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Dapagliflozin prevents vascular ischemia-reperfusion injury in healthy young males: a randomized, placebo-controlled, double-blinded trial

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Ischemia-reperfusion injury (IRI) causes vascular endothelial dysfunction. Preclinical data suggest that the SGLT2 inhibitor dapagliflozin may protect against vascular IRI. This trial has investigated if oral treatment with dapagliflozin can mitigate the transient impairment of IRI-induced-endothelial dysfunction in the forearm resistance vasculature. 32 healthy males ($n = 16$ per group, age: 27 ± 4 yrs) were studied in this randomized, placebo-controlled, parallel-group, double-blinded trial. Acetylcholine (ACh; endothelium-dependent vasodilator) and glyceryltrinitrate (GTN; endothelium-independent vasodilator) were administered into the brachial artery of the non-dominant arm. The response to stepwise increasing doses on forearm blood flow (FBF) was assessed. FBF was measured before and after a cuff-induced 20-minute forearm ischemia at pre-dose and following daily intake of 10 mg dapagliflozin or placebo over 15 days. IRI reduced endothelium-dependent vasodilatation by 29% ($p < 0.001$, paired t-test). After a 15-day treatment period, IRI-induced endothelial dysfunction was abrogated in participants receiving dapagliflozin (FBF ACh_{AUC} ratios post- vs. pre-ischemia: dapagliflozin: 0.93; 95% CI: 0.80–1.29) but unchanged with placebo (0.81; 95% CI: 0.68–0.92; $p = 0.015$ vs. pre-ischemia). GTN-induced vasodilation was not altered by IRI or treatment. Dapagliflozin treatment at standard clinical doses over 15 days prevents IRI-induced vascular endothelial dysfunction in the forearm resistance vasculature of healthy young males. The underlying mechanism and the potential clinical impact remain to be demonstrated.

Clinical trial registration https://clinicaltrials.gov/study/NCT05217654 NCT05217654; EudraCT number: 2021-005002-95 Date of registration: 20/01/2022.

Keywords Ischemia, Vascular function, Ischemia reperfusion injury, SGLT2-inhibitor

Abbreviations

Ach	Acetylcholine
AE	Adverse Event
AMPK	Adenosine Monophosphate-Activated Kinase
AUC	Area Under the Curve
cGMP	Cyclic Guanosine Monophosphate
ED	Endothelial Dysfunction
eNOS	Endothelial Nitric Oxide Synthetase
ESC	European Cardiac Society
FBF	Forearm Blood Flow
GTN	Glyceryltrinitrate
IRI	Ischemia Reperfusion Injury

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LAD	Left Anterior Descend Coronary Artery
MAPK	Mitogen-Activated Protein Kinase
NO	Nitric Oxide
NOX	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
ROS	Reactive Oxygen Species
SD	Standard Deviation
SGLT2	Sodium-Glucose-Cotransporter 2

Vascular ischemia-reperfusion injury (IRI) is characterized by a reduction of endothelium dependent vasodilation and may be reversible within days or weeks depending on ischemia duration[1,2]. IRI occurs paradoxically after restoration of blood flow as it is seen in myocardial infarction (MI) following percutaneous cardiac intervention (PCI) after vessel occlusion. During IRI, cell metabolism and enzymatic reactions are altered, and micro-inflammation is observed. These pathological changes may contribute to myocardial contractile dysfunction[3–5]. While substantial progress was achieved by implementing strategies for catheter-based interventions for reperfusion, clinical evidence on treatment or prevention of IRI is not entirely convincing yet[5,6].

The primary mechanism of vascular dysfunction is a reduced NO bioavailability caused by excessive reactive oxygen species formation(ROS) which may develop into tissue damage and cell death[1,2,7]. A NO deficiency may also result from eNOS "uncoupling" and therefore loss of function[5,8]. It has been demonstrated that stimulation of eNOS may exert positive effects on IRI by restoration of endogenous enzymatic NO production[9,10]. eNOS activity may be influenced by adenosine monophosphate-activated kinase (AMPK) mediated interventions[5,8,11,12].

Dapagliflozin is a selective inhibitor of the sodium-glucose cotransporter 2 (SGLT2) which is mainly expressed in the proximal tubule of the kidney. Long term treatment with dapagliflozin has shown protective effects on proteinuria as well as hearth failure symptoms and reduced mortality[13]. A recent trial has demonstrated improved cardiometabolic outcome in patients with ST-elevation myocardial infarction by dapagliflozin in the absence of effects on overall survival or cardiovascular endpoints[14]. Several investigations in animal models have shown that dapagliflozin attenuates IRI[15–19], which has been associated with AMPK phosphorylation and expression[15]. Dapagliflozin also mitigated the mitogen-activated protein kinase (MAPK) pathway in rats which leads to apoptosis[17]. Pre-ischemia administration of dapagliflozin prior to LAD occlusion in mice reduced infarction size by 16%[16]. In a mouse model of IRI, eNOS activity was reduced and expression of endothelin 1 was increased and these alterations were reversed by dapagliflozin[18]. In diabetic mice, dapagliflozin restored vascular function via eNOS activation, mitigated oxidative stress and attenuated arterial stiffness[19].

To date, there are no data on the effect of dapagliflozin treatment on IRI in humans and it is unclear whether the results from the animal models can be extrapolated. This study aimed to investigate if treatment with dapagliflozin at a standard clinical dose can prevent the transient loss of endothelium-dependent vasodilatation of the forearm resistance vasculature following a short period of ischemia. This could serve as rationale to test the clinical application of dapagliflozin in acute vascular settings.

Methods

Objectives

We hypothesized that treatment with dapagliflozin for two weeks may prevent vascular IRI after forearm ischemia compared to placebo.

The primary objective was to test the effect of dapagliflozin compared to placebo on endothelium-dependent vasodilatation of the forearm resistance vasculature as assessed by forearm blood flow (FBF) measurement before and 10 min after a 20 min forearm ischemia. The area under the dose-effect curve (AUC) of different ACh doses was calculated and compared between treatment groups (dapagliflozin vs. placebo) and different time points (pre-ischemia vs. post-ischemia).

The secondary objective was to test the effect of dapagliflozin or placebo on endothelium-independent vasodilation before and 10 min after a 20 min forearm ischemia.

Study design

This phase I trial was conducted as a randomized, placebo-controlled, double-blind, parallel group study in 32 healthy male subjects. The study was conducted in accordance with the protocol, the principles of the Declaration of Helsinki in its current version and the European and Austrian laws and regulations. The trial was approved by the Ethics Committee of the Medical University of Vienna (EK 1968/2021) and the Austrian Competent Authority and is listed on clinicaltrials.gov (NCT05217654). Informed consent was obtained from all participants prior to study participation.

A detailed time schedule is presented in Fig. 1. A screening visit was scheduled within 3 weeks before the first trial day. Study days were conducted in a quiet room with an ambient temperature of 22° C with complete resuscitation facilities. On study day 1, the effect of local forearm ischemia on FBF reactivity without pharmacological treatment was assessed. The forearm was made ischemic by inflating a cuff to suprasystolic values (> 220mmHg) for 20 min. Before and 10 min after onset of reperfusion FBF reactivity to ACh and GTN were measured. Subjects received the first oral dose of dapagliflozin or placebo at discharge and continued treatment for 14 days. On day 15, subjects received 10 mg dapagliflozin or placebo, and the procedures (FBF reactivity, ischemia) were carried out as described on the first study day. Administration of the medication under study was scheduled 1.5 h prior to ischemia to assess the effect on FBF reactivity at the expected maximum plasma concentration of the drug under study after intake. Urine spot samples were taken to asses glucosuria and monitor therapy adherence. Results remained masked for the investigator until database lock.

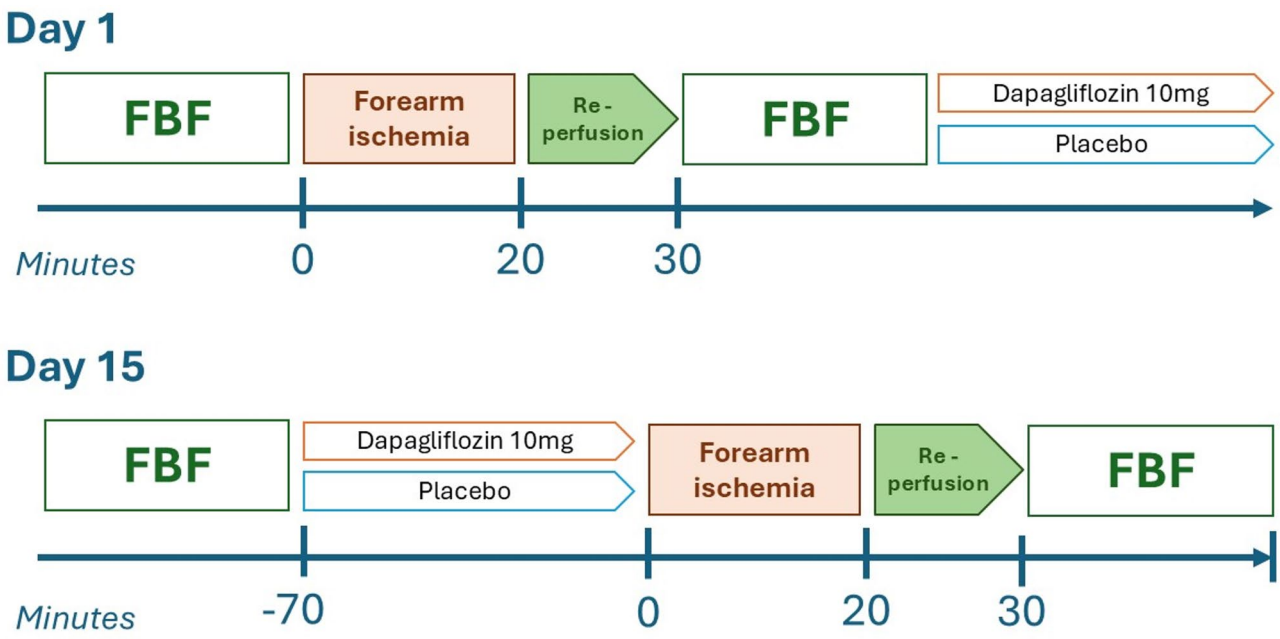

Fig. 1. Study procedure. *FBF* forearm blood flow measurement.

Population

A total of 41 healthy, non-smoking male volunteers without any concomitant medication were enrolled. Inclusion criteria were age between 18 and 40 years, normal findings in medical history and written informed consent. Subjects were excluded if there was any history of vascular disorders, diabetes, ketoacidosis, renal or liver function impairment. 32 participants were randomized and analyzed.

FBF and IRI

FBF measurement is considered as experimental gold standard for the measurement of vascular function. Mercury-filled silastic strain gauges were utilized, connected to a plethysmograph (EC-6, D.E. Hokanson Inc, USA). The strain gauge was attached to the upper part of both forearms, and inflatable cuffs were placed on both upper arms. The distance of the strain gauge from the wrist was measured to allow standardized repeated measurements at different time points.

With the forearm elevated above the level of the right atrium, rapid inflation of the upper arm venous occlusion cuff for 9 s to a supravenous pressure of 45 mmHg at every 30 s caused a linear increase in forearm volume. The following deflation period was sufficiently long to allow emptying of the forearm veins before the next measurement was taken. Upon inflation of the upper arm congesting cuff, the FBF output signal was transmitted to a computer, and traces were analyzed (NIVP 3.0, Hokanson).

Subjects remained in supine position in a quiet, air-conditioned room throughout the study. A 27-gauge needle was inserted into the brachial artery of the non-dominant arm for infusion of saline and vasoactive agents using a constant rate infusion pump (B.Braun Perfusor Compact S, Braun Austria Ges. m. b. H.). To test endothelium-dependent vasodilation, acetylcholine (ACh; Miochol-E®, Bausch & Lomb Swiss AG, Switzerland) was administered at increasing doses of 25, 50 and 100 nmol $*$ min^{-1} each for 3 min. To test endothelium-independent vasodilation, glyceryltrinitrate (GTN; Nitro POHL, G. Pohl-Boskamp GmbH & Co. KG, Germany) was administered at increasing doses of 4, 8 and 16 nmol $*$ min^{-1} each for 3 min. A washout period of 10 min was implemented between the vasoactive drug infusion[20]. Recordings spanning 6 pulse cycles were averaged for FBF analysis, and 10 cycles during saline infusion were averaged for baseline assessment. FBF was measured as mL per minute per 100 mL of forearm tissue volume and expressed as a percentage change of ratios from baseline.

Following baseline/pre-ischemia FBF measurements, the intra-arterial needle was removed, and the forearm was made ischemic as described above. Immediately after cuff release, the intra-arterial cannula was reinserted, followed by the assessment of post-ischemia FBF responses to ACh or GTN at 10 min after the onset of reperfusion.

Study medication

Dapagliflozin tablets (Forxiga® 10 mg Filmtabletten) were from AstraZeneca GmbH, Tinsdaler Weg 183, 22,880 Wedel, Germany. Placebo was produced by Auge Gottes Apotheke, Nußdorfer Straße 79, 1090 Vienna, Austria. Dapagliflozin tablets were repackaged into opaque capsules for oral administration. Dapagliflozin and placebo capsules were manufactured, filled and labeled by Auge Gottes Apotheke. The investigational products (dapagliflozin or placebo) were administered as a single daily oral dose with 250 ml of tap water. To monitor participant's adherence, time of drug intake at home and any deviation were recorded in individual diaries.

Blinding and randomization

Randomization was performed by the Auge Gottes Apotheke using the web application www.randomizer.org. Block randomization with two randomization groups (1:1 ratio between placebo and dapagliflozin) was performed. The sealed and identical looking study medication was provided to blinded staff.

Statistics

All statistical analyses were conducted using GraphPad Prism 9 (GraphPad, 225 Franklin Street. Fl. 26 Boston, MA 02110, USA). Continuous variables were summarized as mean ± standard deviation (SD) if their distributions were consistent with normality. If the distributions were not consistent with normality, the variables were summarized by their median and the 25th to 75th percentile range. The effect of ACh and GTN at FBF (mean of 6 FBF measurements during ACh or GTN infusion) was calculated as relative change of ratios over baseline for each subject individually. FBF AUC were calculated by the linear trapezoidal rule of the active dosing range consisting of 3 data points (i.e. 25, 50, 100 nmol $*$ min^{-1} for ACh, and 4, 8, 16 nmol $*$ min^{-1} for GTN, respectively). The effect of forearm ischemia and the effect of dapagliflozin or placebo after 15-day intake as well as after ischemia was expressed as post- vs. pre- ratio of FBF AUC. Changes between and within groups (dapagliflozin or placebo) were analyzed by ANOVA, post- vs. pre- ratio of FBF AUC were analyzed using two sample t-test. A two-sided P value < 0.05 was considered statistically significant.

The sample size estimation was based on previous own data[21]. Assuming a standard deviation (SD) of 20%, a sample size of 16 subjects per group was estimated to provide an 80% power to detect a mean difference of 20% between dapagliflozin and placebo with a two-sample t-test at a significance level of 5% (two-tailed).

Results

A total of 41 participants were screened to randomize 32 subjects. A study flow chart is provided in Fig. 2. Baseline characteristics of the subjects are presented in Table 2.

Results of FBF measurement are presented in Table 1. Baseline FBF ratios during saline infusion were similar between intervention and control arm ($p = 0.59$, ANOVA) and within groups at any time point ($p = 0.21$, ANVOA for placebo; $p = 0.41$, ANVOA for dapagliflozin).

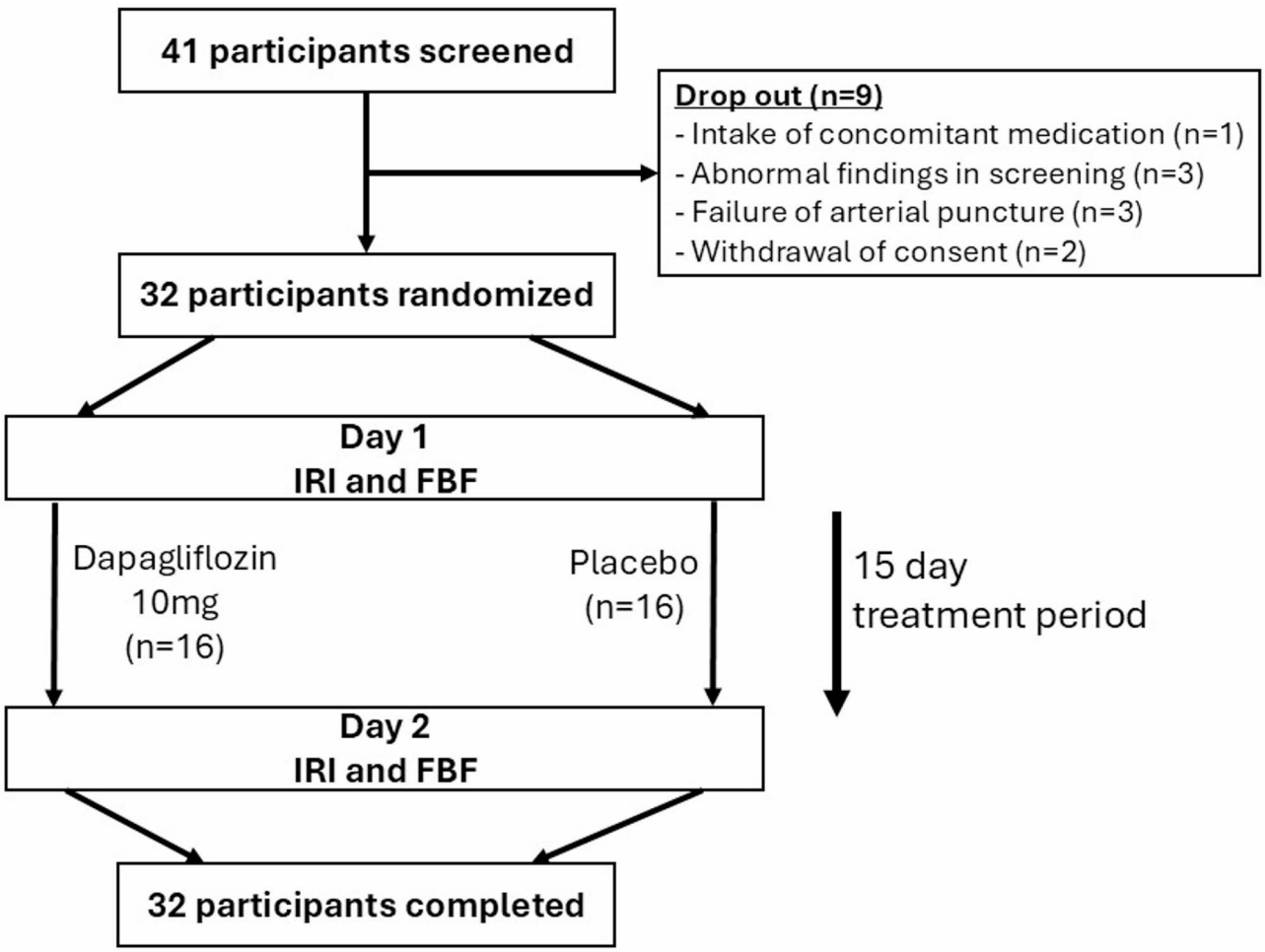

Fig. 2. Study flow chart. *IRI* ischemia reperfusion injury, *FBF* forearm blood flow measurement.

	Day 1		Day 15	
	Placebo	Dapagliflozin	Placebo	Dapagliflozin
AchAUC (% nmol min^{-1})				
Pre	1239 (1149; 1408)	1348 (1043; 1733)	1055 (925; 1391)	1061 (850; 1440)
Post	962 (889; 1143)*	805 (713; 961)*	932 (690; 1015)*†	1058 (873; 1413)
Ratio				
Post/Pre	0.88 (0.70; 0.97)	0.72 (0.50; 0.83)	0.81 (0.69; 0.91)#	0.93 (0.80; 1.27)§
GTNAUC (% nmol min^{-1})				
Pre	1136 (902; 1289)	942 (807; 1466)	1028 (739; 1315)	915 (722; 1200)
Post	1074 (851; 1374)	955 (836; 1165)	1010 (855; 1305)	1151 (951; 1413)
Ratio				
Post/Pre	0.91 (0.80; 1.20)	0.97 (0.73; 1.14)	0.93 (0.84; 1.49)	1.27 (0.99; 1.51)

Table 1. Forearm blood flow (FBF) ratios of interventional vs. control arm in response to acetylcholine (ACh) or glyceryltrinitrate (GTN); vasodilation is calculated as area under the dose versus FBF curve (AUC). AUC are presented before (Pre) and after (Post) forearm ischemia reperfusion injury (IRI) on day 1 and after treatment with Dapagliflozin or placebo for 15 days ($n = 16$ per group). Response to ischemia reperfusion injury (IRI) is given as ratio (Post/Pre). Data are presented as median and interquartile ranges; $^{*}p < 0.05$ vs. Pre (ANOVA). $^{\S}p < 0.001$ vs. Day 1 (paired t-test). $^{\dagger}p = 0.035$ between groups on day 15 (t-test). $^{\#}p = 0.015$ between groups on day 15 (t-test).

Endothelium-dependent response

Differences of ACh induced vasodilation between dapagliflozin and placebo are shown in Fig. 3; Table 1. The pre-ischemia FBF ACh_{AUC} was comparable between both groups ($p = 0.60$, t-test on day 1; $p = 0.48$, t-test on day 15). On the first study day, at 10 min after IRI, endothelium-dependent response was significantly impaired in both groups (placebo= -22% ($p = 0.0065$), dapagliflozin= -34% ($p < 0.001$), vs. pre-ischemia, $p = 0.18$ between groups; ANOVA). On the second study day, following the treatment period with dapagliflozin or placebo, ACh-induced vasodilation was no longer impaired by IRI in participants receiving dapagliflozin (FBF ACh_{AUC} ratios post- vs. pre-ischemia: dapagliflozin: 0.93; 95% CI: 0.80–1.29) but detectable with placebo (0.81 95% CI: 0.68–0.92; $p = 0.015$ vs. pre-ischemia). The post-ischemia FBF ACh_{AUC} in the dapagliflozin group was also significantly greater compared to placebo ($p = 0.035$, t-test).

Endothelium-independent response

Effect of intraarterial GTN on endothelium-independent vasodilation is shown in Fig. 4; Table 1. There were no differences in GTN_{AUC} between placebo and dapagliflozin at any time points as well as in post- vs- pre- ischemia GTN_{AUC} ratios on both study days ($p > 0.05$, ANOVA).

Effect on blood pressure

At baseline, no significant difference in blood pressure measurements were found between groups (Table 2). On the second study day, systolic blood pressure was significantly lower in the dapagliflozin group (126 ± 7 mmHg) compared to placebo (132 ± 9 mmHg) ($p = 0.04$, t-test). No significant difference was found between both study days in the dapagliflozin group ($p = 0.23$, paired t-test). (Table 3)

Effect on glucosuria

Glucosuria was not detectable in any participant on day 1. After treatment with dapagliflozin, glucosuria was found in all but one participant in the dapagliflozin group but not in those receiving placebo (Table 3). The range of glucosuria was between 50 and 300 mg/dl.

Safety

There were no study discontinuations and no replacement of participants. All adverse events (AE) reported were mild and reversible and are shown in Table 4. The study medication was well tolerated. No ketone bodies were found in urine on the second study day. The most common reported AE was sweating ($n = 2$) and polydipsia ($n = 2$) in the dapagliflozin group and common cold ($n = 2$) and tiredness ($n = 2$) in the placebo group.

Discussion

In this randomized, placebo-controlled trial in healthy young males, a 15-day intake of a therapeutic dose of dapagliflozin prevented an IRI-induced transient vascular endothelial dysfunction after forearm ischemia. This treatment was accompanied by an increase in urinary glucose excretion in most participants receiving dapagliflozin.

Endothelial dysfunction (ED) after local IRI has been described previously[21,22] and in response to triggers like inflammation[10], hypertension or metabolic alterations[5]. This was reproduced in our study which showed a substantially reduced endothelial-dependent vasodilation after 20-minute forearm ischemia. This effect was consistent across all participants under study and also seen after placebo treatment. In contrast, the vasodilating

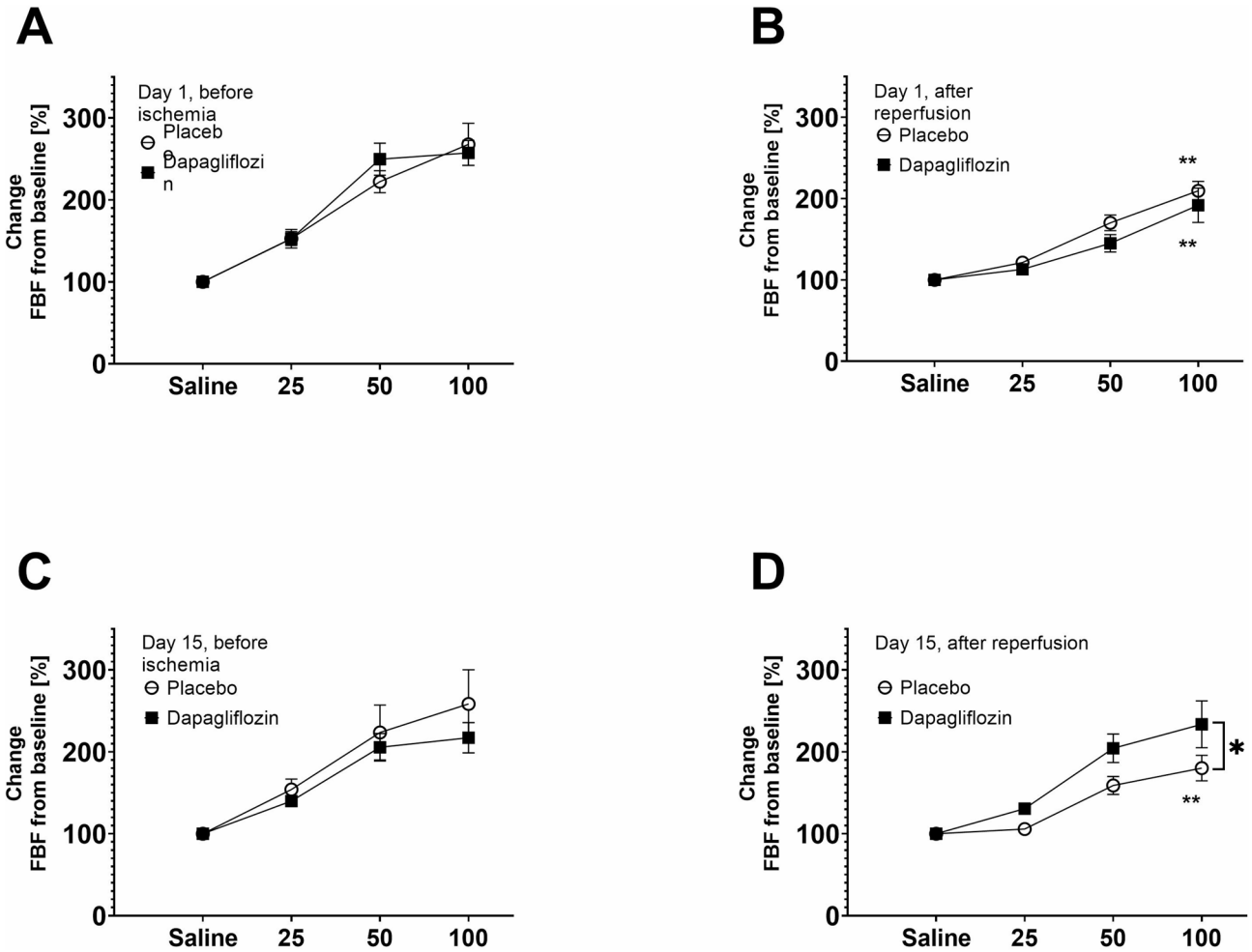

Fig. 3. Forearm blood flow (FBF) response to acetylcholine (ACh) [nmol min^{-1}]: Day 1 before (**A**) and 10 min after reperfusion (**B**) of 20 min forearm ischemia; after a 15 day treatment of dapagliflozin or placebo before (**C**) and 10 min after reperfusion of 20 min forearm ischemia (**D**); FBF ratios between intervention and control arm are presented as mean ± SEM, $n = 16$ per group. *Dapagliflozin vs. placebo, $p = 0.035$, t-test. **ACh_{AUC} after reperfusion vs. before ischemia, $p < 0.05$, ANOVA.

effect of GTN was not affected, indicating that the vasodilatory potential was not impaired. This is also in agreement with previous experiments[1,5,8,23–25].

As this trial represents a proof-of-concept study, it remains unclear if the preservation of endothelial-dependent vasodilation by dapagliflozin in our study may be driven by the AMPK – eNOS pathway without or with direct eNOS activation[5,6]. Animal data and in vitro investigations support this hypothesis: Dapagliflozin increased AMPK activity via AMPK phosphorylation[18]. Further downstream, NOX was inhibited and ROS production decreased consequently[15]. The eNOS activation was induced by phosphorylation of the Ser-1177 binding site leading to higher levels of bioactive NO[19].

Several pharmaceutical substances, for example methotrexate[26], dexamethasone[27], atorvastatin[21], allopurinol[28] or antihypertensive drugs have demonstrated beneficial effects on endothelial dysfunction in a human IRI-setting. These observations concluded that a mitigation of IRI is caused by eNOS activation or prevention of eNOS uncoupling regardless the individual mode of drug action[5].

We did not observe an improvement in pre-ischemic ACh-response after dapagliflozin treatment. These findings were expected as we included healthy participants without evidence of vascular dysfunction only. Chronic diseases like hypertension or metabolic alterations as hyperlipidemia[5,8] or insulin resistance[4,9,29] are associated with vascular endothelial dysfunction and reduced NO bioavailability. In patients with impaired glucose control and insulin resistance, chronic treatment with dapagliflozin resulted in an improved endothelial function assessed by flow-mediated dilation measurements[30]. A different magnitude of pharmacodynamic action is therefore possible in patients with concomitant disease.

Beneficial effects on cardiovascular outcome of dapagliflozin were reported in randomized controlled trials regardless the presence of type 2 diabetes mellitus[14,30–32]. The SGLT2-I AMI PROTECT Registry[33] observed patients with or without ongoing SGLT2 inhibitor therapy at time of acute myocardial infarction and percutaneous coronary intervention (PCI). SGLT2 inhibitor intake was associated with a quicker ST-elevation

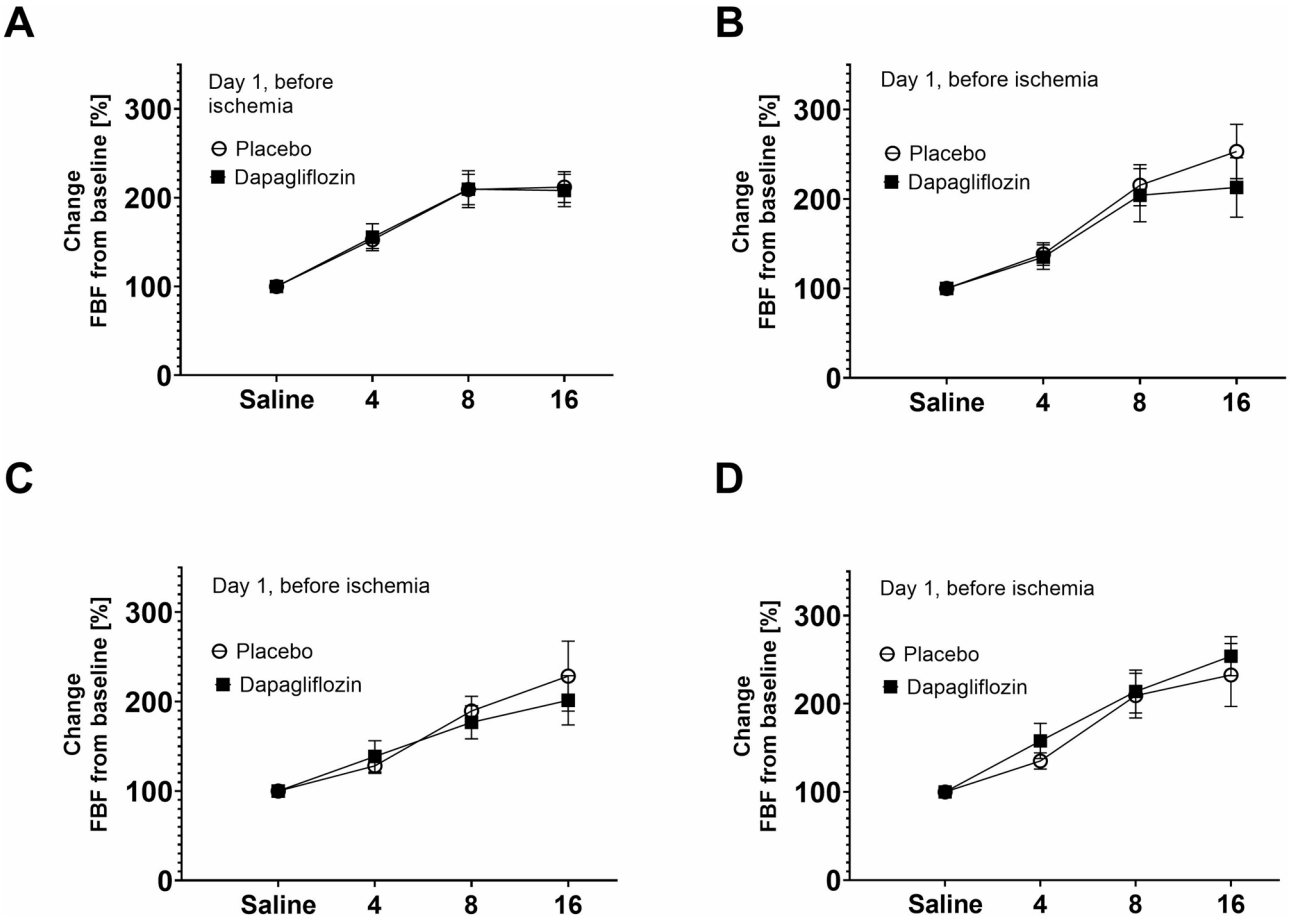

Fig. 4. Forearm blood flow (FBF) response to glyceryltrinitrate (GTN) [nmol*min^{-1}]: Day 1 before (**A**) and 10 min after reperfusion (**B**) of 20 min forearm ischemia; after a 15 day treatment of dapagliflozin or placebo before (**C**) and 10 min after reperfusion of 20 min forearm ischemia (**D**). FBF ratios between intervention and control arm are presented as mean ± SEM, $n = 16$ per group.

	Placebo	Dapagliflozin	*p*-value
Age [years]	26 ± 5	28 ± 5	0.41
BMI [kg m^{-2}]	22.7 ± 2.0	22.9 ± 1.8	0.79
SBP [mmHg]	132 ± 10	129 ± 5	0.34
DBP [mmHg]	77 ± 7	77 ± 9	0.90

Table 2. Baseline characteristics. *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure. Results are mean ± SD; $n = 16$ per group.

resolution, better left ventricular ejection fraction and lower mortality. The DAPA-CKD[32,34] trial also showed beneficial effects on kidney function and renal outcomes. Endothelial dysfunction and abnormal vasomotion as well as reduced regulation of arterial function may contribute to disease progression and mortality. In recent literature, manifold potential mechanisms of SGLT2 inhibition beyond glucose lowering effects are discussed: Inhibition of sodium-proton exchange, attenuation of oxidative stress and influence of the transcription pathways[35]. Combining this evidence with preclinical animal data[15,16,18] and our results may indicate a potential benefit in the prophylactic treatment of patients at increased risk of ischemia before vascular interventions to reduce development of endothelial dysfunction and improve clinical outcome. In particular in conditions like type 2 diabetes, where endothelial dysfunction takes place even without derangements of blood glucose[29], hypertension[36], lipid disorders[37], a SGLT2-targeted treatment approach may prevent vascular complications. Further prospective investigations are needed to evaluate the effect of dapagliflozin on IRI in a real-world cohort.

A reduction of systolic blood pressure in patients with diabetes[38] or renal diseases[39] receiving dapagliflozin was observed. This was also seen in the healthy males under study, where a lower systolic blood pressure with a mean difference of 6 mmHg was recorded after a 15-day treatment with dapagliflozin compared to placebo.

	Placebo	Dapagliflozin	*p*-value
SBP [mmHg]	132 ± 9	126 ± 7	**0.04**
DBP [mmHg]	71 ± 10	72 ± 6	0.74
Urinary ketone bodies [mmol/l]	0	0	
Glucosuria [mg/dl]			
0	16	1	
50	0	7	
100	0	3	
300	0	5	

Table 3. Clinical test results at day 15. *SBP* systolic blood pressure, *DBP* diastolic blood pressure. Glucosuria and urinary ketone bodies were assessed using semi-quantitative urine dipstick test; Glucosuria was found in all but one subject in the dapagliflozin group. Results are mean ± SD; $n = 16$ per group. Significant values are in bold.

	Placebo	Dapagliflozin
Sweating, n	0	2
Tiredness, n	2	0
Common cold, n	2	1
Thorat pain, n	1	1
Polydipsia, n	0	2
Gastrointestinal discomfort, n	1	0
Stomatitis, n	0	1
Nausea, n	1	1
Headache, n	1	0
Grass allergy, n	1	0

Table 4. Adverse events: all adverse events were mild and recovered without intervention; $n = 16$ per group.

However, no difference was detectable within the dapagliflozin group, and this has to be interpreted with caution accordingly. Nevertheless, the difference in systolic blood pressure was comparable to effects in recent trials[38,39].

Strengths and limitations

This trial has employed a robust study design and a validated model of FBF measurement[20,40]. However, effects of dapagliflozin were obtained in healthy individuals, which limits direct conclusions about the effect on vessels with functional or structural abnormalities, such as in diabetes mellitus, hyperlipidemia, high blood pressure or atherosclerotic vascular diseases. Further, the peripheral forearm vasculature can only serve as model and does not necessarily reflect vascular conditions of coronary conduct vessels or cardiac tissue ischemia. The study does not provide a direct mechanistic insight to clarify the mode of action of dapagliflozin on IRI. A direct assessment of oxidative stress and ROS or NO production is not available in vivo, and the mechanism of our observation is therefore unexplained. Further investigations are necessary to determine NO production, ROS reduction as well as clinically relevant outcomes. Finally, it is unclear if a salutary effect of dapagliflozin may be detectable even after a shorter treatment period.

Conclusion

Treatment with dapagliflozin at standard clinical doses over 15 days prevents IRI-induced vascular endothelial dysfunction in the forearm resistance vasculature of healthy young males. While underlying mechanisms need to be explored and clinical applications identified, the present results suggest new therapeutic possibilities in the management of reperfusion injury.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 4 February 2025; Accepted: 6 May 2025
Published online: 13 May 2025

References

1. Seal, J. B. & Gewertz, B. L. Vascular dysfunction in ischemia-reperfusion injury. *Ann. Vasc Surg.* **19** (4), 572–584 (2005).
2. Lefer, A. M., Tsao, P. S., Lefer, D. J. & Ma, X. L. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J. Off Publ Fed. Am. Soc. Exp. Biol.* **5** (7), 2029–2034 (1991).

3. Kleinbongard, P. & Heusch, G. A fresh look at coronary microembolization. *Nat. Rev. Cardiol.* **19** (4), 265–280 (2022).
4. Erkens, R. et al. Endothelium-dependent remote signaling in ischemia and reperfusion: alterations in the cardiometabolic continuum. *Free Radic Biol. Med.* **165**, 265–281 (2021).
5. Janaszak-Jasiecka, A., Płoska, A., Wierońska, J. M., Dobrucki, L. W. & Kalinowski, L. Endothelial dysfunction due to eNOS uncoupling: molecular mechanisms as potential therapeutic targets. *Cell. Mol. Biol. Lett.* **28** (1), 21 (2023).
6. Heusch, G. Myocardial ischemia/reperfusion: translational pathophysiology of ischemic heart disease. *Med. N Y N.* **5** (1), 10–31 (2024).
7. Yellon, D. M. & Hausenloy, D. J. Myocardial reperfusion injury. *N Engl. J. Med.* **357** (11), 1121–1135 (2007).
8. Siragusa, M. & Fleming, I. The eNOS signalosome and its link to endothelial dysfunction. *Pflugers Arch.* **468** (7), 1125–1137 (2016).
9. Shinozaki, K. et al. Oral administration of tetrahydrobiopterin prevents endothelial dysfunction and vascular oxidative stress in the aortas of insulin-resistant rats. *Circ. Res.* **87** (7), 566–573 (2000).
10. Mittermayer, F. et al. Tetrahydrobiopterin corrects Escherichia coli endotoxin-induced endothelial dysfunction. *Am. J. Physiol. Heart Circ. Physiol.* **289** (4), H1752–1757 (2005).
11. Chen, Z. P. et al. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett.* **443** (3), 285–289 (1999).
12. Zippel, N. et al. Endothelial AMP-Activated kinase A1 phosphorylates eNOS on Thr495 and decreases endothelial NO formation. *Int. J. Mol. Sci.* **19** (9), 2753 (2018).
13. Thomas, M. C. & Cherney, D. Z. I. The actions of SGLT2 inhibitors on metabolism, renal function and blood pressure. *Diabetologia* **61** (10), 2098–2107 (2018).
14. James, S. et al. Dapagliflozin in myocardial infarction without diabetes or heart failure. *NEJM Evid.* **3** (2), EVIDoa2300286 (2024).
15. Tsai, K. L. et al. Dapagliflozin attenuates hypoxia/reoxygenation-caused cardiac dysfunction and oxidative damage through modulation of AMPK. *Cell. Biosci.* **11** (1), 44 (2021).
16. Lahnwong, S. et al. Acute Dapagliflozin administration exerts cardioprotective effects in rats with cardiac ischemia/reperfusion injury. *Cardiovasc. Diabetol.* **19** (1), 91 (2020).
17. Chen, W. et al. Dapagliflozin alleviates myocardial ischemia/reperfusion injury by reducing ferroptosis via MAPK signaling Inhibition. *Front. Pharmacol.* **14**, 1078205 (2023).
18. Ma, L. et al. SGLT2 inhibitor Dapagliflozin reduces endothelial dysfunction and microvascular damage during cardiac ischemia/reperfusion injury through normalizing the XO-SERCA2-CaMKII-coffilin pathways. *Theranostics* **12** (11), 5034–5050 (2022).
19. Tai, S. et al. Dapagliflozin impedes endothelial cell senescence by activating the SIRT1 signaling pathway in type 2 diabetes. *Heliyon* **9** (8), e19152 (2023).
20. Benjamin, B. et al. Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertens. Dallas Tex. 1979.* **25** (5), 918–923 (1995).
21. Weisshaar, S., Litschauer, B., Kerbel, T. & Wolzt, M. Atorvastatin combined with Ticagrelor prevent ischemia-reperfusion induced vascular endothelial dysfunction in healthy young males - A randomized, placebo-controlled, double-blinded study. *Int. J. Cardiol.* **255**, 1–7 (2018).
22. Kharbanda, R. K. et al. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. *Circulation* **103** (12), 1624–1630 (2001).
23. Alhejily, W., Aleksi, A., Martin, B. J. & Anderson, T. J. The effect of ischemia-reperfusion injury on measures of vascular function. *Clin. Hemorheol Microcirc.* **56** (3), 265–271 (2014).
24. Eltzschig, H. K. & Eckle, T. Ischemia and reperfusion—from mechanism to translation. *Nat. Med.* **17** (11), 1391–1401 (2011).
25. Karbach, S., Wenzel, P., Waisman, A., Munzel, T. & Daiber, A. eNOS uncoupling in cardiovascular diseases–the role of oxidative stress and inflammation. *Curr. Pharm. Des.* **20** (22), 3579–3594 (2014).
26. Ma, Y. et al. Methotrexate improves perivascular adipose tissue/endothelial dysfunction via activation of AMPK/eNOS pathway. *Mol. Med. Rep.* **15** (4), 2353–2359 (2017).
27. Zhang, J. et al. Glucocorticoid receptor agonist dexamethasone attenuates renal ischemia/reperfusion injury by up-regulating eNOS/iNOS. J Huazhong univ sci technol med sci Hua Zhong Ke Ji Xue Xue Bao Yi Xue Ying Wen ban Huazhong Keji Daxue Xuebao Yixue Yingdewen ban. ;**34**(4):516–520. (2014).
28. Lee, W. Y., Koh, E. J. & Lee, S. M. A combination of ischemic preconditioning and allopurinol protects against ischemic injury through a nitric oxide-dependent mechanism. *Nitric Oxide Biol. Chem.* **26** (1), 1–8 (2012).
29. Abd-Elmoniem, K. Z. et al. Endothelial dysfunction in Youth-Onset type 2 diabetes: A clinical translational study. *Circ. Res.* **135** (6), 639–650 (2024).
30. Shigiyama, F. et al. Effectiveness of Dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. *Cardiovasc. Diabetol.* **16** (1), 84 (2017).
31. Jhund, P. S. et al. Dapagliflozin across the range of ejection fraction in patients with heart failure: a patient-level, pooled meta-analysis of DAPA-HF and DELIVER. *Nat. Med.* **28** (9), 1956–1964 (2022).
32. Bonaca, M. P. et al. Dapagliflozin and cardiac, kidney, and limb outcomes in patients with and without peripheral artery disease in DECLARE-TIMI 58. *Circulation* **142** (8), 734–747 (2020).
33. Paolisso, P. et al. Outcomes in diabetic patients treated with SGLT2-Inhibitors with acute myocardial infarction undergoing PCI: the SGLT2-I AMI PROTECT registry. *Pharmacol. Res.* **187**, 106597 (2023).
34. Wheeler, D. C. et al. Effects of Dapagliflozin on major adverse kidney and cardiovascular events in patients with diabetic and non-diabetic chronic kidney disease: a prespecified analysis from the DAPA-CKD trial. *Lancet Diabetes Endocrinol.* **9** (1), 22–31 (2021).
35. Heusch, G. & Kleinbongard, P. The enigmata of cardioprotection with SGLT2 Inhibition. *JACC Basic. Transl Sci.* **10** (1), 62–64 (2025).
36. Mordi, I., Mordi, N., Delles, C. & Tzemos, N. Endothelial dysfunction in human essential hypertension. *J. Hypertens.* **34** (8), 1464–1472 (2016).
37. Rhee, M., Lee, J., Lee, E. Y., Yoon, K. H. & Lee, S. H. Lipid variability induces endothelial dysfunction by increasing inflammation and oxidative stress. *Endocrinol. Metab. Seoul Korea.* **39** (3), 511–520 (2024).
38. Ghanim, H. et al. Dapagliflozin reduces systolic blood pressure and modulates vasoactive factors. *Diabetes Obes. Metab.* **23** (7), 1614–1623 (2021).
39. Heerspink, H. J. et al. Dapagliflozin and blood pressure in patients with chronic kidney disease and albuminuria. *Am. Heart J.* **270**, 125–135 (2024).
40. Maruhashi, T., Kihara, Y. & Higashi, Y. Assessment of endothelium-independent vasodilation: from methodology to clinical perspectives. *J. Hypertens.* **36** (7), 1460–1467 (2018).

Acknowledgements

We are grateful for the administrative work and assistance of Claudia Eder and Carola Fuchs.

Author contributions

M. L.: drafting of manuscript, acquisition of data, statistical analysis and interpretation of data; S. W.: acquisition of data, proofreading of manuscript, concept and design; B. L.:, proofreading of manuscript, concept and design, statistical analysis; M. B.: critical revision of the manuscript; J. N.: critical revision of the manuscript; M. W. study

concept and design, critical revision of the manuscript;

Funding
The Medical University of Vienna, Austria acted as sponsor of this trial;

Declarations

Competing interests
The authors declare no competing interests.

Statement of human rights
The study was conducted in accordance with the protocol, the principles of the Declaration of Helsinki in its current version and the European and Austrian laws and regulations. The trial was approved by the Ethics Committee of the Medical University of Vienna (EK 1968/2021) and the Austrian Competent Authority and is listed on clinicaltrials.gov (NCT05217654), date of registration: 20/01/2022. Informed consent was obtained from all participants prior to study participation.

Additional information
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