Vasorelaxation of goat mesenteric artery is mediated by endothelial Na\(^+\)-K\(^+\)-ATPase

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**ABSTRACT**

**Objective:** To examine the role of Na\(^+\)-K\(^+\)-ATPase and K\(^+\) channels in mediating vasorelaxation in the superior mesenteric artery of *Capra hircus*. 

**Materials and Methods:** Goat superior mesenteric artery (GSMA) was cut into 1.5–2 mm circular rings and mounted in a thermostatically controlled (37°C ± 0.5°C) organ bath containing 20 ml of modified Krebs-Henseleit saline (MKHS) (pH 7.4), with continuous aeration under 1.5 g tension for 90 min. Endothelium-intact (ED+) or endothelium-denuded (ED−) GSMA ring was contracted with phenylephrine (PE) or 5-hydroxytryptamine (5-HT) (1 μM–0.1 mM) in the absence or presence of ouabain (0.1 μM). KCl (1 μM–10 mM) was added cumulatively to K\(^+\)-free MKHS-pre-contracted (ED+/−) rings in the absence or presence of ouabain (0.1 μM) or barium (1 μM) or 4-aminopyridine (1 μM). 

**Results:** Ouabain did not alter the basal tone of the arterial ring. The contractile response induced by PE (E\(_{\text{max}}\): 50.46 ± 2.68, pD2: 5.53 ± 0.04) and 5-HT (E\(_{\text{max}}\): 30.86 ± 1.33, pD2: 6.17 ± 0.03) in ED+ ring was significantly (P < 0.001) augmented in ED− rings (PE: E\(_{\text{max}}\): 93.30 ± 2.11, pD2: 6.41 ± 0.04; 5-HT: E\(_{\text{max}}\): 95.07 ± 0.99, pD2: 6.27 ± 0.03). The contractile response induced by PE and 5-HT in ED+ or ED− rings in the presence of ouabain was almost identical with that of ED− rings. Vasorelaxation of KCl (E\(_{\text{max}}\): 2.90 ± 1.14, pD2: 3.9 ± 0.03) was significantly attenuated in the presence of ouabain (E\(_{\text{max}}\): 73.8 ± 5.16, pD2: 4.3 ± 0.04), Ba\(^2+\) (E\(_{\text{max}}\): 16.34 ± 4.7, pD2: 3.22 ± 0.02), 4-AP (E\(_{\text{max}}\): 18.16 ± 2.4, pD2: 3.68 ± 0.03), ouabain and Ba\(^2+\) (E\(_{\text{max}}\): 70.09 ± 3.66, pD2: 4.41 ± 0.04), and ouabain and 4-AP (E\(_{\text{max}}\): 66.98 ± 4.61, pD2: 4.13 ± 0.06). 

**Conclusion:** The vasorelaxation in GSMA is mediated by the endothelium-derived hyperpolarizing factor (EDHFS) such as ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase, K\(_{\text{IR}}\) and K\(_{\text{V}}\) channels.

**Key words:** EDHF, goat, K\(_{\text{IR}}\), K\(_{\text{V}}\), mesenteric artery, ouabain, pump, sodium pump

**INTRODUCTION**

Na\(^+\)-K\(^+\)-ATPase is an integral membrane enzyme, ubiquitously distributed in the plasma membrane of all eukaryotic cells.[1] It plays an important role in generation and maintenance of the electrochemical gradient by extrusion of three sodium ions and influx of two potassium ions across the cell membrane by utilizing the energy derived from ATP hydrolysis.[2] Endothelium has also been shown to regulate the Na\(^+\)-K\(^+\)-ATPase activity either by release of nitric oxide (NO) or endothelial diffusible...
factors like leukotrienes and prostaglandins. The role of the endothelium in sodium pump induced vasorelaxation varies between vascular beds and species. The vasorelaxation induced by vascular sodium pump has been reported to be endothelium dependent in human placental vessels, mice femoral artery, and rat aorta and endothelium independent in goat ruminal artery and rabbit aorta. In small arteries like mesenteric bed, where the ratio of endothelium/smooth muscle increases, the endothelium plays a more important role in modulation of the expression of Na+-K+-ATPase.

In addition to the role of sodium pump, the activation of vascular endothelial K+ channels hyperpolarizes and relaxes the underlying smooth muscle; hence, K+ acts as the endothelium-derived hyperpolarizing factor (EDHF). Endothelium-derived K+ is also reported to function as a local intravascular vasodilator as observed in rabbit aorta, rat resistance arteries and rabbit arcuate arteries and rat mesenteric artery, whereas findings from other studies conflicted with the proposed role of K+ ion as an EDHF in porcine coronary arteries and rat mesenteric artery. K+ induced relaxation in vascular smooth muscles may involve activation of sarcolemmal Na+-K+-ATPase or inward rectifier K+ channels or even both the mechanisms.

In view of the contradicting evidences with respect to the activity of Na+-K+-ATPase and K+ channel is linked to the endothelium in different vascular beds, we hypothesized that vasorelaxation in ruminant mesenteric artery could be contributed by endothelium-dependent Na+-K+-ATPase and K+ channels such as K$_{in}$ and K$_{o}$ channels. In order to establish it, we selected superior mesenteric artery of goat (Capra hircus) as a ruminant vascular model and conducted functional studies to characterize the Na+-K+-ATPase in endothelium-intact (ED+) and −denuded (ED−) vessels using K+-induced relaxation in K+-free solution as an activator of vascular Na+-K+-ATPase.

MATERIALS AND METHODS

Preparation of superior mesenteric artery and tension recording

After careful exposure of goat intestinal mesentery, a branch of superior mesenteric artery adjacent to the duodenum and jejunum just before it branches into the inferior branch was dissected out and placed in cold, aerated, modified Krebs-Henseleit saline (MKHS) solution. Arteries were cleared of fat and connective tissues. Endothelium was removed by rubbing it with horse hair. The arterial rings of 1.5–2 mm were then mounted between two fine, L-shaped hooks made of stainless steel and kept under a resting tension of 1.5 g in a thermostatically controlled (37.0°C ± 0.5°C) automatic organ bath (Pan Lab) of 20 ml capacity containing MKHS and aerated continuously with air. The arterial rings were equilibrated for 90 min before recording the muscle tension. During this period, the bathing fluid was changed every 15 min. This experiment was repeated for both ED+ and ED− vessels. The change of isometric tension was measured by a highly sensitive isometric force transducer (Model MLT0201; AD instrument, Sydney, Australia) and analyzed using Chart 7.1.3 software.

Acetylcholine (1 μM) on 5-hydroxy tryptamine–induced sustained contractile response

In order to examine intact functional endothelium, a single sub-threshold concentration of 5-hydroxy tryptamine (5-HT; 0.1 μM) was added to the bath to obtain a sustained contraction and observed for inhibitory effect of ACh (1 μM) for 1 min.

5-HT and phenylephrine (1 nM)–induced contraction in ED+ and ED− goat superior mesenteric artery rings in the absence or presence of ouabain

Experiments with K+-free physiological solution

K+-induced relaxation of arterial rings exposed to K+-free MKHS is an experimental protocol for the functional assessment of vascular sodium pump. In order to study the regulation of sodium pump by vascular endothelium in goat superior mesenteric artery (GSMA), the arterial rings were equilibrated in MKHS for 90 min and then the tissue viability was checked with 5-HT (1.0 μM). The endothelial integrity was examined by applying ACh (1 μM) to the vessels pre-contracted with 5-HT (1 μM). After a period of wash for 30 min in MKHS, tissues were incubated in K+-free solution and all the subsequent experimental protocols were carried out in K+-free solution. This experiment was repeated for both ED+ and ED− vessels.

KCl-induced relaxation in K+-free MKHS in the presence of ouabain or 4-aminopyridine or barium, or ouabain and 4-AP, or ouabain and barium

The arterial ring was incubated in K+-free MKHS pre-incubated with either 0.1 μM ouabain or 1 μM 4-aminopyridine (4-AP) or 1 μM Ba$^{2+}$, or 0.1 μM ouabain and 1 μM 4-AP, or 0.1 μM ouabain and 1 μM Ba$^{2+}$ for 30 min to obtain a sustained contraction and, subsequently, KCl was added (1 μM–10 mM) to the bath cumulatively at an interval of 1 min to record the contractile response.

Data analysis

The results were analyzed by interactive non-linear regression through the computer program GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA). Statistical analysis was done using unpaired Student’s “t” test with GraphPad Software Quick Cales. P values <0.05 and <0.001 were considered statistically significant.

Drugs

Acetylcholine chloride, ouabain, phenylephrine and 5-hydroxy tryptamine (Sigma Aldrich, USA), barium chloride and
4-aminopyridine (Qualigens and HiMedia, India) were employed in this study.

**RESULTS**

**Effect of ACh on 5-HT (0.1 µM)-induced contraction**
5-HT pre-contracted the arterial ring, reaching a steady level (1.28 ± 0.25 g, n = 14) within 2–3 min. ACh (1 µM) inhibited the 5-HT–induced sustained contraction by 44.51 ± 9.37% (n = 7), suggesting the presence of functional endothelium. In contrast, ACh did not inhibit the 5-HT–induced sustained contraction in ED− GSMA rings.

**Effect of KCl (1 µM–10 mM) in ED+ and ED− GSMA rings in K+-free MKHS**
Exposure of arterial ring to K+-free solution caused a rise in basal tone that reached the maximum (1.43 ± 0.27 g, n = 22) in about 25–30 min and, subsequently, a sustained contraction was observed within 2–3 min. Cumulative addition of KCl inhibited sustained contraction in a concentration-dependent manner in both ED+ and ED− rings. The concentration related contractile (CRC) response curve of K+ (pD2 3.9 ± 0.03, Emax 2.90 ± 1.14%) in ED+ rings was shifted to the right with a significant (P < 0.001) decrease in pD2 (3.32 ± 0.02) and increase in Emax (66.78 ± 1.49%) in ED− rings [Figure 1].

**Effect of ouabain in 5-HT and PE (1 nM–0.1 mM)-induced CRC response curves in ED+ and ED− vessels**
5-HT–induced CRC response curve was shifted to the left with insignificant alteration in pD2 (6.27 ± 0.03) and significant (P < 0.05) increase in Emax (95.07 ± 0.99%) in ED− rings as compared to ED+ rings (pD2 6.17 ± 0.03, Emax 30.86 ± 1.33). In the presence of ouabain, 5-HT–induced CRC response curve was shifted to the left with significant (P < 0.05) increase in pD2 (6.82 ± 0.02) in ED− rings as compared to ED+ (pD2 6.25 ± 0.02) rings [Table 1 and Figure 2a–c]. PE-induced CRC response curve was shifted to the left with significant (P < 0.001) increase in pD2 (6.41 ± 0.04) and Emax (93.30 ± 2.11%) in ED− rings as compared to ED+ rings (pD2 5.53 ± 0.04, Emax 50.46 ± 2.68%). In the presence of ouabain, PE-induced CRC response curve was shifted to the left with significant (P < 0.001) increase in pD2 (6.34 ± 0.02) in ED− rings as compared to ED+ (pD2 5.2 ± 0.05) rings [Table 1 and Figure 3a–c].

**Effect of ouabain (0.1 µM), barium (1 µM), 4-AP (1 µM), ouabain (0.1 µM) and barium (1 µM), and ouabain (0.1 µM) and 4-AP (1 µM) on KCl-induced relaxation**
K+-induced vasorelaxation was attenuated with rightward shift of KCl-induced CRC response curve with significant (P < 0.001) increase in Emax in the presence of ouabain (73.8 ± 5.16%), Ba2+ (16.34 ± 4.7%), 4-AP (18.16 ± 2.4%), ouabain and Ba2+ (70.09 ± 3.66%), and ouabain and 4-AP (66.98 ± 4.61%), as compared to that of control (2.90 ± 1.14%). Similarly, pD2 (3.9 ± 0.03) of KCl-induced CRC curve was shifted to the right with a significant (P < 0.001) increase (4.3 ± 0.04), decrease (3.22 ± 0.02), decrease (3.68 ± 0.03), increase (4.41 ± 0.04), and increase (4.13 ± 0.06) in the presence of ouabain, Ba2+, 4-AP, ouabain and Ba2+, and ouabain and 4-AP, respectively [Table 2 and Figure 4a–c].

**DISCUSSION**
The results obtained in the present study showed the following: (1) The vasotonic response of PE or 5-HT in ED+ GSMA rings is potentiated due to the removal of endothelium that results in termination of direct

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**Table 1:** Effect of ouabain (0.1 µM) on 5-HT-induced and PE (1 nM–0.1 mM)-induced CRC response curves in endothelium-intact (ED+) and endothelium-denuded (ED−) vessels

| Agonist | Emax (%) | pD2  |
|---------|----------|------|
| 5-HT (n=8) |          |      |
| Control (ED+) | 30.86±1.33 | 6.17±0.03 |
| Ouabain (ED+) | 99.99±0.00** | 6.25±0.02*  |
| (ED−) | 95.07±0.99** | 6.27±0.03 |
| Ouabain (ED−) | 99.99±0.00** | 6.82±0.02** |

| PE (n=8) |          |      |
| Control (ED+) | 50.46±2.68 | 5.53±0.04 |
| Ouabain (ED+) | 99.99±0.00** | 5.20±0.05** |
| (ED−) | 93.30±2.11** | 6.41±0.04** |
| Ouabain (ED−) | 99.99±0.00** | 6.34±0.05** |

*Significant difference between the sub-rows at the level of P<0.05.
**Significant difference between the sub-rows at the level of P<0.001.

Data of each row (treated) are compared with the data of ED+ (non-treated control) within the corresponding column of each drug. Emax and pD2 values are expressed as means±SEM; n- No. of experiments.
Figure 2: (a) Effect of endothelium removal on 5-HT (1 nM–0.1 mM)-induced concentration-related contractile response presented in curves labeled as 5-HT, ED+ (○) and 5-HT, ED− (□). Effect of pre-incubation of ouabain (0.1 µM) on 5-HT (1 nM–0.1 mM)-induced concentration-related contractile response curves in endothelium-intact (●) and endothelium-removed (■) GMSA rings is presented in 5-HT (ED+) + 0.1 µM Ouab and 5-HT (ED−) + 0.1 µM Ouab. (b) Tracing representing the effect of ouabain (0.1 µM) on the contractile response induced by 5-HT (10 µM) in endothelium-intact goat superior mesenteric artery. (c) Tracing representing the effect of ouabain (0.1 µM) on the contractile response induced by 5-HT (10 µM) in endothelium-denudated goat superior mesenteric artery.

Figure 3: (a) Effect of endothelium removal on PE (1 nM–0.1 mM)-induced concentration-related contractile response presented in curves labeled as PE, ED+ (○) and PE, ED− (□). Effect of pre-incubation of ouabain (0.1 µM) on PE (1 nM–0.1 mM)-induced concentration-related contractile response curves in endothelium-intact (●) and -removed (■) GMSA rings is presented in PE (ED+) + 0.1 µM Ouab and PE (ED−) + 0.1 µM Ouab. (b) Tracing representing the effect of ouabain (0.1 µM) on the contractile response induced by PE (10 µM) in endothelium-intact GMSA rings. (c) Tracing representing the effect of ouabain (0.1 µM) on the contractile response induced by PE (10 µM) in endothelium-denudated GMSA rings.

Table 2: Effect of ouabain (0.1 µM), 4-AP (1 µM), barium (1 µM), ouabain (0.1 µM) and 4-AP (1 µM), and ouabain (0.1 µM) and barium (1 µM) on KCl-induced vasorelaxation

| Parameters       | Eₘₐₓ/Eₘₐₓ (%) | pD₂  |
|------------------|---------------|------|
| Control, ED+ (n=10) | 2.90±1.14     | 3.9±0.03 |
| ED− (n=7)       | 66.78±1.49**  | 3.32±0.02** |
| Ouabain (n=6)   | 73.8±5.16**   | 4.3±0.04** |
| 4-AP (n=6)      | 18.16±2.41**  | 4.6±0.03** |
| Barium (n=6)    | 16.34±4.71**  | 3.22±0.02** |
| Ouabain + 4-AP (n=6) | 66.98±4.61** | 4.13±0.06** |
| Ouabain + barium (n=6) | 70.09±3.66**| 4.41±0.04** |

**Significant difference between the sub-rows at the level of P<0.05. **Significant difference between the sub-rows at the level of P<0.001. Data of each row (treated) are compared with the data of ED+ (non-treated control) within the corresponding column. Eₘₐₓ and pD₂ values are expressed as mean±SEM; n=No. of experiments.

vasorelaxation of endothelial NO; (2) ouabain augmented identically the PE/5-HT–induced maximal vasotonic effect in ED+ rings, but not in ED− rings. This augmentation of agonist-induced vasotonic response in ED+ ring by ouabain could be due to abolition of vasorelaxation effect of endothelium-dependent Na+-K+-ATPase; (3) K+-induced vasorelaxation in K+-free medium of ED+ rings was attenuated by ouabain (70%), which is identical to that in ED− rings (64%) and further supports that Na+-K+-ATPase is endothelium dependent in this tissues; and (4) 4-AP, a Kᵥ channel blocker, and Ba²⁺, a Kᵢ₅ channel blocker, inhibited KCl-induced vasorelaxation in ED+ rings only by 16–18%, which was augmented fourfold in conjunction with ouabain, but did not exceed the attenuation of maximum vasorelaxation caused by ouabain only (70%).
The net augmentation of vascular contraction induced by different agonists may be due to loss of a tonic release of vasorelaxant factors such as endothelium-dependent relaxing factors (EDRF), viz. NO. In addition, EDHF can also modulate vasorelaxation via NO, prostaglandin I\textsubscript{2}, or arachidonic acid and its metabolites, which in turn opens big conductance calcium-activated K-channels (BKCa) or Na\textsuperscript{+}, K\textsuperscript{+} ions on vascular smooth muscle cells. The significant augmentation of the maximal contractile responses to both 5-HT and PE in ED\textsuperscript{−} rings clearly supports the active involvement of EDRF or/and EDHF in GSMA rings, which is the same as that reported in human placental vessels. Interestingly, endothelium removal in goat mesenteric artery augmented the maximal contractile response to 5-HT and PE by threefold and twofold, respectively. This result clearly explains that endothelium-derived vasorelaxation factors possibly exhibited greater inhibitory effect on vasotonic response to 5-HT than PE. In several vascular beds, endothelium-derived factors such as EDRF and endothelium-dependent contracting factor (EDCF) differentially regulated the vasotonic responses to different agonists such as nor-adrenaline (NA) and 5-HT in rat, canine, and porcine coronary arteries, Angiotensin II in rat aorta, and histamine in human internal mammary artery. PE in rat aorta and superior mesenteric artery.

Furthermore, to assess the role of endothelium on the contractile response to ouabain, 5-HT/PE–induced contractile response was elicited in the presence of ouabain either in ED\textsuperscript{+} or ED\textsuperscript{−} vessels. In the presence of ouabain, the maximal contractile response to either 5-HT or PE was increased by about threefold in ED\textsuperscript{+} but not in ED\textsuperscript{−} GSMA rings. Thus, 5-HT- or PE-induced contractile responses were not enhanced further by ouabain in ED\textsuperscript{−} rings. This clearly demonstrates that the vasodilatory effect of endothelium is ouabain sensitive in goat mesenteric artery. Endogenous ouabain may play an important role in regulating arterial tone and peripheral vascular resistance. Increase in maximal contractile response to either 5-HT or PE by ouabain observed in ED\textsuperscript{+} GSMA rings, does not agree with the myogenic action of this glycoside. The reduction of vasotonic effect by ouabain is due to the release of NO from the functional endothelium in coronary artery or stimulation of inducible NO synthase expression in rat vascular smooth muscle cells and rat aorta, rat tail artery, and rat mesenteric artery. Our present finding clearly contradicts this action of ouabain and demonstrates that in GSMA, reduction of maximal contractile response to either 5-HT or PE in ED\textsuperscript{+} vessels is due to direct or indirect activation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and this is endothelium dependent.

We observed that only in ED\textsuperscript{+} mesenteric artery, there was significant influence of extracellular K\textsuperscript{+} on the activation of sodium pump, and that small increase in the extracellular concentration of K\textsuperscript{+} caused concentration-dependent relaxation in K\textsuperscript{+}-free medium, confirming the function of endothelium-derived K\textsuperscript{+} as a local intravascular vasodilator as observed in rabbit aorta, rat resistance arteries and rabbit arcuate arteries, and rat mesenteric artery, whereas findings from other studies conflicted with the proposed role of K\textsuperscript{+} ion as an EDHF in porcine coronary arteries and rat mesenteric artery. In addition, the endothelium has also been shown to regulate the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity either by release of NO or endothelial diffusible factors like leukotrienes and prostaglandins. Our results confirm that K\textsuperscript{+}-induced vasorelaxation in goat mesenteric artery is predominantly endothelium dependent and this is due to activation of endothelial Na\textsuperscript{+}-K\textsuperscript{+}-ATPase as evidenced in human placental vessels, mice femoral artery, and in rat aorta. In small arteries like mesenteric bed, where the ratio of endothelium to smooth muscle increases, the endothelium plays a more important role in modulation of the expression of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase.
KCl-induced relaxation in vascular smooth muscles may involve activation of sarcolemmal Na‘-K‘-ATPase or inward rectifier K‘-channels or even both the mechanisms. One of the distinguishing features of vascular relaxation by K‘ involving Na‘-K‘-ATPase is that the extracellular concentration of K‘ is less than 5 mM, whereas inward rectifier K‘ channels primarily mediate K‘-induced relaxation above the physiological K‘ concentration (>5 mM). In goat mesenteric arteries, we found that extracellular K‘ between 1 μM and 5 mM produced graded relaxation of the vessels bathed in K‘-free solution and it was significantly inhibited by ouabain, a Na‘-K‘-ATPase inhibitor, suggesting that vasorelaxation is mediated by endothelial Na‘-K‘-ATPase and is ouabain sensitive.

The Na‘-K‘-ATPase α isoforms contain a highly conserved ouabain-binding site that mediates sensitivity to inhibition by cardiotonic steroids, such as ouabain and digoxin. In most mammals, all the α subunits are sensitive to ouabain inhibition with notable exceptions, i.e. guinea pig small intestine and human cardiac muscle, in which ouabain-resistant α subunits have been reported. Attenuation of Na‘-K‘-ATPase activity by about 70% by ouabain in ED+ GMSA rings clearly suggests that vasorelaxation in this artery could be mediated predominantly by ouabain-sensitive αi isoforms of Na‘-K‘-ATPase. In ovine pulmonary artery, both α1 and α2 isoforms of Na‘-K‘-ATPase have been reported to be present and possess low and high affinity, respectively, for ouabain. The low, but not the high, affinity isoforms of Na‘-K‘-ATPase by ouabain significantly increased the basal tone and 5-HT–induced contractility in this vessel.

Activation of the KIR channel and conduction of an outward K‘ current in response to small increases in extracellular K‘ is thought to occur because of unique gating properties of KIR channels, and larger increases in K‘ (by < 30 mM) cause smooth muscle depolarization and subsequent constriction of several arteries due to marked membrane depolarization and Ca2+ entry via voltage-operated Ca2+ channels. In the present study, we observed that K‘-induced maximal vasorelaxation in goat mesenteric artery was inhibited (16–18%) by either 4-AP or Ba2+ only. This suggests that there is an independent involvement of KIR or KIR channels in addition to Na‘-K‘-ATPase in the vasorelaxation, as observed in goat ruminal artery. Conversely, in rat basilar artery, it was reported that Ba2+ completely blocked K‘-induced vasorelaxation. This discrepancy in the sensitivity to Ba2+ to K‘-induced vasorelaxation versus hyperpolarization could be related to either altered vascular response specific to mesenteric artery or an influence of several mediators that participate in ruminal contraction.

**CONCLUSION**

In conclusion, the vasorelaxation of goat mesenteric artery is mediated (i) predominantly by endothelium-dependent, ouabain-sensitive Na‘-K‘-ATPase, which could be an EDHF in goat mesenteric artery, and (ii) by activation of KIR and KIR channels.

**Ethical clearance**

This work has been approved by institutional animal ethical committee (IAEC) and registered under Registration No. 433/CPCSEA for conducting animal tissue experiment. It was funded by a university research grant.

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**Conflicts of interest**

There are no conflicts of interest.

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