Interval exercise, but not endurance exercise, prevents endothelial ischemia-reperfusion injury in healthy subjects

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Seeger JP, Lenting CJ, Schreuder TH, Landman TR, Cable NT, Hopman MT, Thijssen DH. Interval exercise, but not endurance exercise, prevents endothelial ischemia-reperfusion injury in healthy subjects. Am J Physiol Heart Circ Physiol 308: H351–H357, 2015. First published November 21, 2014; doi:10.1152/ajpheart.00647.2014.—Endothelial ischemia-reperfusion injury (I/R) initiates a remote protective response that results in the absence of I/R injury in the myocardium. This protective effect, termed ischemic preconditioning (IPC), was originally described in a nonvascular system, the brain, and later revealed that (acute) exercise reduces cardiovascular injury associated with prolonged (potentially lethal) ischemia (4, 5, 19). Recently, Michelsen and co-workers (20) provided further support that exercise may possess preconditioning effects. They found that interval running exercise (4 × 2 min) and IPC (4 × 5 min) in humans, followed by blood withdrawal and perfusion through isolated rabbit hearts, were similarly effective in reducing infarct size in the rabbit hearts (20). These effects of exercise on cardiac I/R injury may also be present in humans adopting an in vivo model of endothelial I/R injury.

To date, little is known about the possible preconditioning effects of (acute) exercise in humans. Therefore, the aim of the present study was to investigate the ability of an acute bout of exercise to prevent endothelial I/R injury in healthy, young humans. To study endothelial I/R injury, we adopted a frequently used and validated human in vivo model (14, 17, 32). Second, we hypothesized that a single bout of interval exercise represents a more potent preconditioning stimulus than endurance exercise. We speculated that interval exercise would lead to short periods of local deoxygenation of the working muscle mass, thereby mimicking “mechanical” IPC application. Therefore, our second aim was to compare the ability of a single bout of interval exercise versus endurance exercise to prevent endothelial I/R injury in healthy, young humans.

METHODS

Subjects

A total of 17 healthy volunteers were included to participate in this study (Table 1). All subjects performed regular physical activity, with an average sport participation of 6.7 ± 5.0 h (range: 1.5–17.5 h). Subjects were nonsmokers and were free of any cardiovascular disease, diabetes mellitus, hypertension (diastolic blood pressure > 90 mmHg and/or systolic blood pressure > 140 mmHg), and hypercholesterolemia (total cholesterol > 6.5 mmol/l). In addition, obese subjects (body mass index ≥30 kg/m²), and those on medication potentially influencing the cardiovascular system were excluded. All subjects signed an informed consent. The study was conducted according to the Declaration of Helsinki (2000) and approved by the
Interval exercise prevents endothelial ischemic injury

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Subjects</th>
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<tr>
<td>Men/women</td>
<td>10/7</td>
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<tr>
<td>Age, yr</td>
<td>23 ± 4</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>21.8 ± 1.5</td>
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<tr>
<td>DBP, mmHg</td>
<td>73 ± 8</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>57 ± 9</td>
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<tr>
<td>Cholesterol, mmol/l</td>
<td>4.3 ± 0.5</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>3.6 ± 0.5</td>
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Maximal incremental cycling test

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<tr>
<td>Maximal O₂ uptake, ml O₂·kg⁻¹·min⁻¹</td>
<td>52.4 ± 8.8</td>
</tr>
<tr>
<td>Maximal heart rate, beats/min</td>
<td>193 ± 11</td>
</tr>
<tr>
<td>Maximal workload, W</td>
<td>309 ± 74</td>
</tr>
<tr>
<td>Postexercise blood lactate, mmol/l</td>
<td>13.0 ± 1.6</td>
</tr>
<tr>
<td>Maximum respiratory exchange ratio</td>
<td>1.20 ± 0.06</td>
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Values are means ± SD; n = 17 subjects. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Experimental Design

First, subjects performed an incremental cycling test to determine maximal O₂ consumption (V̇O₂max) and maximum workload (in W). Subsequently, participants visited our laboratory on three different occasions (separated by at least 7 days, with a maximum of 1 mo; Fig. 1). During these visits, brachial artery endothelial function [using flow-mediated dilation (FMD)] was examined under resting conditions, after the intervention (interval exercise, endurance exercise, and control), and after I/R (20 min of arm ischemia and 20 min of reperfusion). To examine local tissue oxygenation in the physically active lower limbs during the exercise bouts, near-infrared spectrometry (NIRS) was placed on the thigh during exercise. All measurements for a subject were performed at the same time of day, and the order of testing (interval exercise, endurance exercise, and control) was randomized between subjects. The randomization procedure was performed using SPSS (version 20).

Day 1: Incremental cycling test. Subjects completed an incremental exercise test on a stationary bike (Lode Excalibur, Groningen, The Netherlands) to determine maximal V̇O₂ (in ml O₂·kg⁻¹·min⁻¹), maximum workload (in W), and maximum heart rate (in beats/min). Exercise was performed in a temperature controlled room (19°C). O₂ uptake was measured continuously by analyzing the expired ventilation with a continuous gas analyzer (COSMED Quark CPET, Pavona di Albano, Italy). Dependent on sex and expected fitness level, workload was increased every minute with 20 or 25 W. Workload increased every minute by volitional exhaustion, typically demonstrated by an inability to cycle at 60–70 revolutions/min. Heart rate was measured continuously with a 12-lead ECG. Blood pressure was obtained every 3 min to monitor subjects’ health (data not reported). Blood lactate levels were measured with a finger stick (Lactate Pro LT-1710, ArkRAY) before and within 2 min after the test was finished. All exercise tests were performed under supervision of a laboratory technician and physician. All tests included in our study met at least three of the four most often used quality assessment points: ± 10 beats/min of the predicted maximum heart rate (208 — age × 0.7), leveling off V̇O₂ (<150 ml O₂ during the last minute), postexercise respiratory exchange ratio of >1.1, and postexercise lactate level of >8.0 mmol/l (21).

Days 2–4: experimental protocol. Subjects were instructed to abstain from caffeine, chocolate, alcohol, and high doses of vitamin C for at least 18 h before testing and were instructed to not perform strenuous exercise 24 h before testing. Before each experiment, subjects refrained from food ingestion for at least 4 h and were instructed to have a standardized meal on the measurement days (sandwiches with jam without butter). All subjects were tested at the same time of day to prevent diurnal variations in the FMD response, and the measurements were performed in temperature-controlled testing rooms and using recent guidelines of FMD (29). Women were consistently tested in the luteal phase of their menstrual cycle (33).

The experimental protocol started with a 15-min rest period in the supine position followed by an assessment of blood pressure using a manual sphygmomanometer (Welch Allyn pressostabil) at the left upper arm. Subsequently, brachial artery endothelial function was examined using FMD on the right arm. This was followed by a 43-min intervention that consisted of 1) interval exercise, 2) endurance exercise, or 3) a control period of rest in the supine position. Both exercise bouts were isocaloric, and workload was individually calculated based on the maximum incremental test on day 1. This means that the total work performed by individuals was the same between the endurance and interval exercise bout, excluding the possibility that differences in caloric expenditure could affect our outcomes. During exercise, we continuously monitored heart rate and local oxygenation of the right thigh using NIRS. Blood lactate concentration was measured immediately before and after the exercise bouts. Ratings of perceived exertion were obtained at the end of the exercise intervention, just before the cool down period using a Borg 6–20 scale. After exercise, subjects rested for 30 min in the supine position, and another FMD measurement was performed at the end of this period. Then, 20 min of upper limb ischemia and 20 min of reperfusion were applied, after which brachial artery FMD was measured again to determine the impact of I/R injury on the right arm.

I/R injury. A rapid inflation/deflation pneumatic cuff (E10 rapid cuff inflator, Hokanson) was positioned proximally around the right upper arm. I/R was induced by 20 min of occlusion (cuff inflation to 220 mmHg) of the brachial artery followed by 20 min of reperfusion. This method represents a method to assess endothelial changes to I/R. Assessment of prolonged ischemia (40–60 min), as applied in animal studies to the myocardium, is for obvious reasons not possible in humans. Given the correlation between brachial and coronary artery responses to assess endothelial function (28), this technique is hypothesized to provide in vivo insight into ( endothelial) I/R injury. This method has been frequently used by others to examine endothelial changes to I/R (14, 16, 32).

Interventions

Interval exercise. After a 10-min warmup period at 30% of maximum workload, subjects performed ten 1-min cycle exercise bouts at 100% of maximum workload. These 100% bouts were separated by 2-min recovery periods at with cycle exercise at 25% of maximum workload. The interval exercise session was finished with a 5-min cooldown period at 30% of maximum workload.

Endurance exercise. Cycle endurance exercise consisted of a 10-min warmup period at 30% of maximum workload followed by a...
28-min exercise at 50% of maximum workload. The cycle endurance exercise session concluded with a 5-min cooldown period at 30% of maximum workload. We ensured that subjects performed the same amount of total workload during both exercise bouts.

Control. Subjects rested in the supine position for 43 min.

Measurements

FMD. Brachial artery diameter and blood flow velocity were determined using noninvasive echo-Doppler equipment. Subjects rested in a supine position with the right arm extended. For the assessment of FMD, a rapid inflation/deflation pneumatic cuff (E10 rapid cuff inflator, Hokanson) was placed around the right arm distal to the olecranon process to provide an ischemic stimulus distal from the brachial artery to provoke vasodilation and subsequent shear stress. The right arm was extended to the side and positioned at the heart level and was supported with towels to provide a stable position for recordings. A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine was used to perform imaging (T3000, Terason, Aloka, UK). The brachial artery was imaged in the distal third of the upper arm. Ultrasound parameters were set to optimize longitudinal B-mode images of the luminal-arterial wall interface. A continuous Doppler velocity assessment was obtained simultaneously, and data were collected using the lowest possible insonation angle (always <60°), which did not vary during each study. After a resting period of at least 15 min, 1 min of baseline recording of the arterial diameter and blood flow velocity was performed. Subsequently, the occlusion cuff was inflated to 220 mmHg for 5 min. Arterial diameter and blood flow velocity recordings were restarted at least 30 s before cuff deflation and continued for at least 3 min after deflation.

Brachial artery diameter and blood flow analysis. Analysis of the brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias (34). Baseline data were calculated across the 1-min preceding cuff inflation. After cuff deflation, peak diameter was automatically detected according to an algorithm previously described in detail elsewhere (3). Within-subject reproducibility of FMD using this semiautomated software has a coefficient of variation of 6.7–10.5% (30). Postdeflation shear rate data, derived from velocity and diameter measures, were used to calculate the shear rate area under the curve (SR_{AUC}).

NIRS. NIRS was used to measure local oxygenation in the lower limb tissue during exercise by assessing regional concentration changes in oxyhemoglobin (O$_2$Hb) and deoxyhemoglobin (HHb) using a continuous-wave near-infrared spectrophotometer (OXY-MON, Artinis Medical Systems). NIRS optodes were positioned on the right thigh, 12 cm proximal to the fibular head, on the vastus lateralis muscle parallel to the long axis of the muscle. NIRS measurements were performed continuously. An interrogate distance of 35 mm was used, resulting in a penetration depth of ~15–20 mm (6). Briefly, this technique is based on the relative transparency of tissue for light in the near-infrared region and on the changes in O$_2$-dependent absorption of hemoglobin and myoglobin. As one cannot distinguish between myoglobin and hemoglobin, the combined effect of these two substances is studied. Changes in absorption measured by NIRS are converted into estimates of concentration changes of O$_2$Hb and HHb. The sum of O$_2$Hb and HHb reflects changes in blood volume, represented by the total hemoglobin signal (Fig. 2).

NIRS analysis. The baseline O$_2$Hb level was determined by averaging the 7-min baseline period during warmup. This typically represented a stable recording of O$_2$Hb (Fig. 2). Subsequently, for each intervention, total O$_2$Hb area under the curve (HbO$_2$ total area) was calculated. Second, local oxygenation status was determined by calculating the positive area under the curve above baseline (“positive area”) and local deoxygenation/hypoxia as the negative area under the curve below baseline (“negative area”).

Statistical Analysis

All statistical analyses were performed using SPSS (version 20, SPSS, Chicago, IL) software. Data are reported as means ± SD unless stated otherwise. Statistical significance was set at $P < 0.05$. Baseline characteristics were compared with paired Student’s t-tests. To evaluate the effect of (different types of) exercise on endothelial I/R injury, we used a linear mixed model with a random factor of “subject” and fixed factors of “intervention” (interval exercise, endurance exercise, or control), “time” (preintervention, postintervention, and post-I/R), and the interaction of “intervention × time.” In an additional analysis, SR_{AUC} and baseline diameter were added as covariates. A recent study (2) has described that inadequate scaling for FMD would be present if the upper confidence limit of the regression slope of the relationship between logarithmically transformed base diameter and peak diameter is <1. In such an event, FMD (in %) may not be an appropriate measure to estimate endothelial function. Therefore, we repeated the analysis for FMD using allometric modeling (2).

Fig. 2. Near-infrared spectroscopy data for one subject. A: interval exercise. B: endurance exercise. The green line indicates total hemoglobin, the blue line indicates deoxyhemoglobin, and the red line indicates oxyhemoglobin. The x-axis shows time (in s), and the y-axis shows hemoglobin concentration (in μM). Vertical lines represent markers given every 3 min for additional data analysis afterward.
RESULTS

Interval exercise resulted in a higher postexercise blood lactate, average heart rate, and postexercise Borg score compared with endurance exercise (Table 2). There were no significant differences in (pre- and postexercise) blood pressure between both exercise bouts (Table 2). During the endurance exercise bout, heart rate and estimated VO₂ uptake were 152 ± 19 beats/min and 33.9 ± 10.6 ml O₂·kg⁻¹·min⁻¹ (i.e., 64 ± 14% of peak VO₂). During high-intensity exercise, heart rate and estimated VO₂ uptake of the first bout of high-intensity exercise was 167 ± 11 beats/min and 38.8 ± 9.7 ml O₂·kg⁻¹·min⁻¹ (i.e., 73 ± 11% of peak VO₂). The last bout of high-intensity exercise was performed at 188 ± 11 beats/min and 49.4 ± 9.7 ml O₂·kg⁻¹·min⁻¹ (i.e., 95 ± 6% of peak VO₂). Heart rate and estimated VO₂ uptake during the rest intervals were 147 ± 14 beats/min and 30.0 ± 8.7 ml O₂·kg⁻¹·min⁻¹ (i.e., 58 ± 13% of peak VO₂).

Impact of Exercise on Endothelial I/R

We found a significant interaction effect for the change in brachial artery FMD (in %) after I/R (P < 0.001; Table 3). While post hoc analysis revealed no change in brachial artery FMD immediately after the intervention for all three conditions, a significant decrease was observed in FMD after I/R during the endurance and control conditions (linear mixed model for time, P < 0.001). In marked contrast, the decline in FMD after I/R was prevented by interval exercise (post hoc P = 0.56; Fig. 3). Repeating this analysis using allometric scaling to control for the potential impact of (inter- and intraindividual differences in) baseline diameter confirmed the presence of a significant intervention (P = 0.034), time (P < 0.001), and intervention × time effect (P < 0.001).

The I/R procedure did not change brachial artery diameter (Table 3). No change in diameter was found after the control and endurance exercise session, whereas a significant increase in diameter was observed immediately after interval exercise (P < 0.05; Table 3). The eliciting shear rate stimulus SR_AUC was significantly higher after interval exercise (Table 3) but returned to baseline levels during the post-I/R measurement. The control and endurance exercise interventions showed no changes in SR_AUC. To statistically control for the potential influence of these parameters, we repeated our linear mixed model analysis with baseline diameter and SR_AUC as covariates. Our analysis confirmed our earlier findings and revealed
a significant interaction effect between time and intervention ($P < 0.001$), with a decline in FMD after I/R for control and endurance exercise sessions but not when interval exercise preceded I/R.

**Impact of Exercise on Local Oxygenation Status**

NIRS data showed no significant differences between endurance and interval exercise bouts for the total area under the curve for HbO$_2$ ($63.311 \pm 18.048$ and $60.008 \pm 27.941$ arbitrary units, respectively, $P = 0.21$) and the area below baseline ($-1.24 \pm 1.33$ and $-1.72 \pm 0.96$ arbitrary units, respectively, $P = 0.13$). Endurance exercise was associated with a significantly smaller area above baseline compared with the interval exercise bout ($1.28 \pm 1.67$ and $3.72 \pm 2.38$ arbitrary units, respectively, $P = 0.03$). Nonetheless, clear differences in the pattern of local HbO$_2$ could be observed between endurance and interval exercise bouts (Fig. 2).

**DISCUSSION**

To the best of our knowledge, this is the first study examining whether an acute bout of exercise is able to protect the vascular endothelium against I/R injury in humans in vivo and whether such preconditioning effect of exercise depends on the modality of exercise. The major finding of this study is that a single bout of interval exercise is able to protect the brachial artery endothelium against I/R injury in vivo in healthy young subjects. The observation that lower limb exercise (i.e., cycling) induced protective effects in the upper limb (i.e., brachial artery) suggests the presence of a remote preconditioning effect of interval exercise. Interestingly, when I/R was preceded by a single bout of moderate-intensity endurance exercise, post-I/R brachial artery endothelial function was impaired to a similar extent compared with the control condition. These findings indicate that a single, short-duration bout of interval exercise, but not endurance exercise, possesses remote preconditioning effects in healthy, young subjects.

In our study, we used a model of ischemia and reperfusion in the human forearm to simulate I/R injury, a method that has been extensively used in previous studies (14, 16, 32). Brachial artery endothelial function was chosen, since a strong correlation between brachial artery and coronary artery endothelial function has been described (1, 31). In our study, I/R resulted in a significant decrease of $\sim 40\%$ in brachial artery endothelial function, measured as FMD. This reduction of $40\%$ during the control condition is in line with previous studies from our laboratory as well as those from others who adopted a similar protocol ($38\text{--}65\%$) (16–18, 32). We observed a decrease in brachial artery FMD after I/R, which may reflect changes in tissue damage after I/R. Furthermore, our results indicate that interval exercise is able to prevent endothelial I/R injury, which suggests that interval exercise induces preconditioning effects. An interesting observation is that exercise was performed using the lower limbs, whereas protection against endothelial I/R was observed in the upper limbs. This observation provides support for a remote preconditioning effect of interval exercise, which suggests that also other vascular beds are protected against I/R injury.

In contrast to the observations during interval exercise, we found that the reduction in FMD after endothelial I/R injury remains present after performance of a bout of moderate-intensity endurance exercise in healthy subjects. Since the total workload was similar between both exercise bouts, the distinct impact on endothelial I/R injury may relate to differences in tissue oxygenation. More specifically, we hypothesized that interval exercise in contrast to endurance exercise would reveal similarities with “mechanical” IPC, as both interval exercise and IPC induce repeated, short periods of tissue deoxygenation. While our NIRS data revealed no differences in total deoxygenation between interval and endurance exercise, we indeed demonstrated that interval exercise, but not endurance exercise, induced repeated, short periods of ischemia (Fig. 2). This suggests that the pattern, rather than the total amount, of oxygenation contributes to the protective effects of a single bout of exercise on endothelial I/R injury. Future studies are warranted to better understand these potential underlying protective mechanisms of exercise on endothelial I/R.

A recent report (20) studying the effects of exercise preconditioning found that preconditioning effects of exercise are mediated by a blood-borne factor. Moreover, they found that the preconditioning effects of a single bout of (interval) exercise are, at least partly, mediated through opioid receptors (20). Exercise induces the release of $\beta$-endorphins, especially when performed at higher intensity levels that lead to significant elevations of blood lactate (7, 8, 27). Since $\beta$-endorphins contribute to the cardioprotective effects of IPC through their effects on opioid receptors, distinct release of $\beta$-endorphins between endurance and interval exercise (such as evident for blood lactate levels; Table 2) may contribute to our findings. Future studies are warranted to explore the potential role of $\beta$-endorphins and opioid receptors in the preconditioning effects of exercise.

Another explanation for the preconditioning effects of interval exercise may relate to the nitric oxide pathway. Although endurance exercise training improves endothelial nitric oxide synthase expression (12), some recent evidence suggests a superior upregulation of the nitric oxide pathway after interval training (26). Another explanation may relate to the rapid depletion of ATP during interval exercise compared with endurance exercise. Depletion of ATP activates ATP-dependent K$^+$ (K$_{ATP}$) channels to maintain the resting potential of the cells and to prevent cell death (15, 35). Mitochondrial K$_{ATP}$ channels are activated via a molecular cascade in which adenosine stimulates a G protein-coupled receptors transmitting the signal to PKC and, more importantly, have been suggested to play a significant role in IPC (15, 36). Whether (interval) exercise has protective preconditioning effects through endo-
thelial nitric oxide synthase and/or KATP channels remains unclear and should be subject of future research.

Study Limitations

A limitation of our study is that we did not include measures to explore the potential mechanisms involved underlying the protective effects of (high-intensity interval) exercise on endothelial I/R. However, the study was designed to explore whether different forms of exercise could prevent endothelial I/R injury. Another limitation is that we have included healthy young individuals only and did not assess exercise bouts of different duration and/or intensity. Ischemic events typically occur in an elderly population with cardiovascular risk and/or diseases. Whether exercise has similar preconditioning effects in groups with cardiovascular disease or risk is currently unknown and should be subject for future research. In this light, it is important to realize that recent papers have questioned the efficacy of “traditional” IPC in older subjects and heart failure patients (11, 32). Exercise may represent a suitable, effective, and safe alternative as a preconditioning stimulus in these groups. Furthermore, previous work has highlighted the impact of duration and intensity of exercise on the vasculature. Therefore, we cannot exclude the possibility that endurance exercise of different intensity (i.e., above lactate threshold) or duration has preconditioning effects. Finally, we used brachial artery endothelial function as a model to study I/R injury in the forearm. Although this model has been frequently used by others (14, 16, 32) and strong correlations have been reported between brachial artery FMD and coronary endothelial function (1, 31), caution should be taken when extrapolating our findings to other vascular beds, such as the coronary circulation. Future studies are recommended to further validate this technique.

Clinical Relevance

Exercise training has strong cardioprotective effects. Approximately 40% of the beneficial effects of exercise training can be explained by improvement in cardiovascular risk factors (22). Possibly, the preconditioning effects of a single bout of (interval) exercise may contribute to the cardioprotective effects of exercise. Moreover, repeatedly performing this type of exercise (and therefore preconditioning) may protect against I/R injury. Previous work found that habitual endurance (9) or resistance (10) exercise (partly) prevented endothelial I/R injury. A potential implication of our results is that exercise training may possess preconditioning effects that ultimately protect against I/R injury. Future studies are needed to explore this hypothesis.

Conclusions

Our study showed the ability of interval exercise to prevent endothelial I/R injury in a group of healthy young subjects, whereas this effect of exercise was absent when I/R injury was preceded by a single bout of short-duration, moderate-intensity endurance exercise. These protective effects of lower limb interval exercise were observed in upper limb arteries, which suggests that the preconditioning effects represent a remote, rather than local, effect of interval exercise. Our findings may have potential clinical relevance for the protection of the heart and other tissues against I/R injury by (interval) exercise.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


