Colchicine enhances adrenoceptor-mediated vasodilation in men with essential hypertension

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Colchicine enhances β adrenoceptor-mediated vasodilation in men with essential hypertension

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Aims: The aim of this study is to examine whether colchicine improves β adrenoceptor-mediated vasodilation in humans by conducting a double-blinded, placebo-controlled intervention study. Colchicine treatment has known beneficial effects on cardiovascular health and reduces the incidence of cardiovascular disease. Studies in isolated rodent arteries have shown that colchicine can enhance β adrenoceptor-mediated vasodilation, but this has not been determined in humans.

Methods: Middle-aged men with essential hypertension were randomly assigned firstly to acute treatment with either 0.5 mg colchicine (n = 19) or placebo (n = 12). They were subsequently re-randomized for 3 weeks of treatment with either colchicine 0.5 mg twice daily (n = 16) or placebo (n = 15) followed by a washout period of 48–72 h. The vasodilator responses to isoprenaline, acetylcholine and sodium nitroprusside were determined as well as arterial pressure, arterial compliance and plasma inflammatory markers.

Results: Acute colchicine treatment increased isoprenaline (by 38% for the highest dose) as well as sodium nitroprusside (by 29% main effect) -induced vasodilation but had no effect on the response to acetylcholine. The 3-week colchicine treatment followed by a washout period did not induce an accumulated or sustained effect on the β adrenoceptor response, and there was no effect on arterial pressure, arterial compliance or the level of measured inflammatory markers.

Conclusion: Colchicine acutely enhances β adrenoceptor- and nitric oxide-mediated changes in vascular conductance in humans, supporting that the mechanism previously demonstrated in rodents, translates to humans. The results provide novel translational evidence for a transient enhancing effect of colchicine on β adrenoceptor-mediated vasodilation in humans with essential hypertension.

Thomas S. Ehlers and Jennifer van der Horst shared first authorship.

Thomas S. Ehlers and Jennifer van der Horst are 2 first authors who contributed equally to the manuscript.

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The authors confirm that the Principal Investigator for this paper is Dr Thomas Svare Ehlers and that he had direct clinical responsibility for patients.
1 | INTRODUCTION

Colchicine is an anti-inflammatory drug, first described in the Ebers Papyrus (1500 BCE), which has since been isolated from its plant source (Colchicum autumnale) for the treatment of inflammatory diseases, such as gout and Mediterranean fever. More recently, low-dose, daily colchicine treatment has been shown to lower the risk of cardiovascular disease, including myocardial infarction, stroke and acute coronary syndrome. Although the cardiovascular effects of long-term, low-dose colchicine treatment have been attributed to its anti-inflammatory effects, the drug may also improve cardiovascular health by a direct effect on vascular resistance, as demonstrated in animal models. Colchicine is a disruptor of the microtubule network and can therefore affect trafficking of proteins to and from the membrane. Through this microtubule depolymerising mechanism, recent studies on rodent arteries found that colchicine enhanced vasodilatation through increasing the membrane abundance of the voltage-gated Kv7.4 potassium channel in arterial smooth muscle, as well as promoting the functional role of the β2 adrenoceptor. In isolated rat mesenteric arteries, the increased Kv7.4 membrane abundance following colchicine treatment led to enhanced relaxations to Kv7 channel-specific activators and contributed to the enhanced vasodilatation to the β adrenoceptor agonist isoprenaline. Importantly, in hypertensive rodents (the spontaneously hypertensive rat [SHR]), treatment of colchicine enhanced the attenuated Kv7 channel function and promoted β1 adrenoceptor function, thereby restoring the impaired isoprenaline-mediated relaxation in arteries of the SHR.

The abovementioned animal studies demonstrate that colchicine improves β adrenoceptor-mediated vasodilatation through Kv7.4 channels in isolated rat mesenteric arteries, but, to date, there are no translational studies demonstrating this effect in human resistance arteries. This effect of colchicine may be particularly important in essential hypertension as the condition is associated with reduced β adrenoceptor-mediated vasodilatation, and colchicine treatment could potentially reverse this impairment.

Therefore, based on our findings in rodents, we hypothesized that colchicine would enhance β adrenoceptor-mediated vasodilatation in humans. Herein, we evaluated the effect of low-dose colchicine acutely (within 75 min) and after a 3-week period of daily treatment, on microvascular function in men with essential hypertension, with focus on β adrenoceptor-mediated vasodilatation. To this end, brachial artery blood flow and intra-arterial pressure were determined during control conditions and during arterial infusion of the β adrenoceptor specific agonist isoprenaline. To evaluate the potential effect of colchicine on endothelial-dependent and endothelial-independent vasodilatation, we also examined the effect of acetylcholine and the nitric oxide (NO)-donor, sodium nitroprusside, respectively.
2 | METHODS

2.1 Study design and participants

This translational study was conducted as a double-blinded, placebo-controlled intervention study. The study included men with essential hypertension, to determine whether colchicine treatment could be a feasible strategy to enhance vascular function. Middle-aged men, \( n = 31 \), with essential hypertension (25 of 31 were medicated; see Table 1) were randomly assigned, first to a single acute dose of 0.5 mg of colchicine or placebo and then 3 weeks of either colchicine 0.5 mg twice daily or placebo. Before and after the acute treatment, vascular reactivity to arterially infused vasodilators was determined. Before and after the 3-week period, vascular reactivity to arterially infused vasodilators, arterial pressure and arterial compliance was determined.

Inclusion criteria were as follows: men, age 40–65 years; no chronic diseases apart from essential hypertension; resting systolic and diastolic blood pressures of >130 and/or >85 mmHg without antihypertensive medication or ≥120 and/or ≥80 mmHg with antihypertensive medication, respectively.

Exclusion criteria were as follows: glycated haemoglobin ≥48 mmol/mol; systolic and diastolic blood pressure of >160 mmHg and/or >110 mmHg, respectively; alcohol consumption >14 units per week; smoking; liver disease; or neutropenia.

2.2 Ethical approval and clinical trials

The study was approved by The Danish National Committee on Health Research Ethics, Capital Region of Copenhagen (H-19088649) and was conducted in accordance with the latest guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects before enrolment. Prior to recruitment, the study was registered at clinicaltrials.gov with identifier NCT04303689.

2.3 Pharmacological intervention

2.3.1 Acute colchicine treatment

The first 5 subjects were part of a pilot study for the acute effect of colchicine on vascular function. These subjects received 1 0.5 mg oral (tablet form, Hovedstadens apotek, Herlev, Denmark) dose of colchicine unblinded. The remaining subjects were randomly assigned, standard double-blinded placebo-controlled, to either acute oral colchicine 0.5 mg (\( n = 19 \)) or oral placebo (\( n = 12 \)) for the assessment of the acute effect. The placebo consisted of oral calcium tablets.

2.3.2 Three-week colchicine treatment

Subjects were randomly assigned to either 0.5 mg of colchicine twice daily (COL; \( n = 16 \)) or placebo (PLAC; \( n = 15 \)) for a 3-week period. Primary investigators were blinded to the assignments of placebo/colchicine. Daily telephone reminders and pill-count after the intervention period were used to improve compliance. The 3-week treatment period was fully carried out as standard double-blinded placebo-controlled intervention, and the period was followed by a 48–72-h washout period.

2.4 Screening

As part of meeting inclusion and exclusion criteria, the subjects underwent physical examination by a medical doctor including electrocardiogram and resting blood pressure, and a fasting blood sample was obtained to determine whether the following screening markers were within the normal range: haemoglobin, glycated haemoglobin, creatinine, C-reactive protein (CRP), alanine aminotransferase, aspartate aminotransferase, \( \gamma \)-glutamyltransferase, activated partial thromboplastin time, international normalized ratio and coagulation factors II

### Table 1 Subject medication.

<table>
<thead>
<tr>
<th>Group</th>
<th>Acute colchicine</th>
<th>Acute placebo</th>
<th>3-week colchicine</th>
<th>3-week placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicated</td>
<td>14/19</td>
<td>11/12</td>
<td>12/16</td>
<td>13/15</td>
</tr>
<tr>
<td>≥3 antihypertensive medications</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Unmedicated</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table of Subject and Type of Medication

<table>
<thead>
<tr>
<th>Subject/type of medication</th>
<th>Acute colchicine</th>
<th>Acute placebo</th>
<th>3-week colchicine</th>
<th>3-week placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE-inhibitors</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Angiotensin-II-receptor antagonists</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Thiazide</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Aldosterone receptor antagonist</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>( \beta ) blocker</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: ACE, angiotensin-converting enzyme.
+ VII + X. No subjects were excluded based on these criteria. After the physical evaluation, subjects underwent whole body Dual X Ray Absorption scan.

### 2.5 Experimental day

Subjects arrived at the laboratory at ~8 AM and placed supine on a bed to rest. Subjects were, prior to the experimental day, instructed to eat their normal breakfast. After sterilization and local anaesthesia (lidocaine, 20 mg mL⁻¹; Astra Zeneca, Denmark) a catheter (20 gauge, Becton Dickinson Infusion Therapy Systems, UT, USA) was placed in the brachial artery and an antecubital vein (Venflon, 20 gauge, Becton Dickinson Infusion Therapy Systems, UT, USA) of the experimental arm. A 3-port connector (Connecta, Becton Dickinson Infusion Therapy Systems, UT, USA) was connected to allow simultaneous infusion and measurement of blood pressure. Thirty minutes after placement of the catheters, 2D ultrasound images (20 s) were recorded to assess arterial compliance. After 2D-measurements, subjects received continuous infusions of acetylcholine (ACh; Miochol-E, Bausch & Lomb Inc., Berlin, Germany; 10, 25 and 100 μg min⁻¹ [kg arm-mass]⁻¹), sodium nitroprusside (SNP; Meda, Ballerup, Denmark; 1.5, 3 and 6 μg min⁻¹ [kg arm-mass]⁻¹) and isoprenaline (ISO, Macure Pharma, Copenhagen, Denmark; 10, 30 and 60 ng min⁻¹ [kg arm-mass]⁻¹). ACh and isoprenaline were dissolved in isotonic saline and SNP in 200 mg/mL glucose solution. Thirty-second recordings of brachial arterial blood flow and arterial and venous pressure were carried out 2 min after the effect of the first infusion dose for each vasodilator and again 2 min after each increase in continuous infusion. Between the different vasodilator drug infusions, subjects rested for 15 min to ensure washout.²⁷,²⁸

The order of infusions was the same for all subjects and experimental days, before and after the intervention. After completion of the infusions, subjects received either 0.5 mg of colchicine or placebo and rested for 1 h and 15 min after which the infusion protocol was repeated. After the 3-week colchicine treatment, a washout period of 48–72 h was implemented to avoid a potential acute effect of colchicine. The timing of measurements was based on previous findings of colchicine pharmacokinetics.²⁷,²⁸

### 2.6 Blood flow, invasive blood pressure and vascular conductance

Blood flow was measured ~2 cm proximal to the placement of the catheter-tip by ultrasound Doppler (GE Vivid E9; GE Healthcare, Chicago, Illinois, USA), L-9 linear probe, Doppler frequency of 5.6 MHz and an imaging frequency of 12.0 MHz. Intra-arterial and intravenous pressure recordings were obtained via a pressure transducer positioned at the level of the catheters (Pressure Monitoring Set; Edwards Lifesciences, Irvine, CA, USA) and recorded and stored in LabChart 8 (ADinstruments, Sydney, Australia). For detailed description of the measurements and analysis, see Rytter et al. ²⁹

### 2.7 The 2D ultrasound vascular compliance

Brachial artery diameter was continuously recorded for a period of 32 s using an ultrasound device (GE Vivid E9; GE Healthcare, Chicago, Illinois, USA) equipped with a 12.0 MHz L-11 linear array transducer. The ultrasound DICOM files were imported into the Brachial Analyzer software (Medical Imaging Applications LLC, Coralville, Iowa, USA), and a region of interest was set for a single frame, in which the best definition of the edges of the brachial artery was detectable. The region of interest included both sides of the lumen, and the inner vessel wall was marked to measure brachial artery diameter. The analysis was launched, and a continuous measurement of brachial artery diameter in all frames was determined for the entire recording. Borders were adjusted manually per frame for images where vessel border was not computed correctly. The diameter changes over time were imported into LabChart 8 (ADinstruments, Sydney, Australia) for alignment with the intra-arterial blood pressure recordings, where arterial compliance was calculated as follows:

\[
\text{Compliance} = \left[ \pi \left( \frac{\text{diameter max}}{2} \right)^2 - \pi \left( \frac{\text{diameter min}}{2} \right)^2 \right] / \Delta P
\]

where \(\Delta P\) is the intra-arterial pulse pressure.

To determine the stiffness of the arterial wall, \(\beta\)-stiffness was calculated as follows:

\[
\beta - \text{stiffness} = \frac{(\ln(\Delta P))}{\left[ \left( \pi \left( \frac{\text{diameter max}}{2} \right)^2 - \pi \left( \frac{\text{diameter min}}{2} \right)^2 \right) / \pi \left( \frac{\text{diameter min}}{2} \right)^2 \right]}
\]

A minimum of 5 cardiac cycles were selected to determine the arterial compliance.

### 2.8 Brachial artery pressure measurements

Subjects carried out resting measurements of brachial artery pressure at home after careful instruction. Subjects measured blood pressure with automated upper-arm blood pressure monitor (Cuff size: 22–45 cm Intelli Wrap Cuff, Omron Healthcare, Kyoto, Japan) on 3 separate mornings and 3 separate evenings after at least 10 min of supine rest in quiet conditions. Resting blood pressure was calculated as a mean over a total of 36 measurements. Subjects continued their antihypertensive medication throughout the study. Therefore, the reported results represent subject blood pressure while on antihypertensive medication.

### 2.9 Analysis of inflammatory markers in plasma

Analysis of plasma levels of high-sensitivity CRP (hsCRP), interleukin (IL)-18 and tumour necrosis factor (TNF)-α was conducted at the University Hospital of Copenhagen, Rigshospitalet.
2.10 | Statistical analyses

All statistical analyses were performed using RStudio (version 1.3.1073; Boston, MA). ANOVA was used to determine overall effects of group (COL and PLAC), time (pre vs. acute/3 week) and infusion (ISO, SNP and ACh). A linear mixed-model approach was used to analyse differences in the effect of colchicine vs. placebo (acute and 3-week treatment) on the response to ISO, SNP and ACh infusions. Group, time and infusion were set as fixed factors and subject as a random factor with Tukey’s post hoc test and Bonferroni–Holm adjustments applied. Residual and Q-Q plots were used to confirm the homogeneity of covariance and normal distribution. *P* < .05 was considered statistically significant. Graphs were made using GraphPad Prism 9 (GraphPad Software; La Jolla, CA, USA).

### 3 | RESULTS

#### 3.1 | Baseline subject characteristics

Baseline subject characteristics are included in Table 2. There were no statistical differences between the groups.

#### 3.2 | Resting vascular conductance and intra-arterial blood pressure

At baseline, there were no differences in resting vascular conductance between the acute colchicine (1.29 ± 0.62 mL/min/mmHg) and the placebo (1.07 ± 0.52 mL/min/mmHg) groups (Figure 1A; *P* = .58) or between the 3-week colchicine (1.36 ± 0.64 mL/min/mmHg) and 3-week placebo (1.28 ± 0.69 mL/min/mmHg) groups (Figure 1B; *P* = .92). There was no change in resting conductance in either group following the acute (Figure 1A, *P* = .84) or the 3-week intervention (Figure 1B, *P* = .74).

There were no differences in the baseline resting mean intra-arterial pressure between the acute colchicine (103 ± 14 mmHg) and the placebo (103 ± 13 mmHg) groups (Figure 1C, *P* = .73) or between the 3-week colchicine (102 ± 7 mmHg) and the 3-week placebo (103 ± 12 mmHg) groups (Figure 1D, *P* = .22). There was no change in blood pressure within either group following the acute (Figure 1C), *P* = .82) or the 3-week intervention (Figure 1D, *P* = .67). It may be noted that, after the 3-week colchicine treatment period, 11 out of 16 subjects displayed lower mean intra-arterial pressure, whereas, in the placebo group, 8 out of 15 subjects displayed lower mean arterial pressure. The nonsignificant mean changes in blood pressure were −3 mmHg in the colchicine group (102 ± 8 mmHg vs. 99 ± 9 mmHg, baseline and 3 weeks of colchicine treatment, respectively), whereas in the placebo group, the difference was +2 mmHg (103 ± 12 vs. 105 ± 16 in baseline vs. 3 weeks of placebo treatment, respectively).

#### 3.3 | Acute colchicine treatment improves vascular conductance to isoprenaline and SNP but not ACh

Following the single, acute colchicine/placebo treatment, the isoprenaline-mediated change in vascular conductance was overall higher (time effect *P* < .00012) than before treatment. Within the colchicine treatment group, there was an overall isoprenaline-mediated change in conductance after the acute intervention compared with before (pre; time effect *P* < .00045) but not within the placebo group (P = .059 Figure 2A). Paired comparison between before and after the acute intervention of the different infusion doses post intervention revealed a significant (P < .05) difference at the 60 ng min⁻¹ (kg arm-mass)⁻¹ isoprenaline dose in the colchicine group (Figure 2A). At this dose, every subject, after receiving colchicine, displayed an increase in vascular conductance, compared with their baseline change (pre).

The acute treatment resulted in a significantly greater overall (both acute colchicine and placebo treatment groups) vasodilator response to SNP (time effect: *P* = .032) compared with the baseline changes (pre). There was an SNP-mediated change in conductance after the acute intervention compared with baseline changes within the colchicine group (time effect *P* = .017) but not within the placebo group (P = .85; Figure 2B).

Acute colchicine treatment had no effect overall (P = .23) on the change in arm vascular conductance in response to ACh infusion (Figure 2C).

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Baseline characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute colchicine</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.1 ± 4.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 2.7</td>
</tr>
<tr>
<td>SYS BP (mmHg)</td>
<td>135.3 ± 14.6</td>
</tr>
<tr>
<td>DIA BP (mmHg)</td>
<td>84.3 ± 8.3</td>
</tr>
<tr>
<td>Fat%</td>
<td>30.8 ± 5.7</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>60.5 ± 5.0</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>37.5 ± 3.1</td>
</tr>
<tr>
<td>Invasive MAP (mmHg)</td>
<td>102.9 ± 13.9</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index; SYS BP, systolic blood pressure; DIA BP, diastolic blood pressure; FFM, fat-free mass; HbA1c, glycated haemoglobin, MAP, mean arterial pressure.
3.4 | After 3 weeks of treatment, followed by a washout period, colchicine had no effect on isoprenaline-, SNP- or ACh-induced vascular conductance changes

After the 3-week period of low-dose colchicine or a placebo treatment, a 48–72-h washout period was allowed prior to measurements. This washout period allowed us to determine whether the colchicine treatment had resulted in a sustained, enhanced vasodilator-stimulated change in vascular conductance, in the absence of colchicine in plasma (the acute effect). Following the 3-week colchicine/placebo treatment, there was no overall change in the isoprenaline, SNP or ACh-mediated changes in vascular conductance when compared with the baseline changes (time effect \( P = .21, P = .88 \) and \( P = .75 \), respectively, Figure 3). When comparing the vascular conductance changes, both at baseline and after 3-week treatment, to SNP between colchicine and placebo groups, a significant difference was observed (group effect: \( P < .001 \), COL vs. PLAC at 3-week, \( P = .038 \)); however, there was no difference in the time-effect (pre vs. 3 weeks) within either group (\( P = .59 \) and \( P = .60 \); Figure 3B).

3.5 | Vascular compliance

There were no differences in \( \beta \)-stiffness, distensibility or EP pressure strain modulus between or within either group at baseline, after acute colchicine treatment or after 3 weeks of daily colchicine treatment (see Table 3).

3.6 | Resting blood pressure—home measurements

At baseline, there were no differences between the colchicine and the placebo groups in home measurements of systolic, diastolic or mean arterial pressure (Table 4). The 3-week colchicine treatment did not affect resting blood pressure, and the levels were not different from placebo (see Table 4).

3.7 | Inflammatory markers

At baseline, there were no differences between the colchicine and the placebo group in plasma levels of either hsCRP, IL-18 or TNF-\( \alpha \)
**FIGURE 2**  Acute changes in arm vascular conductance. Changes in arm vascular conductance in the colchicine (left) and the placebo group (right) during infusions of isoprenaline (A), sodium nitroprusside (B) and acetylcholine (C), before and after the acute intervention. ¥ indicates differences of within group time-effect \( P < .05 \), ### indicates differences of within group time-effect \( P < .001 \), * indicates Tukey’s post hoc test with Bonferroni–Holm adjustment of \( P < .05 \).

**FIGURE 3** Three-week changes in arm vascular conductance. Changes in arm vascular conductance in the colchicine (left) and the placebo group (right) during infusions of isoprenaline (A), sodium nitroprusside (B) and acetylcholine (C), before and after the 3-week intervention. £ indicates overall group difference \( P < .05 \).
There was no change in either group for any of the inflammatory markers after the 3-week intervention period (Table 5). Not all inflammatory markers were detectable for all subjects: for IL-18 and hsCRP \( n = 14 \) and \( n = 11 \) COL and PLAC, respectively. For TNF-\( \alpha \) \( n = 8 \) and \( n = 5 \) COL and PLAC, respectively.

### 4 | DISCUSSION

In this study, we investigated the effect of colchicine on vascular reactivity to isoprenaline, SNP and Ach in humans after a single, acute dose and after 3 weeks of daily, low-dose treatments. Furthermore, the effects of colchicine on mean arterial pressure, arterial compliance and inflammatory markers associated with hypertension were determined. Our data provide the first evidence that colchicine can improve \( \beta \) adrenoceptor-mediated as well as NO-mediated arterial vasodilatation in middle-aged men with essential hypertension. Treatment with colchicine twice daily for a 3-week period, followed by a 48–72-h washout period, had no effect on microvascular function, blood pressure, inflammatory markers or vascular compliance.

In the current study, we report that the isoprenaline-induced increase in vascular conductance was enhanced in humans with hypertension after a single dose of colchicine, with colchicine improving the vascular conductance in every patient at the highest dose of isoprenaline, compared with baseline. In previous studies on rodents, our laboratory showed that colchicine enhanced the isoprenaline-mediated relaxation in isolated third-order mesenteric arteries and conduit renal arteries. These enhanced relaxations were associated with an increased functional contribution of the voltage-gated Kv7 channels, which are known to be activated following agonist binding to various Gs protein-coupled receptors, including the \( \beta \) adrenoceptor.\(^{13,21-25}\) Further analysis revealed that membrane abundance of the Kv7.4 channel was increased in vascular smooth muscle cells following colchicine treatment, and recently, a dynein-dependent mechanism was established to underlie the observed colchicine effect on Kv7.4 trafficking.\(^{26}\) More recently, we investigated the effect of colchicine on isolated mesenteric arteries from the SHR.\(^{10}\) In this study, we found that colchicine could restore the Kv7 activator- and isoprenaline-dependent relaxations,\(^{10}\) which are attenuated in these arteries from the SHR compared to the normotensive control rats.\(^{13,14}\)
Interestingly, this study found that colchicine not only improved Kv7.4 channel function in the SHR arteries but also promoted β2 adrenoceptor membrane abundance and function, which replaced attenuated β1 adrenoceptor function to reinstate the isoprenaline relaxation. Our current study shows that this previous evidence in rodents, on colchicine improving β adrenoceptor vasodilatation, translates to humans, but whether this effect was due to an increase in Kv7.4 channel and β2 adrenoceptor function remains to be determined. Our finding of improved isoprenaline-mediated vasodilatation by oral administration of colchicine provides a novel mechanism in humans that likely contributes to the known cardiovascular benefits of colchicine treatment.

To assess the effect of acute colchicine on endothelial-dependent vasodilation and on smooth muscle cell sensitivity to NO, the changes in vascular conductances to ACh and SNP infusion were also determined. These experiments revealed, unexpectedly, that acute colchicine treatment enhanced the vasodilatation in response to arterial infusions of the NO donor SNP. We have not investigated the effect of colchicine on SNP-induced vasodilations in vitro; therefore, the mechanism underlying this effect can only be speculated on. We have reported previously that certain cGMP-mediated relaxations occur partially through the activation of Kv7 channels in different rodent arteries. In the rat aorta, SNP-relaxations were impaired by the Kv7 channel inhibitor, linopirdine, but not in the renal artery. It is possible that, as described previously, colchicine treatment enhanced Kv7 channel function in the smooth muscle cells of the arterial wall, which could lead to enhanced vascular conductance changes with SNP. Additionally, Kv7 channels have been implicated in the vasodilator response to NO donors in pulmonary hypertension. Further studies are required to understand this mechanism. The finding that colchicine improved the vascular conductance changes to SNP but had no effect on ACh-induced changes may, at first, appear contradictory. However, in resistance arterioles, ACh induces vasodilatation through several mediating mechanisms, such as prostacyclin formation and endothelial hyperpolarization; thus, the vasodilatation by ACh depends only partly on NO. Therefore, direct infusion of SNP provides a more direct and sensitive means of assessing the effect of colchicine on smooth muscle cell sensitivity to NO.

To determine whether a short-term period colchicine treatment would induce a sustained enhancement of β adrenoceptor-mediated vasodilation, the vascular measurements were repeated after 3 weeks of 0.5 mg colchicine treatment twice daily. The measurements were conducted after a washout period of 48–72 h to avoid interference with the acute effect of treatment. After 3 weeks of treatment, we observed no significant difference in vascular conductance changes to isoprenaline infusion nor were the vasodilator responses to ACh or SNP infusions different. This finding indicates that colchicine may have 2 separate effects on vascular function: a short-lasting effect by improving β adrenoceptor and NO responsiveness and a long-lasting effect involving the reduction of inflammatory mediators associated with cardiovascular disease. In the present study, we deliberately chose a short treatment period to minimize the potential anti-inflammatory effect of colchicine, thereby allowing us to study the mechanism we have previously described in rodents. Accordingly, although an effect of colchicine on inflammation cannot be completely excluded, there was no change in the inflammatory markers hsCRP, IL-18 and TNF-α after the 3-week intervention.

Although several trials have been undertaken to investigate the cardiovascular protective effect of daily, low-dose colchicine, none have included blood pressure as an outcome. Therefore, it remains unclear whether colchicine can be used to lower blood pressure in hypertension, a major risk factor for cardiovascular disease. A study from Lagrue et al. showed that colchicine treatment for 3–4 months increased the time of the dicrotic notch in 51 hypertensive patients, suggesting that colchicine treatment improved the elasticity of the arteries. No placebo group was used in the study, and no significant change in blood pressure was detected. In our study, the 3-week treatment period had no significant effect on arterial compliance or on blood pressure; the latter was measured at home over 3 days and also measured intra-arterially on the experimental day. This lack of effect may have been due to the relatively short duration of the treatment period combined with the washout period. It should be noted that arterial pressure and arterial compliance were not primary outcomes of this study. Additionally, the intervention period was deliberately brief to allow us to determine a potential accumulated and lasting effect of colchicine in the absence of its anti-inflammatory effects. However, it would be interesting for future studies to determine whether the vascular conductance effects observed in the acute study are maintained and enhanced in patients after 3 weeks, without a washout period. Further studies have been initiated to investigate whether daily, low-dose colchicine over a longer period than just 3 weeks can improve arterial stiffness and blood pressure in hypertensive patients (https://clinicaltrials.gov/ct2/show/NCT04916522).

5 | CONCLUSION

In conclusion, colchicine acutely increases β adrenoceptor and NO-mediated vasodilation in humans, supporting that the mechanism we have demonstrated previously in rodent studies translates to humans. This is the first translational evidence for this mechanism, and the findings suggest that at least part of the cardiovascular effect of colchicine may be mediated by an acute effect on vascular resistance. Future studies should provide insight to dose-responses and more precise time frames of the effect, as well as direct evidence of the dynamics of vascular Kv7 channels in human tissue before and after colchicine treatment. Furthermore, larger scale clinical trials should provide insight into the clinical relevance of longer-term colchicine treatment on vascular health in hypertensive patients.

CONTRIBUTORS
The study was carried out at the University of Copenhagen, Department for Nutrition, Exercise and Sports. Thomas S. Ehlers, Jennifer...
van der Horst, Thomas A. Jepps, Christian Aalkjær, Peter K. Piil and Ylva Hellsten designed and planned the study. Thomas S. Ehlers, Jennifer van der Horst and Lasse Gliemann were responsible for data acquisition. Thomas S. Ehlers, Jennifer van der Horst, Sophie Møller, Lasse Gliemann, Thomas A. Jepps and Ylva Hellsten analysed, interpreted and prepared data. Thomas S. Ehlers, Jennifer van der Horst, Thomas A. Jepps and Ylva Hellsten drafted the work. Thomas S. Ehlers, Jennifer van der Horst, Sophie Møller, Peter K. Piil, Lasse Gliemann, Christian Aalkjær, Thomas A. Jepps and Ylva Hellsten all did critical revision of the work for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All listed persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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COMPETING INTERESTS

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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