Exercise Training Lowers Arterial Blood Pressure Independently of Pannexin 1 in Men with Essential Hypertension

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ABSTRACT

MØLLER, S., C. C. HANSEN, T. S. EHLERS, A. TAMARIZ-ELLEMANN, S. Á. R. TOLBORG, M. E. KURELL, J. PÉREZ-GÓMEZ, S. S. PATRZALEK, C. MAULITZ, Y. HELLSTEN, and L. GLIEMANN. Exercise Training Lowers Arterial Blood Pressure Independently of Pannexin 1 in Men with Essential Hypertension. *Med. Sci. Sports Exerc.*, Vol. 54, No. 9, pp. 1417–1427, 2022. **Introduction:** Regular exercise training reduces arterial blood pressure, but the underlying mechanisms are unclear. Here, we evaluated the potential involvement of pannexin 1, an ATP releasing channel, in the blood pressure–reducing effect of training. **Methods:** Middle-age men, 13 normotensive and 14 nonmedicated stage 1 hypertensive, completed 8 wk of intensive aerobic cycle training. Before and after training, blood pressure and changes in leg vascular conductance, induced by femoral arterial infusion of tyramine (induces endogenous noradrenaline release), acetylcholine, or sodium nitroprusside, were measured during control conditions and after acute pannexin 1 inhibition by probenecid. A skeletal muscle biopsy was obtained from the thigh, pre- and posttraining. **Results:** Exercise training reduced mean systolic and diastolic blood pressure by ~5 ($P = 0.013$) and 5 mm Hg ($P < 0.001$), respectively, in the hypertensive group only. The reduction in blood pressure was not related to changes in pannexin 1 function because mean arterial blood pressure and tyramine-induced vasoconstriction remain unaltered by pannexin 1 inhibition after training in both groups. After training, pannexin 1 inhibition enhanced leg vascular conductance in the normo- and hypertensive groups at baseline (41.5%, $P = 0.0036$, and 37.7%, $P = 0.024$, respectively) and in response to sodium nitroprusside infusion (275%, $P = 0.038$, and 188%, $P = 0.038$, respectively). Training did not alter the pannexin 1 protein expression in skeletal muscle. Training enhanced the vasodilator response to acetylcholine infusion and increased the expression of microvascular function-relevant proteins. **Conclusions:** The exercise training-induced lowering of arterial blood pressure in nonmedicated hypertensive men does not involve an altered function of pannexin 1. **Key Words:** HIGH BLOOD PRESSURE, PANNEXIN 1, PHYSICAL ACTIVITY, PROBENECID, VASCULAR CONDUCTANCE

Essential hypertension is associated with physical inactivity (1), and it is well known that even short 8- to 16-wk interventions with regular physical activity can substantially lower both systolic and diastolic blood pressure in hypertensive individuals (2,3). Nevertheless, despite the well-known blood pressure–lowering effect of exercise training, the mechanisms underlying the effect remain unresolved. Blood pressure is governed by cardiac output and resistance of the vascular network. Resistance of the vascular network is determined by vascular tone which, in turn, is set by a tight balance between vasoconstrictor signals from sympathetic noradrenaline release and locally produced vasodilator substances like nitric oxide. An altered balance toward more constriction can thus affect blood pressure.

Increased sympathetic vasoconstriction has been proposed to be a factor in the elevated blood pressure in essential hypertension (4), and alterations in the vasoconstrictive effect of sympathetic activity may be involved in the blood pressure–lowering effect of exercise training (5). However, recent findings suggest that exercise training reduces blood pressure only in hypertensive individuals, whereas sympathetic activity is reduced in both normotensive and hypertensive individuals, indicating that a reduced sympathetic activity is not the

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underlying cause (5). An alternative mechanism behind the effect of exercise training on blood pressure could be a reduced sensitivity to noradrenaline, as previously shown (6). However, the mechanism underlying this reduced sensitivity remains unknown. In this regard, an interesting preclinical finding has been that the pannexin 1 (Panx1) channel enhances the vasoconstrictor effect of noradrenaline (7), indicating that this could be a potential mechanism of the change in noradrenaline sensitivity with training and thereby in blood pressure control (8). Studies in rodents have shown that noradrenaline-induced opening of Panx1 channels in smooth muscle cells leads to ATP release (7,9). The released ATP, in turn, binds to purinergic receptors on the smooth muscle cells, which leads to vasoconstriction and, thus, a potentiation of the direct effect of noradrenaline (7). Accordingly, the inhibition of Panx1 has been shown to reduce blood pressure in hypertensive rodents (10) and reduces α-adrenergic vasoconstriction in isolated arterioles from hypertensive humans (8), suggesting a role in hypertension.

In a recent study, we verified an involvement of Panx1 in the regulation of vascular tone in physically active healthy young men, where the inhibition of Panx1 channel reduced vascular resistance, both at rest and with induced sympathetic vasoconstriction (11). In the study, Panx1 was inhibited by oral intake of probenecid, and an enhanced sympathetic vasoconstriction was achieved by arterial infusion of tyramine, a compound that induces an endogenous release of noradrenaline (12). Although the study did not observe a reduction in blood pressure, this may have been due to a reduction in heart rate compensating for the increased vascular resistance, a compensatory mechanism, which may not be as prominent in older individuals with elevated blood pressure. Combined, the findings from previous hypertensive experimental models suggest a role for Panx1 in blood pressure regulation, with our data showing a role for Panx1 in regulation of vascular tone in young humans, suggest that Panx1 potentially could provide a mechanism underlying the effect of training in lowering the sensitivity to noradrenaline and blood pressure in hypertensive humans.

Skeletal muscle microvascular endothelial function, assessed by intra-arterial acetylcholine infusion, is improved by exercise training with little or no effect on smooth muscle cell sensitivity to nitric oxide (NO) (13,14). Although some of the improvement in microvascular function with training is likely to be due to enhanced formation of NO and prostacyclin (15–18), this enhancement is unlikely to explain all of the effect. On the assumption that individuals with essential hypertension present an exaggerated vasoconstrictive effect through Panx1, as previously demonstrated in animals (8,10), a training-induced reduction of this effect could be a factor contributing to the improved microvascular function in this population.

Thus, the main aim of the present study was to assess whether the potentiating effect of Panx1 on noradrenaline-induced vasoconstriction could be reduced by exercise training in hypertensive individuals. A secondary objective was to elucidate whether Panx1 inhibition influenced the microvascular vasodilator response to acetylcholine and sodium nitroprusside (SNP) before and after the training period. The main hypothesis was that exercise training would lower blood pressure concurrently with a reduction in the Panx1-mediated vasoconstrictive effect in men with essential hypertension, with no effect in normotensive men. To this end, a group of normotensive men and a group of nonmedicated men with stage 1 essential hypertension, completed 8 wk of intensive aerobic exercise training. Blood pressure, noradrenaline-induced vasoconstriction, and microvascular function were determined with and without acute Panx1 inhibition, before and after the training period. The role of Panx1-mediated vasoconstriction was evaluated at baseline and with arterial infusion of tyramine.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Capital Region of Copenhagen (H-18057185) and conducted in accordance with the guidelines of the Declaration of Helsinki; ClinicalTrials.gov identifier: NCT03778489. Written informed consent was obtained from all subjects before enrolment into the study.

Participants. Fourteen physically inactive nonmedicated hypertensive men (systolic and diastolic blood pressures of >130 and >80 mm Hg, respectively) 45–65 yr old and 13 age-matched normotensive controls (systolic and diastolic blood pressures of <120 and <80 mm Hg, respectively) were included in the study. Physical inactivity was defined as less than 2 h of moderate-intensity exercise per week for the last 10 yr. The study participants were nonsmokers, did not consume an excessive amount of alcohol, were not on any medication, including NSAIDs, and had no history or symptoms of cardiovascular disease, renal dysfunction, insulin resistance, diabetes mellitus, or hypercholesterolemia.

All subjects were screened via medical examination, 12-lead ECG, blood sampling from an antecubital vein, and 3 d of home-based blood pressure measurements. Baseline data from some of the included participants have previously been reported (19).

Home blood pressure measurements. Study participants were given a blood pressure monitor equipped with a cuff, sized 22–42 cm (OMRON M3; OMRON Healthcare, Køge, Denmark) to take home, where they were instructed to place the cuff on their bare upper arm, and to measure while in supine position after 20 min of rest. Participants were instructed to take six measurements on three mornings and three evenings. These measurements were completed before the main experimental day, before and after the training intervention.

Although circadian rhythm influences blood pressure, we have found that combining morning and evening measurements provides a better overall measure of an individual's blood pressure level. Therefore, a combination of morning and evening blood pressure measurements was used in the analysis.

Body composition and determination of maximal oxygen uptake ($\dot{V}O_{2peak}$). Body composition assessment and performance tests were conducted before and after the training intervention. Subjects reported to the laboratory after an overnight fast. Body composition assessment was performed via dual-energy x-ray absorptiometry scan (Lunar iDXA; GE

Medical Systems, Brøndby, Denmark). A light breakfast was served, and $\dot{V}O_{2peak}$ was determined 45 min later.

The determination of $\dot{V}O_{2peak}$ was achieved by continuous measurements of gas exchange (Oxycon Pro; Intramedic, Gentofte, Denmark) during a graded exercise test on a cycle ergometer (Monarch 839E, Varberg, Sweden). The exercise protocol consisted of a 4-min warm-up period at 75 W followed by increasing workload of 25 $W \cdot min^{-1}$ until voluntary exhaustion. Verbal encouragement was given. To improve the probability of reaching a true $\dot{V}O_{2peak}$, the participants rested for 10 min after reaching exhaustion and then cycled to exhaustion again at 110% of the maximal workload attained during the first test (20,21).

Experimental design. The main experimental day was conducted before and between 48 and 96 h after the last training session. The participants were asked to refrain from caffeine, alcohol, and exercise for 24 h before reporting to the laboratory at 8 AM on the experimental day. The participants were instructed to consume the same light breakfast before both test days.

Under local anesthesia (lidocaine, 20 mg mL^{-1}; AstraZeneca, Copenhagen, Denmark), catheters (20 gauge; Arrow International, Reading, PA) were placed in the femoral artery and vein of the experimental leg. A skeletal muscle biopsy was also obtained from *m. vastus lateralis* of the experimental leg by use of the Bergstrom technique (22). After 30 min of supine rest, blood flow and intra-arterial blood pressure were measured in the supine position, at baseline and after 3 min of the following femoral arterial infusions (in the order it was conducted): acetylcholine (Miochol-E; Bausch & Lomb Inc., Berlin, Germany) at 10 and 50 μg min^{-1} kg leg $mass^{-1}$, SNP (Meda, Ballerup, Denmark) at 3 μg min^{-1} kg leg $mass^{-1}$, and tyramine (Sigma-Aldrich, Søborg, Denmark) at 0.1 μmol min^{-1} kg leg $mass^{-1}$. The infusions induce changes in microvascular resistance and thus reflect microvascular function. The effect of the infused drugs on conduit artery diameter is negligible. Each infusion was separated by 15 min rest. The participants then ingested probenecid (3000 mg; Meda), and after 2.5 h, intra-arterial blood pressure and blood flow were determined, and the exact infusion protocol with acetylcholine, SNP, and tyramine was repeated. The resting time after probenecid administration was determined from a pilot study, where peak plasma concentration was observed at 2.5 h. The protocol was repeated in the exact manner posttraining intervention.

Femoral arterial blood flow was measured with ultrasound Doppler (Vivid E9; GE Healthcare) as previously described (11). Mean arterial pressure (MAP) and venous blood pressure measurements were obtained by a pressure transducer connected to a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO) and viewed using LabChart 8 (ADInstruments). Postexperimental evaluations were performed on LabChart 8. Microvascular function was assessed as changes in leg vascular conductance (LVC), calculated as LVC = leg blood flow/(MAP − venous blood pressure).

Exercise training. All participants trained 3 times per week on indoor bikes (BodyBike, Body Bike International, Frederikshavn, Denmark) for 8 wk (23 ± 1.3 training sessions). Participants followed a graded training program up to 60 min of high-intensity aerobic cycle training. Sessions consisted of four to six blocks (bouts) of cycling at varying intensities (60%–95% of each participant's calculated HR_{peak} determined from the $\dot{V}O_{2peak}$ test and throughout training), as shown in Table 1.

Training were offered to the study participants 3 $d \cdot wk^{-1}$ at 7 AM and at 4 PM, where they were given the opportunity to fit the training into their usual daily schedule. All training sessions were supervised, and training intensity was strictly controlled. Intensity levels were individually calculated from each test participant's measured peak heart rate (HR_{peak}) from their $\dot{V}O_{2peak}$ performance tests. Heart rate during all exercise sessions was recorded and monitored in real time using the Polar Team2 Pro heart rate sensors and software (Polar, Kempele, Finland).

Protein determination by Western blot. Protein expression of Panx1, endothelial nitric oxide synthase (eNOS), prostacyclin synthase (PGI_2S), cyclooxygenase 1 (COX-1), superoxide dismutase 2, SOD2, and NADPH oxidase ($gp91^{phox}$) were analyzed in skeletal muscle lysates. Freeze-dried muscle biopsies were dissected free from connective tissue, fat, and blood. Cleaned muscle (5 mg d.w.) was homogenized (Qiagen Tissuelyser II; Retsch, Haan, Germany) in a lysis buffer. The samples were centrifuged, and the supernatant was collected. BSA standards (Pierce Reagent; Thermo Fisher Scientific, Roskilde, Denmark) were used to determine protein concentrations. Samples were run in duplicates on 4%–15% precasted Tris–HCl gels (Bio-Rad Laboratories, Copenhagen, Denmark). Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon Transfer Membrane; Merck Millipore, Søborg, Denmark) and blocked in Tris-buffered saline–Tween with 3% BSA, 5% fish gelatin, or 2% milk and incubated overnight with primary antibody and 1 h with secondary antibody. Primary antibodies used were as follows: rabbit anti-Panx1

TABLE 1. Eight-week high-intensity aerobic cycle training program for both normotensive and hypertensive test participants.

	Interval Training Sessions Completed in Blocks					
	Interval Block 1	**Interval Block 2**	**Interval Block 3**	**Interval Block 4**	**Interval Block 5**	**Interval Block 6**
Week 1: weekly training time, 135 min ($min \cdot wk^{-1}$)	5 min 60%–70% + 5 min 70%–80%	5 min 80% + 5 min 85%	5 min 85% + 3 min 90% + 2 min 95%	5 min 85% + 3 min 90% + 2 min 95%		
Week 2–3: weekly training time, 153 min ($min \cdot wk^{-1}$)	5 min 60%–70% + 5 min 70%–80%	5 min 80% + 5 min 85%	5 min 85% + 3 min 90% + 2 min 95%	5 min 85% + 3 min 90% + 2 min 95%	4 min 90%–95%	
Week 4–8: weekly training time, 171 min ($min \cdot wk^{-1}$)	5 min 60%–70% + 5 min 70%–80%	5 min 80% + 5 min 85%	5 min 85% + 3 min 90% + 2 min 95%	5 min 85% + 3 min 90% + 2 min 95%	4 min 90%–95%	4 min 90%–95%

Intensity levels are individualized to each test participant's HR_{peak} obtained from their maximal oxygen consumption cycle test. Week 1 consisted of blocks 1–4 and weeks 2 and 3 added block 5. In weeks 4–8, all six blocks were completed. All blocks were separated by 2-min breaks.

TABLE 2. Characteristics of normotensive and hypertensive participants before and after 8 wk high-intensity aerobic cycle training intervention.

	Normotensive, $n = 13$		Hypertensive, $n = 14$	
Variables	**Pretraining**	**Posttraining**	**Pretraining**	**Posttraining**
Anthropometrics				
Age (yr)	54.5 ± 5.4	–	60.1 ± 3.5***	–
Height (m)	1.8 ± 0.07	–	1.8 ± 0.05	–
Weight (kg)	85.5 ± 8.7	84.6 ± 8.6	88.4 ± 9.6	86.3 ± 10.2
BMI ($kg \cdot m^{-2}$)	26.6 ± 2.3	26.3 ± 2.3	26.4 ± 2.0	25.9 ± 2.2
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.9 ± 0.5	3.4 ± 0.5###	2.9 ± 0.4	3.3 ± 0.4#
Fitness ($mL \cdot min^{-1}\ kg^{-1}$)	34.5 ± 6.7	40.5 ± 6.2##	33.5 ± 3.6	38 ± 5.3#
Body composition				
Lean body mass (kg)	58.8 ± 5.3	59.5 ± 5.1	58.3 ± 5.6	58.5 ± 5.6
Visceral fat mass (kg)	1.5 ± 0.7	1.3 ± 0.7	1.9 ± 0.5	1.6 ± 0.6
Total fat (%)	28.2 ± 6.3	26.5 ± 6.2	31.4 ± 3.9	29.5 ± 4.3
BMD ($g \cdot cm^{-2}$)	1317 ± 85.5	1313.8 ± 82.4	1317.6 ± 125.8	1307.2 ± 138.2
Blood pressure (home measurements)				
All measurements				
Systolic BP (mm Hg)	117.2 ± 5.5	119.7 ± 5.8	135.7 ± 6.2***	130.3 ± 8.0***,#
Diastolic BP (mm Hg)	72.2 ± 3.5	72.8 ± 3.3	83.7 ± 3.7***	79.1 ± 3.3***,###
Morning measurements				
Systolic BP (mm Hg)	116 ± 7.4	120 ± 6.2	135 ± 6.4***	130 ± 7.2**
Diastolic BP (mm Hg)	71.8 ± 4.8	73.9 ± 2.7	84.6 ± 3.2***	81.2 ± 4.1***
Evening measurements				
Systolic BP (mm Hg)	119 ± 5.3	116 ± 9.4	136 ± 6.9***	130 ± 11.5***
Diastolic BP (mm Hg)	72.6 ± 3.5	71.7 ± 4	83.1 ± 5.1***	77.4 ± 7.3**,#
HR_{rest} (bpm)	62.4 ± 7.5	60.5 ± 8.5	64.4 ± 6.6	61.3 ± 6.6
HR_{peak} (bpm)	177 ± 9	178 ± 8.5	172 ± 9.8**	174 ± 7.2*
Blood lipids				
Hemoglobin ($mmol \cdot L^{-1}$)	9.3 ± 0.6	–	9.3 ± 0.6	–
Total cholesterol ($mmol \cdot L^{-1}$)	5.4 ± 1	–	5.8 ± 1	–
HDL ($mmol \cdot L^{-1}$)	1.3 ± 0.3	–	1.4 ± 0.4	–
LDL ($mmol \cdot L^{-1}$)	3.6 ± 0.9	–	4.1 ± 1.1	–
Triglycerides ($mmol \cdot L^{-1}$)	2.6 ± 3	–	1.8 ± 0.7	–
HbA1c, IFCC ($mmol \cdot mol^{-1}$)	35.3 ± 2.9	–	35.8 ± 3.2	–

Values are presented as mean ± SD.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significant difference between groups; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ significant effect of training.
BMI, body mass index; BMD, bone mineral density; BP, blood pressure; HR, heart rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c IFCC, glycohemoglobin A1c using the International Federation of Clinical Chemistry units; $\dot{V}O_{2peak}$, maximal oxygen consumption.

(conc. 1:1000; abcam, Cambridge, UK), rabbit anti-eNOS (conc. 1:500, abcam), rabbit anti-PGI_2S (conc. 1:250, abcam), rabbit anti-COX1 (conc. 1:600, abcam), rabbit NOX/anti-$gp91^{phox}$ (conc. 1:500, abcam), and rabbit anti-SOD2 (conc. 1:5000, Merck Millipore). Secondary antibody was goat anti-rabbit (conc. 1:5000; Jackson Immunoresearch, Skanderborg, Denmark).

Statistical analyses. The sample population size was calculated *a priori* to detect a physiologically relevant difference of 10% in LVC during tyramine infusion, at a power of 0.8, and $P < 0.05$ was considered statistically significant. Data are reported as means ± SD unless otherwise stated.

A linear mixed-model approach was used to analyze differences between groups (normotensive vs hypertensive) and time (pre- vs posttraining) as fixed factors and subject as a random factor, with Tukey's *post hoc* test with FDR adjustments applied. Residual and Q-Q plots were used to confirm the homogeneity of covariance and normal distribution. Statistical analyses were performed using RStudio (version 1.3.1073; RStudio, Boston, MA). Graphical presentation was performed using GraphPad Prism 9 (GraphPad Software, La Jolla, CA).

RESULTS

Baseline Characteristics

Baseline subject characteristics are presented in Table 2. The hypertensive group were significantly older and, as intended by design, had a significantly higher systolic and diastolic blood pressure. Combined morning and evening blood pressure measurements were used. Statistical analysis indicated no significant difference between the morning and the evening home blood pressure measurements in our subject population for systolic ($P = 0.99$) or diastolic blood pressure ($P = 0.26$). All other measured characteristics (lean body and fat mass) and $\dot{V}O_{2peak}$ were similar between groups.

Training Compliance and Intensity of Training

The normotensive and hypertensive participants had similar training compliance. They completed 98.7% and 97% of the training sessions, respectively. Both groups had a similar distribution of training intensities and trained at a heart rate of >80% of maximum heart rate, 68% of the time (Table 3).

TABLE 3. Heart rate response during training and compliance of both normotensive and hypertensive test participants.

	Percent of Training Time Spent in Heart Rate Zones (%)	
Training Zones (% HR_{max})	**Normotensive, $n = 13$**	**Hypertensive, $n = 14$**
>90%	21.2 ± 7.9	23.8 ± 8.2
80%–90%	46.4 ± 6.6	44 ± 5.3
70%–80%	17.8 ± 2.7	16.4 ± 3.2
60%–70%	16.5 ± 23.3	9.3 ± 2.1
50%–60%	2.0 ± 0.8	1.8 ± 1.1
Training compliance (%)	98.7	97.0

Values are presented as mean ± SD.

Effect of Training on Maximal Oxygen Uptake, Resting Heart Rate, and Body Composition

Training increased absolute $\dot{V}O_{2peak}$ to a similar extent, by 14.7% and 12.1%, in the normotensive and hypertensive groups, respectively (Table 2). There was no training effect on resting heart rate, lean body mass, visceral fat mass, or total fat.

Effect of Training on Home-Based Blood Pressure Measurements

In the hypertensive group, training induced a reduction in systolic blood pressure by 5.4 ± 2.8 mm Hg ($P = 0.013$) and diastolic blood pressure by 4.6 ± 1.3 mm Hg ($P < 0.001$), whereas there was no change in the normotensive group (Table 2).

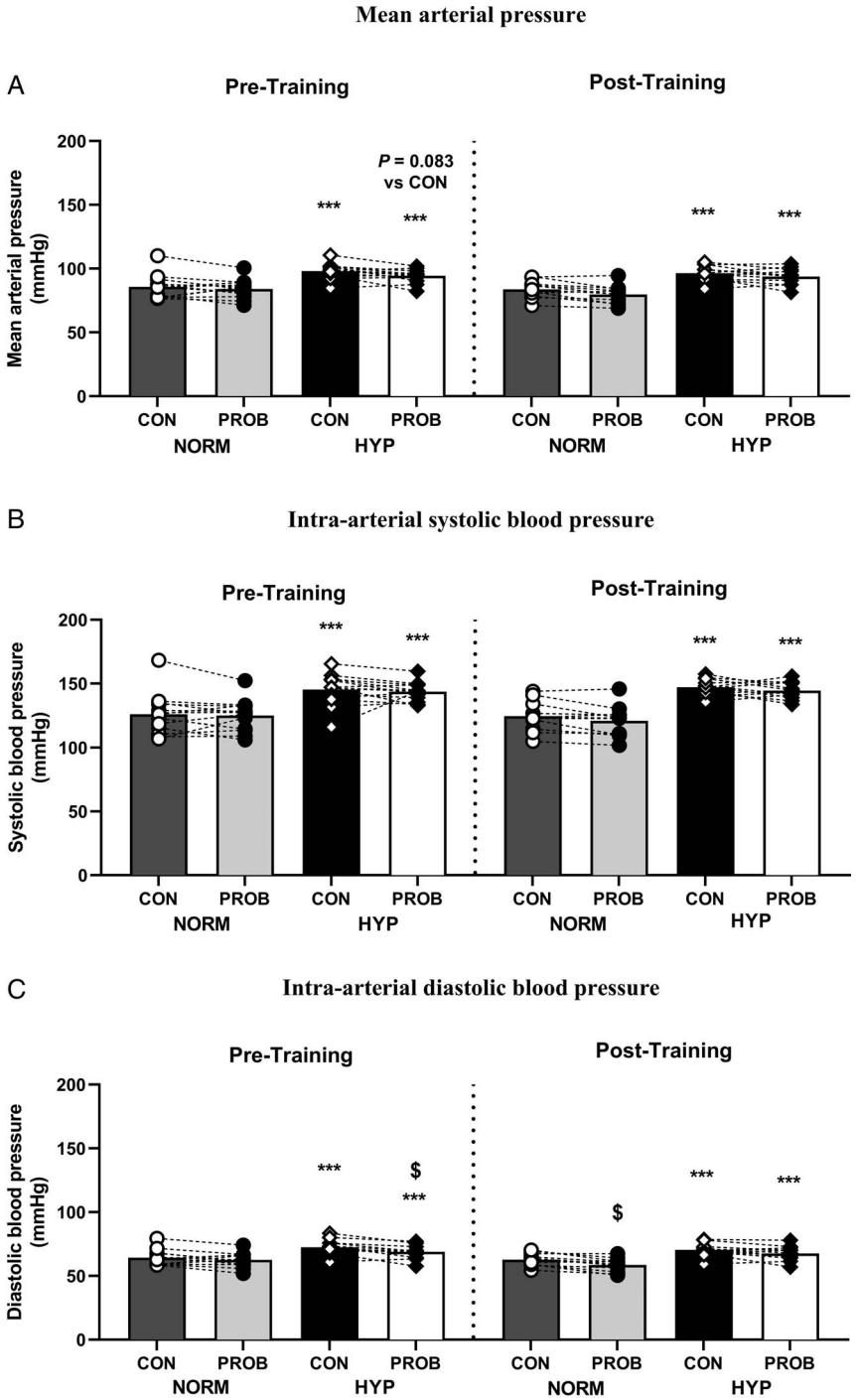

FIGURE 1—Femoral arterial blood pressure measured in normotensive an essential hypertensive men before and after 8 wk of aerobic cycling training. Measurements were made during control conditions and at 2.5 h after oral probenecid intake to inhibit Panx1. A, MAP. B, Systolic blood pressure. C, Diastolic blood pressure. CON, control; PROB, probenecid administration; NORM, normotensive; HYP, hypertensive. $\$P < 0.05$ denotes significant effect of Panx1 inhibition; $***P < 0.001$ denotes significant difference between normotensive and hypertensive.

Effect of Panx1 Inhibition by Probenecid

Arterial blood pressure. Before training, probenecid administration reduced the diastolic blood pressure (by 4.8%, $P = 0.032$) in the hypertensive but not in the normotensive group (Fig. 1C). After training, probenecid reduced the diastolic blood pressure (by 6.7%, $P = 0.028$) in the normotensive but not in the hypertensive group (Fig. 1C). Probenecid did not affect systolic or MAP in either group before or after training (Fig. 1A and B).

Baseline LVC. After training, probenecid induced an increase in baseline LVC in both the normotensive (by 41.5%, $P = 0.0036$) and the hypertensive (by 37.7%, $P = 0.024$) group (Fig. 2A).

Vasoconstrictor response to tyramine. Tyramine infusion induced a similar decrease in LVC of 11.9% and 32.8% in the normotensive and hypertensive groups, respectively, as previously reported (19). The change in LVC was not affected by training or by probenecid (Fig. 2B). As control, noradrenaline levels were assessed at baseline and after tyramine infusion and were found to be similar for both groups and conditions (Fig. 2C).

Vasodilator response to acetylcholine and SNP. Before the training intervention, there were no differences in the change in LVC with femoral arterial infusion of acetylcholine (Fig. 3A) or SNP (Fig. 3B) between the groups. After training, there was a significant increase (by 175%) in LVC to the higher dose of acetylcholine in ($P = 0.0007$; Fig. 3A) in the hypertensive group only. The change in LVC in response to SNP infusion after training was not different between the groups (Fig. 3B). Probenecid had no effect on the change in LVC in response to acetylcholine in either group, before or after training (Fig. 3C). After training, probenecid increased the response to SNP infusion in both the normotensive (by 275%, $P = 0.038$) and the hypertensive (by 188%, $P = 0.038$) groups (Fig. 3D).

Skeletal Muscle Content of Proteins Related to Microvascular Function

The protein content of all measured proteins was similar in the normotensive and hypertensive individuals at baseline. Training had no effect on Panx1 (Fig. 4A) in either group; however, training significantly increased the contents of SOD2 (Norm 27%, $P = 0.00025$; Hyp 26%, $P < 0.001$) and eNOS (Norm 17%, $P = 0.0062$; Hyp 18%, $P = 0.0062$) in both groups (Fig. 4B and C). However, training only significantly increased the contents of COX1 (31%, $P = 0.03$) and NADPH oxidase, $gp91^{phox}$ (17%, $P = 0.034$), in the hypertensive group (Fig. 4D and E). There was no effect of training on PGI_2S (Fig. 4F) content in either group.

DISCUSSION

The main finding of the present study was that Panx1 did not account for the exercise training-induced reduction in blood pressure in stage 1 hypertensive subjects. Moreover, there was no effect of Panx1 inhibition on the vasoconstrictive effect of enhanced noradrenaline, induced by tyramine infusion. By contrast, after training, Panx1 inhibition enhanced LVC in response to an NO donor, suggesting a stronger vasoconstrictive effect of Panx1 on the skeletal muscle arterioles after training. A secondary finding was that intense aerobic exercise training was effective in improving the vasodilator

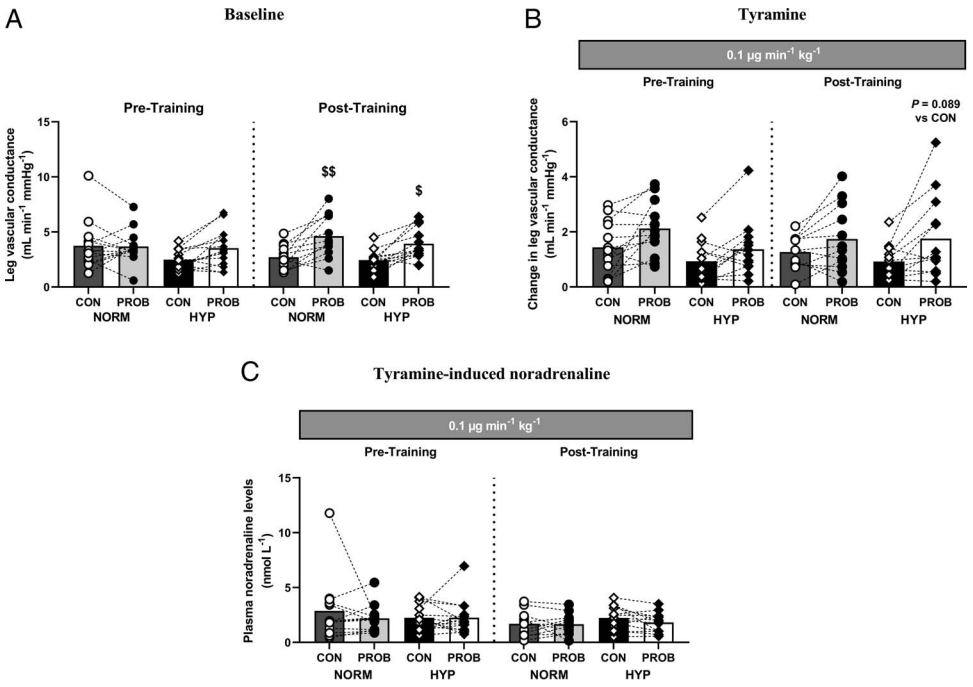

FIGURE 2—The effect of Panx1 inhibition by probenecid on LVC in normotensive and essential hypertensive men before and after 8 wk of aerobic training. A, The effect of probenecid on baseline LVC. B, The effect of probenecid on LVC during infusion of 0.1 µg·min^{-1}·kg^{-1} tyramine. C, Plasma noradrenaline levels with infusion of 0.1 µg·min^{-1}·kg^{-1} tyramine. CON, control; PROB, probenecid administration; NORM, normotensive; HYP, hypertensive. $$P < 0.05$, $$$P < 0.01$ denotes a significant effect of Panx1 inhibition.

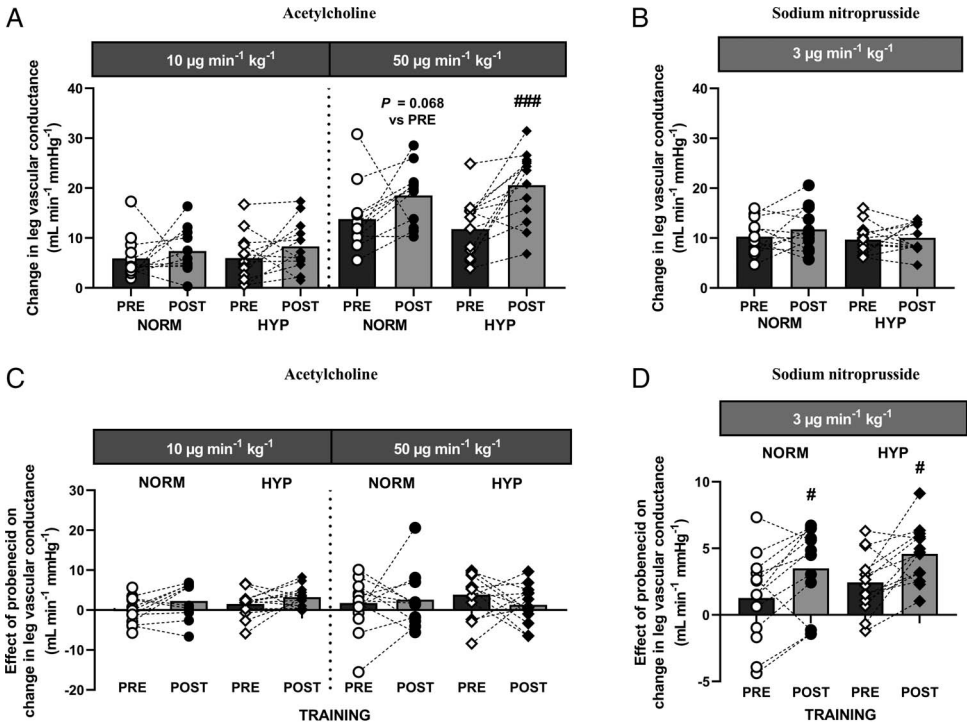

FIGURE 3—Change in LVC in response to femoral arterial infusion of acetylcholine and sodium nitroprusside (SNP), as well as the effect of Panx1 inhibition via probenecid on the change in LVC, in normotensive and essential hypertensive men. The measurements were conducted before and after 8 wk of aerobic exercise training. A, Acetylcholine infusion. B, SNP infusion. C, Effect of probenecid on the change in LVC during acetylcholine infusion. D, Effect of probenecid on the change in LVC during SNP infusion. NORM, normotensive; HYP, hypertensive; PRE, pretraining; POST, posttraining. #$P < 0.05$; ###$P < 0.001$ denotes a significant effect of training.

response to acetylcholine and in enhancing the expression of proteins related to microvascular function, supporting previous findings in medicated stage 2 hypertensive men.

Role of Panx1 in the blood pressure–reducing effect of exercise training. Evidence from rodents and isolated human arteries points to an involvement of Panx1 in the control of vascular resistance and blood pressure in hypertension (8,10). Here we sought to determine whether the blood pressure–lowering effect of exercise training in part could be explained by a less prominent constrictive effect of Panx1 in nonmedicated stage 1 essential hypertension. To assess the involvement of Panx1 in the exercise-induced reduction in blood pressure, we studied the influence of probenecid, a well-known Panx1 inhibitor (11,23,24), on resting blood pressure, resting LVC, enhanced noradrenaline-induced vasoconstriction by tyramine infusion and microvascular function assessed by acetylcholine, and SNP-induced vasodilation. As expected, exercise training reduced the mean, systolic, and diastolic blood pressure in the hypertensive group. On the premise that the blood pressure–lowering effect of training occurred because of a reduction in the potentiating vasoconstrictive effect of Panx1, then a greater effect of probenecid on resting blood pressure would have been expected before, compared with after the training period. However, although probenecid lowered diastolic blood pressure slightly before, but not after, the training period in the hypertensive group, there was no effect on systolic or mean blood pressure, suggesting a negligible involvement of Panx1 in the training effect. In further support of a lack of Panx1 involvement, after, but not before, the training period, probenecid increased baseline LVC as well as the vasodilator response to an NO donor in the hypertensive group, indicating a greater, rather than reduced, constrictive effect by Panx1 after training. There was also no effect of Panx1 inhibition when endogenous noradrenaline levels were raised by tyramine infusion, either before or after training in the hypertensive group. This latter finding contrasts our previous finding in young healthy men (11) and suggests that the involvement of Panx1 in noradrenaline-induced vasoconstriction may be reduced with age. The lack of functional evidence for an exercise training-induced change in Panx1 constriction in the hypertensive group was also supported by a lack of change in Panx1 protein in skeletal muscle with training. It should also be mentioned that the above-described results for the hypertensive group were similar in the normotensive group, which did not present an exercise training-induced reduction in arterial blood pressure.

Exercise training improves vascular endothelial function. The intensive aerobic training intervention was found to improve microvascular endothelial function in the hypertensive group. This was evident by less of an increase in vascular conductance with acetylcholine infusion without an effect on smooth muscle sensitivity to NO. This improvement in microvascular function in hypertensive men was accompanied by an increased expression of several proteins of relevance for microvascular function. Two enzymes of direct importance for the regulation of vascular tone, eNOS, and COX-1, leading to the formation of NO and prostaglandins,

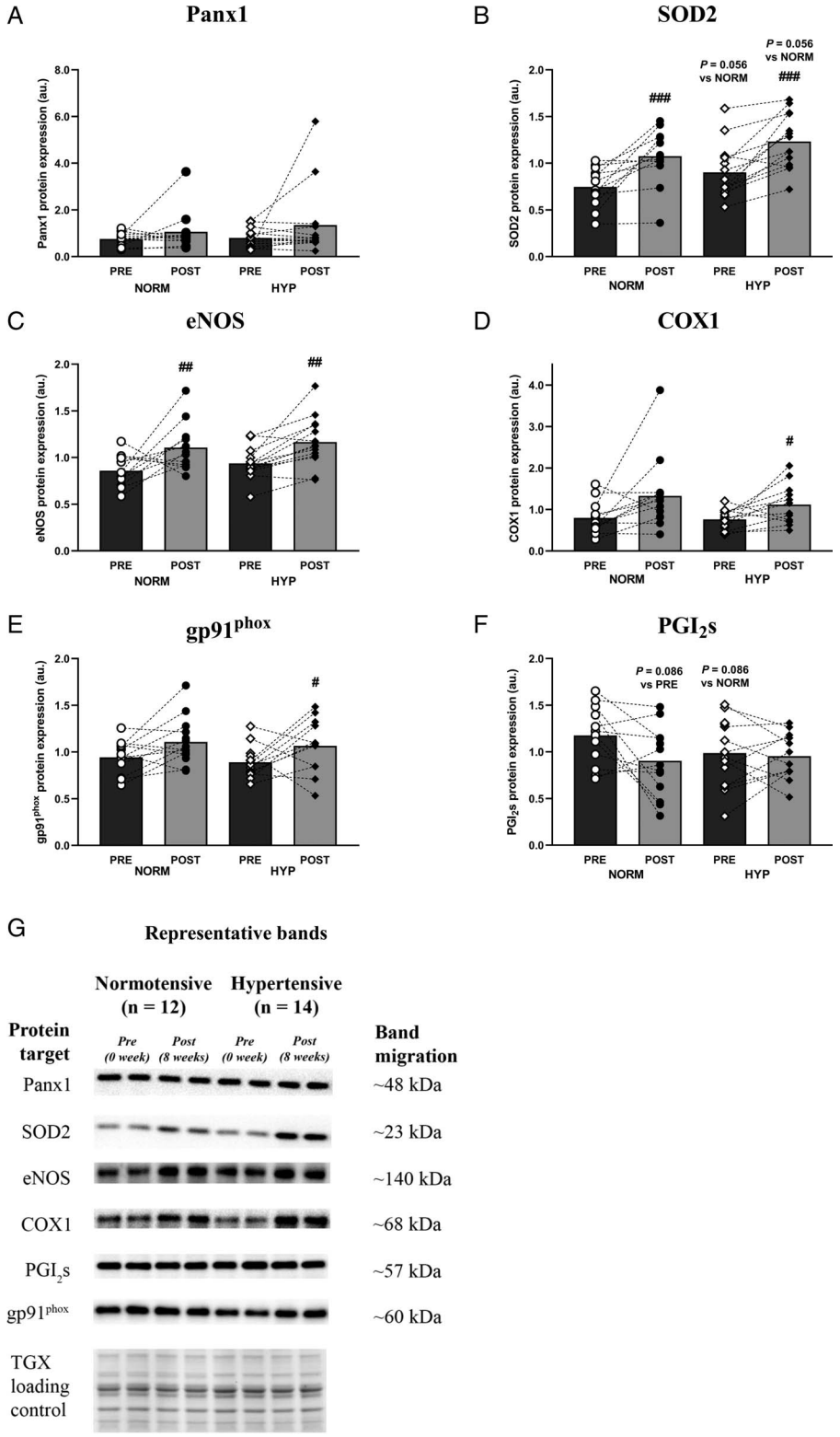

FIGURE 4—Skeletal muscle content of proteins related to vascular function. Measurements were made on skeletal muscle biopsies obtained before and after 8 wk of training. A, Panx1. B, Superoxide dismutase 2 (SOD2). C, eNOS. D, Cyclooxygenase 1 (COX1). E, NADPH oxidase-gp91^{phox}. F, PGI_2S. G, Representative blots. NORM, normotensive; HYP, hypertensive; PRE, pretraining; POST, posttraining. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ denotes significant effect of training.

respectively, were enhanced by training. Additionally, there was an increase in the expression of SOD2, a mitochondrial antioxidant enzyme, relevant for ROS removal and thus NO bioavailability (25). An interesting observation was that NADPH oxidase (NOX) subunit gp91^{phox} also was increased, suggesting a greater potential for ROS formation. Although

this finding would appear contradictory, it is well-known that ROS is important for physiological signaling (26), and an increase in this context may be beneficial for training adaptations. The finding agrees with previous reports of a greater expression of NOX2-p67^{phox} after training in human skeletal muscle tissue in several populations (15,27), although some studies have also reported no change (28,29) or a reduction (30). Combined, our study clearly shows that intense aerobic cycle training has a prominent effect on microvascular endothelial function in nonmedicated stage 1 essential hypertension.

Intensive exercise is highly effective in reducing blood pressure in nonmedicated hypertensive men. To achieve a reduction in blood pressure, we made use of an intense aerobic cycling exercise training regime, previously shown to be highly feasible and effective in lowering blood pressure in middle-age and age men (14,15). Accordingly, both systolic and diastolic blood pressures were reduced by ~5 mm Hg after training in the present hypertensive group with no change observed in the normotensive group, despite similar training compliance and training intensities between groups. This reduction in blood pressure is within the expected range as the meta-analysis of 30 studies with a total of 500 subjects has shown that exercise training interventions lower systolic and diastolic blood pressures by 7 and 5 mm Hg, respectively, in hypertensive subjects (31). Furthermore, large-scale analysis of randomized trials has found that a relatively small reduction (as small as 5 mm Hg) in systolic and/or diastolic blood pressures is sufficient to reduce major cardiovascular events (32,33). Our finding is similar to the notion that the magnitude of change in blood pressure with exercise training depends on the population, where individuals with high blood pressure seem to experience greater reductions (5,14,34,35). The magnitude of change observed in the current nonmedicated hypertensive group was similar to that of other studies applying moderate to high-intensity exercise interventions in middle-age men (36,37), suggesting that similar beneficial effects of intense aerobic training can be seen in mild nontreated and moderate treated hypertension. The findings also suggest that intensive exercise is effective in reducing arterial blood pressure and may be even more effective than moderate-intensity exercise, although meta-analysis has suggested negligible differences (38).

Study limitations. Probenecid is known to be highly effective in inhibiting the Panx1 channel (11,23,24). In terms of other targets, Probenecid has been described to inhibit anion transporters (39,40) involved in the export of cAMP from smooth muscle cells (41). Reduced cAMP secretion could potentially increase intracellular cAMP (42) and cause vasodilation, but as cAMP is a source of the vasodilator adenosine, reduced secretion could also have a constrictive effect (43). Thus, the extent by which this potential side effect of probenecid could have influenced the data is unclear. It is also worth noticing that endothelial cells also express purinergic receptors, which upon activation induces release of nitric oxide and prostacyclin that causes smooth muscle cell relaxation. However, ATP half-life is very short in the extracellular space due to ectonucleotidases; thus, a Panx1 mediated vasodilatory effect via endothelial purinergic receptors is unlikely.

Probenecid is approved for human use to treat gout, and in the present study, we used the maximum approved dose of 3000 mg, known to be safe in humans. Although it is not possible to establish direct evidence for the degree of Panx1 inhibition in humans, our previous work has demonstrated this dose to be effective in inducing vascular effects (11).

Probenecid inhibits the Panx1 channel in all cells; therefore, it cannot be excluded that the lack of effect of blockade on tyramine-induced constriction and blood pressure was due to an opposing effect of blocking ATP release from cells, such as red blood cells (44). However, conclusive evidence in humans for the physiological relevance of red blood cell-mediated ATP release is currently lacking.

In the current study, no change in skeletal muscle Panx1 was observed with training. The most likely exercise stimuli influencing the Panx1 channels is the increased presence of noradrenalin released from sympathetic nerve endings under physical activity but to what extend this translate into changed Panx1 function or expression remains to be determined. However, it should be noted that the measurements were made in whole muscle lysates and, as Panx1 is present in many cell types, a potential change specifically in the smooth muscle cells could have been undetectable.

In this study, the men in the hypertensive group were on average 4 yr older than the normotensive control group. Aging is known to affect adrenergic responsiveness, and therefore this difference in age could be a potential limitation. However, including age as a cofactor in the statistical model had no influence on the outcome.

Furthermore, this study only included male volunteers. It should be noted that we, therefore cannot make any conclusions with regards to the effect of the Panx1 channel on blood pressure regulation in women.

CONCLUSIONS

The present study confirms the efficacy of high-intensity aerobic training in lowering arterial blood pressure in individuals with essential hypertension. However, our data do not support an involvement of Panx1 in this blood pressure–lowering effect of exercise training. By contrast, the probenecid-induced enhancement of basal LVC as well as SNP-induced vascular conductance after training suggests a more prominent role for Panx1 after exercise training. Preclinical work in rodents and isolated human arteries has indicated that Panx1 is involved in the development of essential hypertension. However, the current findings on the effect of probenecid on blood pressure and vascular conductance in humans suggest a negligible involvement of Panx1 in essential hypertension. Therefore, Panx1 is unlikely to be a useful pharmacological target for the reduction of blood pressure in essential hypertension.

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Nutrition, Exercise and Sports, University of Copenhagen. The results of the present study do not constitute endorsement by American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Y. H. and L. G. conceived and designed research; S. M., C. C. H., T. S. E., A. T.-E., S. R. T., M. E. K., S. S. P., and C. M., performed experiments; S. M., C. C. H., A. T.-E., S. R. T., M. E. K., and J. P.-G., analyzed data; S. M., C. C. H., Y. H., and L. G. interpreted results of experiments; S. M. and C. C. H. prepared figures; S. M. and Y. H. drafted manuscript; S. M., C. C. H., Y. H., and L. G. edited and revised manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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