack (1400 mm × 700 mm × 2000 mm, L × W × H) with four floors was set; the distribution of the 50-Hz MF in the rack was uniform. (E) The flux density of the sham exposure system was measured at ~100 nT (close to the background level).
in the sham-exposure device. When powered on, the device of the 50-Hz MF exposure group would produce a uniform 50-Hz MF, whereas the sham group did not produce a MF inside the system. Further, the two systems were located in separated and non-adjacent rooms, which had the same room conditions (lacking other electric devices, e.g. computers). Moreover, the daylight lamps were kept far away from the system. An alternating current power supply (220 V/50-Hz) and a TDGC2 transformer (Mushidq, Shanghai, China) were used. The voltage of the exposure device was adjusted to 35 V, which maintained the intensity of the 50-Hz MF at 100 μT, and the distribution was determined with Narda efa-300 (Narda, Pfullingen, Germany); the degree of the uniform magnetic field in the plastic rack space [140 cm × 70 cm × 200 cm (L × W × H) turned out to be 5.2%]. In total, 24 plastic cages (45 cm × 20 cm × 35 cm, L × W × H) were placed on the rack, and each cage housed two or three rats.

Echocardiography
After 24 weeks of exposure, echocardiography detections were performed. Briefly, after anaesthesia, the rats were tied, and the fur on their chests was removed with a pet razor. Then, echocardiography was carried out using a Vivid 7 ultrasound machine (GE Medical Systems, Milwaukee, WI) with a 3-MHz probe, as described previously [28]. The thickness of the left ventricular wall and the left ventricular internal diameter both in the systole and diastole, as well as the ejection fraction and the fractional shortening, were recorded.

Haemodynamics
Cardiac haemodynamics were evaluated with the Millar pressure–volume system (MPVS) (ADInstruments, New South Wales, Australia). The protocols were described previously [29]. In brief, after anaesthesia, the animals were tied, and the right common carotid artery was separated. Then, the distal was obligated and the proximal was clamped. An appropriate incision was made, and the cardiac catheter was inserted into the artery and put forward into the heart after the clamp was loosened. The data were recorded for at least 15 min. The maximum rate of the left ventricular diastolic pressure change (Dp/Dt maximum), the minimum rate of the left ventricular diastolic pressure change (Dp/Dt minimum), the maximum pressure, the end-diastolic pressure, and the contractility index were recorded.

Electrocardiography
The electrocardiography was carried out after 24 weeks of exposure. The rats were anaesthetized and tied. The limb leads were placed on
the right forelimb (negative electrode), left forelimb (positive electrode) and right hind leg (neutral electrode). Electrocardiographic changes were recorded using an 8-channels Power Lab System (ADInstruments) [30].

**Blood pressure measuring**
The blood pressure was recorded every 4 weeks using the Softron BP-98A system (Softron Biotechnology, Beijing, China). Before measuring, the rats were fixed using the rat holder, which kept the tail outside and maintained the temperature at 37°C. When the animals were calm, the blood pressure was measured. For each rat, the measurements were repeated at least three times. The systolic pressure, the diastolic pressure, the mean pressure and the pulse rate were recorded.

**Haematoxylin–eosin staining**
After the rats were sacrificed, their hearts were separated, fixed with 4% neutral formalin, dehydrated and prepared as paraffin sections. The haematoxylin–eosin (HE) staining kit (Jiancheng Bioengineering Institute, Nanjing, China) was employed, and the protocol complied with the manufacturer's instructions. The images were taken using the Nikon TE2000-U microscope (Nikon, Tokyo, Japan).

Quantitative reverse-translation real-time PCR
The total RNA was extracted from the cardiac tissues with the Trizol reagent (Invitrogen, Carlsbad, CA), and reverse translated to cDNA by using the reverse-translation PCR kit (Thermo Fisher Scientific, Waltham, MA). Subsequently, the cDNA was used as the template, and the specific primers, as well as SYBR Green (Thermo Fisher Scientific, Waltham, MA), were used to detect the expression of the targets on the 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA). We used five primers: β-MHC, α-MHC, BNP (brain natriuretic peptide), ANP (atrial natriuretic peptide) and GAPDH. The sequences of these primers are listed in Table 1.

**Statistical analysis**
All data are presented as mean ± standard distribution (S.D.). For the statistical analyses of blood pressure and pulse rate, repeated-measures analysis of variance (ANOVA) was used, whereas one-way ANOVA was used for other data via PASW Statistics 19 software (IBM Corporation, Armonk, NY). A P value of <0.05 was considered statistically significant.

**RESULTS**
The distribution of the 50-Hz MF was uniform
Before the start of the study, we constructed two exposure system, the 100 μT/50-Hz MF exposure system and the sham exposure system. The size of the system was 2000 mm × 1000 mm × 2000 mm (L × W × H), and the layer to layer distance was 350 mm (Fig. 1A and B). Moreover, the distribution of the 50-Hz MF in the rack (140 cm × 70 cm, L × W) of both systems was measured. As shown in Fig. 1C and 1D, the flux density of the 100 μT/50-Hz MF exposure system was ~100 μT and the deviation was 4.2 μT (<5%), suggesting that the distribution of the 50-Hz MF was uniform. In addition, the intensities of the 50-Hz MF were similar among floors, and the deviation among floors was 1.7 μT against the mean. Another exposure system without the 50-Hz MF was also constructed as the 'sham exposure system'. The intensity of the MF was 103 ± 8.2 nT, which was close to the background field intensity (Fig. 1E).

Exposure to a 50-Hz MF had no effects on blood pressure
We included 128 rats in the study. The rats were randomly assigned to one of two groups. For 24 weeks, one group was subjected to a 50-Hz MF and the other received sham exposure. Every 4 weeks, the blood pressure and pulse rate of all rats were recorded. Results showed that the systolic blood pressure, diastolic blood pressure, mean blood pressure (Fig. 2A–C) and pulse rate (Fig. 2D) did not differ significantly between the two groups.

Exposure to a 50-Hz MF had no effects on cardiac rhythm
Cardiac electrical activity is important in maintaining normal cardiac function. Malignant arrhythmia may cause sudden death, whereas chronic cardiac arrhythmia may cause ventricular remodelling, leading to heart failure. Thus, the rats were subjected to electrocardiogram examination. As shown in Fig. 3A, the electrocardiographic waves were similar between the two groups, and no obvious arrhythmia was observed in either group. In addition, the heart rate of the rats from...
the 50-Hz MF exposure group showed no significant differences compared with the heart rate of the control group (Fig. 3B).

**Exposure to a 50-Hz MF had no effects on cardiac function**

After 24 weeks of exposure, the rats’ cardiac functions were assessed by using echocardiography and cardiac catheterization. As shown in Fig. 4A–C, neither the ejection fraction nor fractional shortening were affected by exposure to a 50-Hz MF. Moreover, we analysed the data on wall thickness, including the interventricular septum (IVS), left ventricular posterior wall (LVPW), and left ventricular interior diameter (LVID) in both systole and diastole. Results revealed that compared with control rats, rats exposed to a 50-Hz MF showed no significant differences in the LVID, IVS or LVPW in either systole (Fig. 4D–F) or diastole (Fig. 4G–I).

![Fig. 4](image-url)

Fig. 4. Exposure to a 50-Hz MF showed no effects on echocardiography. (A) The rats from both groups showed similar manifestations of echocardiography. (B) The ejection fraction of the heart from the two groups was similar. (C) Similarly, no significant differences were observed in the fractional shortening of the heart. (D–F) Neither the left ventricular internal diameter (LVID) nor the thickness of the interventricular septum (IVS) or the posterior wall (PW) in the end systole were affected by exposure to a 50-Hz MF. (G–I) Neither the LVID nor the thickness of the IVS or the PW in the end diastole were influenced by a 50-Hz MF exposure. Error bars indicate the standard deviation (S.D.) of the mean, n = 64.
Moreover, cardiac catheterization was carried out to further explore the effects of a 50-Hz MF on cardiac function. Consistently, as shown in Fig. 5A and B, the Dp/Dt maximum and Dp/Dt minimum showed no significant differences between the two groups. Similarly, the maximum left ventricular pressure, as well as the end-diastolic pressure, showed no significant differences between the two groups (Fig. 5C and D). The contractility index of the exposed rats calculated by the MPVS did not differ statistically compared with the sham rats (Fig. 5E).

**Exposure to a 50-Hz MF had no effects on the cardiac histological morphology**

After 24 weeks of exposure, the rats were sacrificed and the hearts were collected and weighed. The results showed that the ratio of heart weight to body weight exhibited no significant differences between the two groups (Fig. 6A and B). Moreover, morphology in the coronal section and cross-section of the hearts showed that a 50-Hz MF had no effects on the tissue structure of hearts (Fig. 6C and D). Further, the morphology of the cardiac myocytes and their arrangement were not affected by the exposure to a 50-Hz MF, as was observed in the HE staining (Fig. 6E). In addition, the expressions of the genes involved in cardiac hypertrophy were determined. The results showed that there was no statistical difference in the expressions of ANP, BNP, β-MHC and α-MHC in the hearts of the exposed rats compared with in the hearts of the sham-exposed rats (Fig. 6F).

**DISCUSSION**

In the present study, we evaluated the effects of a 50-Hz MF at 100 μT exposure on the cardiovascular system in rats, including the blood pressure, cardiac rhythm, cardiac function, cardiac histological morphology and expressions of cardiac hypertrophy–related genes. The results showed that exposure to a 50-Hz MF had no effects on the blood pressure or pulse rate. Moreover, the results from the electrocardiography showed that a 50-Hz MF exposure did not influence the cardiac rhythm. Similarly, no significant difference was observed in the cardiac function between the two groups. Most importantly, neither the cardiac histological morphology nor the expression of cardiac hypertrophy–related genes were affected by exposure to a 50-Hz MF.

In our study, we constructed an exposure system with five-rectangle coils by ourselves; it was well designed and the MF generated by our system was uniform. Actually, various systems have been designed for the MF generation. First of all, most of the systems were designed with three to five coils. Although it was suggested that the optimal four-coil design is superior to the three- and

![Fig. 5. Exposure to a 50-Hz MF had no effects on cardiac haemodynamics. (A, B) The maximum (A) and the minimum (B) rates of the left ventricular diastolic pressure change (Dp/Dt) were not affected by exposure to a 50-Hz MF. (C) Exposure to a 50-Hz MF had no effects on the maximum left ventricular pressure. (D) The left ventricular end-diastolic pressure showed no differences between the two groups. (E) The contractility index of the heart was not influenced by exposure to a 50-Hz MF. Error bars indicate the standard deviation (S.D.) of the mean, n = 64.](image-url)
five-coil systems [31], five-coil systems were also suitable for scientific research. For example, Tsukasa Shigemitsu et al. reported a five-coil system in 1993, which produced a uniform MF (2%) [32].

Second, it was reported that most of the exposure systems were circular or square. However, there were other studies that applied rectangular exposure systems. It has also been reported that the Electric Power Research Institute (EPRI) used a rectangular system in 1993, which was more flexible and easier to construct [33].

The effects of MF on the blood pressure and heart rate have been explored previously by other studies [14]. In 1996, Korpinen and Partanen were the first investigators to report the effects of 50-Hz MF on human blood pressure [19]. After 1 h exposure to MF with a flux density of 1.4–6.6 μT, the blood pressure was not altered as compared with the pre-exposure pressure. Following that, Ghione et al. exposed human participants to 50-Hz MF with a flux density of 40 or 80 μT for 90 minutes, and no effects on heart rate was observed [18]. These data are consistent with the present study.

However, there are several studies which report that exposure to 50-Hz MF may induce harmful effects on the cardiovascular system. For example, Korpinen et al. studied the effects of 3 h of exposure to 50-Hz/1.4–6.6 mT MF on the human heart. Their findings suggested that a small decrease in heart rate was observed in some cases after field exposure [34].

Interestingly, we observed that the flux density of a 50-Hz MF might be the explanation for the conflicts among these studies. For those that reported positive results, the flux densities of MFs were always very high, even higher than the occupational limitation (1 mT) recommended by the ICNIRP in 2010 [3]. In the present study, the flux density of the MF we used was set at 100 μT. As expected, exposure to a 50-Hz MF showed no effects on the blood pressure or heart rate of any of the rats. More importantly, no arrhythmia was observed in either group.

More importantly, we explored the effects of 50-Hz MFs at 100 μT on cardiac function for the first time. To make our results

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**Fig. 6.** Exposure to a 50-Hz MF showed no effects on the histological morphology of the heart. (A) The globe view of the hearts; the scale bar is 1 cm. (B) The ratio of the heart weight to the body weight was calculated, and there was no difference between the two groups. (C, D) The coronal and cross-section views of the hearts. (E) HE staining of the hearts was conducted and showed that the structure of the heart was similar in both groups. The images were taken with a magnification of ×200. (F) Gene expression of ANP, BNP, α-MHC and β-MHC in mRNA levels were not influenced by exposure to a 50-Hz MF. Error bars indicate the standard deviation (S.D.) of the mean, n = 64.
more convincing, we employed both echocardiography and cardiac catheterization in assessing the cardiac function. The echocardiography was used to detect alteration in the structure of the heart, whereas cardiac catheterization focused on the haemodynamics of the heart. Notably, the results revealed that the cardiac function from both examinations showed no differences between the 50-Hz MF exposure group and the sham-exposure group. This is important evidence for function evaluation of the cardiovascular system because the heart is the most important component of the cardiovascular system.

Taken together, our work suggests that long-term exposure to a 50-Hz MF at 100 μT has no effects on the cardiovascular system in rats. The results from our study are better supported and generalizable than those of the previous studies for the following reasons. First, the materials we used to construct the device and animal cages were well insulated, and the generated 50-Hz MF was stable and uniform; thus, our experimental method would have been more rigorous compared with the methods in the earlier literature. Further, unlike other studies [35], the inner space of our device was large enough for the animals to move freely. In addition, the flux density of the 50-Hz MF in our study was 100 μT, which is considered a limit for human exposure. Thus, our results contribute important information to public health.

However, some issues remained to be solved. First, the present study was mainly focused on the heart; more work should be done to reveal the effects of a 50-Hz MF on the vascular system (e.g. on microvascular function). Second, the flux density of 50-Hz MF we used was 100 μT, which is the limit for general public exposure in China. However, the limit recommended by the ICNIRP has been raised to 200 μT, and even 1 mT for occupational exposure. The effects on the cardiovascular system of 200 μT, or even higher MFs, should be investigated further.

CONCLUSION

In conclusion, exposure to a 50-Hz MF at 100 μT shows no effects on cardiovascular parameters (including blood pressure, cardiac rhythm, cardiac function and cardiac histological morphology) in rats.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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