SciMedCentral

Annals of Sports Medicine and Research

Research Article

Heparin Priming Enhances Exercise-Induced Vascular Remodelling in Hindlimb Ischaemia

Taishi Hirahara[1,2], Yasuhiro Maejima[3], Yuma Tamura[4], Tomoki Tsurumi[4], Masanobu Kawakami[1,5], and Takanori Yasu[3]*

[1]*First Department of Integrated Medicine, Saitama Medical Center, Jichi Medical University, Japan*

[2]*Department of Cardiovascular Medicine, Japan Community Health Care Organization Saitama Medical Center, Japan*

[3]*Department of Cardiovascular Medicine and Nephrology, Dokkyo Medical University Nikko Medical Center, Japan*

[4]*Department of Rehabilitation, Dokkyo Medical University Nikko Medical Center, Japan*

[5]*Japan Association for Development of Community Medicine, Japan*

***Corresponding author**

Takanori Yasu, Department of Cardiovascular Medicine and Nephrology, Dokkyo Medical University Nikko Medical Center, 145-1 Moritomo, Nikko, Tochigi 321-1298, Japan, Tel: +81-288-23-7000; Fax: +81-288-23-5054

Submitted: 15 October 2026

Accepted: 12 November 2026

Published: 05 November 2026

ISSN: 2379-0571

Copyright

© 2026 Hirahar T, et al.

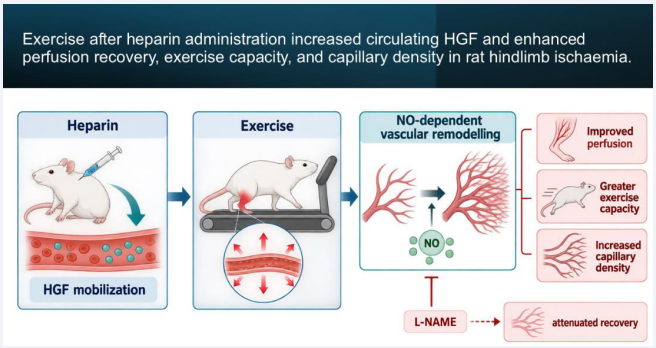
OPEN ACCESS

Keywords

- Exercise
- Heparin
- Hepatocyte growth factor
- Nitric oxide
- Peripheral arterial disease

Abstract

Exercise therapy is central to peripheral artery disease (PAD) management, but its efficacy may be limited by impaired vascular remodelling. We investigated whether exercise after heparin administration enhances perfusion recovery through nitric oxide (NO)-dependent mechanisms in a rat hindlimb ischaemia model. Serum hepatocyte growth factor (HGF) levels were measured before and after subcutaneous heparin administration in Wistar rats. Unilateral hindlimb ischaemia was induced by ligation and excision of the right external iliac and distal femoral arteries and ligation of all visible side branches. Rats were assigned to control, heparin, exercise, heparin plus exercise, or corresponding Nω-nitro-L-arginine methyl ester (L-NAME)-treated groups. Heparin (300 U/kg) was administered subcutaneously, followed by 30-min treadmill exercise twice daily for 14 days. Hindlimb perfusion, exercise capacity, and capillary density were evaluated. Heparin increased circulating HGF approximately fivefold. Combined therapy significantly improved all outcomes compared with control or either intervention alone. L-NAME markedly attenuated improvements in perfusion and exercise capacity with combined therapy but only partially attenuated those with exercise alone. Capillary density remained elevated despite L-NAME, suggesting distinct regulatory mechanisms for angiogenesis and collateral vessel growth. Exercise after heparin administration enhanced vascular remodelling and functional recovery, potentially through HGF mobilization and NO-dependent mechanisms. This strategy may augment exercise therapy for PAD.



Graphical Abstract Exercise after heparin administration increased circulating HGF and enhanced perfusion recovery, exercise capacity, and capillary density in rat hindlimb ischaemia. L-NAME attenuated these benefits, supporting a role for nitric oxide-dependent vascular remodelling in the response to combined therapy.

Cite this article: *Hirahara T, Maejima Y, Tamura Y, Tsurumi T, Kawakami M, et al. (2026) Heparin Priming Enhances Exercise-Induced Vascular Remodelling in Hindlimb Ischaemia. Ann Sports Med Res 13(2): 1241.*

INTRODUCTION

Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis and is associated with increased cardiovascular morbidity, mortality, and functional impairment [1,2]. Intermittent claudication reduces walking ability, exercise capacity, and quality of life, leading to progressive physical deconditioning [2-5]. Accordingly, supervised exercise therapy is recommended as the first-line treatment for symptomatic PAD because it improves walking performance, functional status, and cardiovascular health [4-7].

The benefits of exercise therapy are mediated through multiple mechanisms, including improvements in endothelial function, skeletal muscle metabolism, mitochondrial function, nitric oxide (NO) bioavailability, and vascular remodelling rather than through increased limb blood flow alone [8]. Exercise also improves lower-extremity muscle strength, including both knee extensor and flexor strength, in patients with PAD [7]. At the molecular level, exercise-induced skeletal muscle adaptation is mediated in part by activation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which promotes mitochondrial biogenesis, suppresses inflammatory signalling, and contributes to vascular and metabolic homeostasis [9].

Despite these benefits, the clinical efficacy of exercise therapy remains limited in many patients with PAD. Endothelial dysfunction is now recognized as a central feature of PAD pathophysiology and contributes to impaired vasodilation, chronic inflammation, thrombosis, microvascular dysfunction, and defective angiogenic responses [8-10]. Reduced NO bioavailability further impairs adaptive vascular remodelling and collateral vessel development in response to ischaemia, thereby limiting perfusion recovery despite exercise therapy [8,10].

Experimental studies have demonstrated that exercise-induced vascular remodelling is regulated by coordinated activation of hypoxia-inducible factor-1 (HIF-1), vascular endothelial growth factor (VEGF), and NO-dependent signalling pathways [10-13]. Among these, endothelium-derived NO plays a critical role in vasodilation, endothelial function, arteriogenesis, and skeletal muscle perfusion during exercise [14,15]. Consistent with this concept, pharmacological inhibition of NO synthase markedly attenuates collateral vessel development and blood flow recovery in experimental hindlimb ischaemia [16].

Heparin, beyond its anticoagulant activity, has also been shown to promote vascular regeneration. Previous studies demonstrated that heparin rapidly increases circulating hepatocyte growth factor (HGF) levels and that HGF is a major mediator of heparin-induced angiogenesis [17,18]. HGF is a multifunctional cytokine that promotes endothelial cell proliferation, migration, and neovascularization while exerting anti-inflammatory, anti-apoptotic, and endothelial-protective effects [17,18]. Furthermore, Yang et al., demonstrated that heparin enhances exercise-induced collateral vessel development and collateral blood flow in experimental hindlimb ischaemia, suggesting that heparin facilitates arteriogenesis in addition to angiogenesis [19].

These experimental findings are supported by clinical evidence. Long-term low-dose calcium-heparin therapy improved walking performance and claudication symptoms in patients with PAD [20]. Furthermore, we previously demonstrated that exercise performed after heparin administration improved walking ability more effectively than either treatment alone in patients with non-option PAD [21]. However, the mechanisms responsible for the synergistic effects of heparin and exercise have not been fully elucidated.

We hypothesized that heparin-induced HGF mobilization enhances exercise-induced vascular remodelling through NO-dependent mechanisms. We therefore investigated whether exercise performed after heparin administration improves perfusion recovery, exercise capacity, and capillary formation in a rat hindlimb ischaemia model using the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME).

RESULTS

Protocol 1

Serum HGF levels increased rapidly after subcutaneous heparin administration (Figure 1). At 60 min, HGF levels were approximately 5.5-fold higher than baseline in the 300 U/kg group and approximately 7.5-fold higher in the 600 and 1,200 U/kg groups; these increases were significant compared with the NaCl group ($P < 0.001$). Serum HGF levels remained elevated at approximately five-fold or more above baseline at 180 min in all heparin-treated groups, whereas they remained unchanged after NaCl administration. Because 300 U/kg produced a substantial and sustained increase in serum HGF levels, this dose was selected for Protocol 2.

Protocol 2

Body weight after 14 days of treatment did not differ among the groups. Mean arterial pressure was slightly higher in the L-NAME-treated groups, whereas heart rate was similar among all groups. Exercise capacity

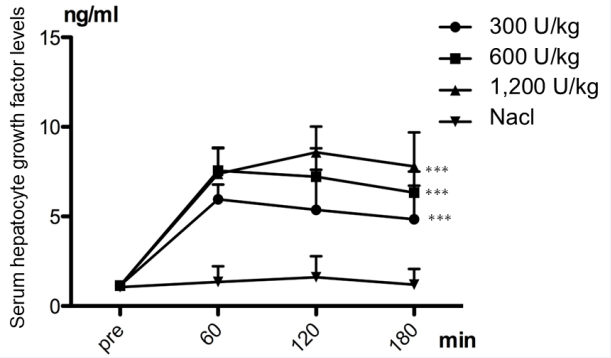


Figure 1 Serial changes in serum hepatocyte growth factor (HGF) levels after subcutaneous administration of heparin sodium.

Serum HGF levels increased rapidly after subcutaneous heparin administration. At 60 min, HGF concentrations were approximately 5.5-fold higher than baseline in the 300 U/kg group and approximately 7.5-fold higher in the 600 and 1,200 U/kg groups. Serum HGF levels remained elevated at approximately five-fold or more above baseline at 180 min in all heparin-treated groups. In contrast, serum HGF levels remained essentially unchanged after NaCl administration. Data are presented as the mean ± SEM. ***P < 0.001 versus NaCl.

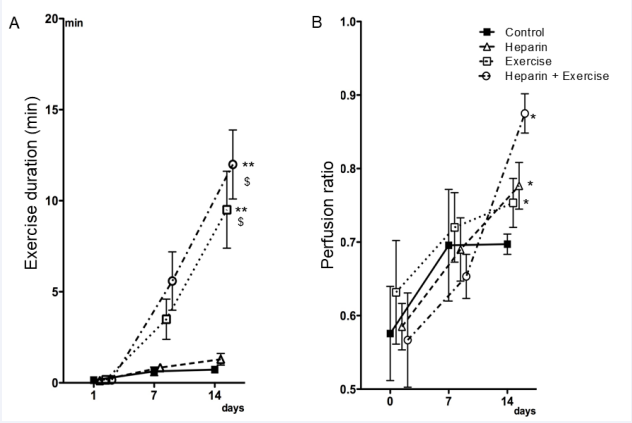


Figure 2 Effects of heparin and exercise on exercise capacity and hindlimb perfusion after induction of hindlimb ischaemia.

(A) Serial changes in exercise capacity on days 1, 7, and 14 after induction of hindlimb ischaemia. Closed squares represent the Control group; open triangles, the Heparin group; open squares, the Exercise group; and open circles, the Heparin + Exercise group. Exercise capacity was significantly greater in the Exercise and Heparin + Exercise groups than in the Control group and the Heparin group. (B) Serial changes in the hindlimb perfusion ratio (ischaemic/non-ischaemic limb), assessed using laser Doppler perfusion imaging, on days 0, 7, and 14 after surgery. The symbols are the same as those in panel A. Perfusion recovery was significantly greater in the Heparin + Exercise group than in the Control group. Data are presented as the mean ± SEM. *P < 0.05 and **P < 0.01 versus Control; #P < 0.05 versus Exercise; and $P < 0.05 versus Heparin.

progressively improved during the 14-day treatment period in both the Exercise and Heparin + Exercise groups, with the greatest improvement observed in the Heparin + Exercise group (Figure 2A). At day 14, exercise duration was significantly longer in the Exercise group (9.51 ± 5.18 min) and the Heparin + Exercise group (11.99 ± 4.64 min) than in the Control (0.73 ± 0.54 min) and Heparin groups (1.29 ± 0.78 min) (P < 0.001). Similarly, hindlimb perfusion progressively recovered during follow-up (Figure 2B). On day 14, the Heparin + Exercise group (0.88 ± 0.07) had the highest perfusion ratio, followed by the heparin group (0.78 ± 0.08), the exercise group (0.75 ± 0.08), and the control group (0.70 ± 0.04) (P < 0.05).

Figure 3 shows representative laser Doppler perfusion images acquired on days 0, 7, and 14. In the Heparin + Exercise group, perfusion in the ischaemic hindlimb gradually improved, but this improvement was suppressed by L-NAME administration. Since laser Doppler perfusion imaging reflects functional blood flow throughout the entire vascular network, including collateral arteries and the microcirculation, these findings are consistent with enhanced functional vascular remodelling, potentially including collateral vessel development.

Representative tissue sections are shown in Figure 4A–D. Capillary density in the ischaemic gastrocnemius muscle was higher in the heparin group (306 ± 21/mm^2), the exercise group (339 ± 30/mm^2), and the Heparin + Exercise group (639 ± 51/mm^2), with the highest value observed in the Heparin + Exercise group (Figure 4E, P < 0.001). Since capillary density directly reflects new capillary formation

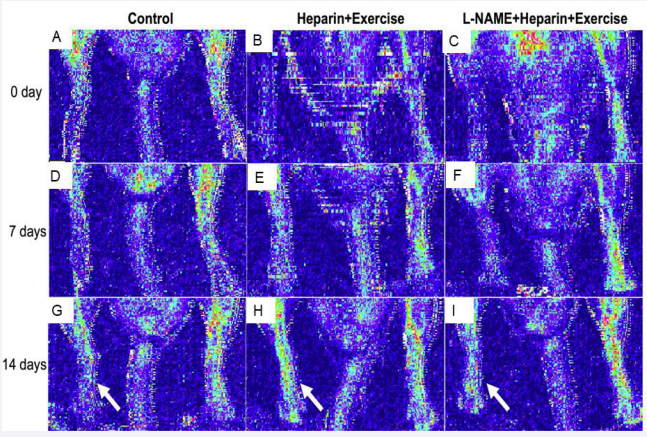


Figure 3 Representative laser Doppler perfusion images of both hindlimbs on days 0, 7, and 14 after induction of hindlimb ischaemia.

Representative images from the Control (A, D, G), Heparin + Exercise (B, E, H), and L-NAME + Heparin + Exercise (C, F, I) groups are shown. In the Control group, perfusion of the ischaemic right hindlimb remained markedly reduced, with minimal recovery by day 14 (G, white arrow). In contrast, perfusion of the ischaemic right hindlimb progressively recovered during the 14-day treatment period in the Heparin + Exercise group (H, white arrow). This recovery was attenuated by L-NAME administration (I, white arrow), suggesting that the beneficial effect of combined heparin and exercise treatment was at least partly nitric oxide dependent.

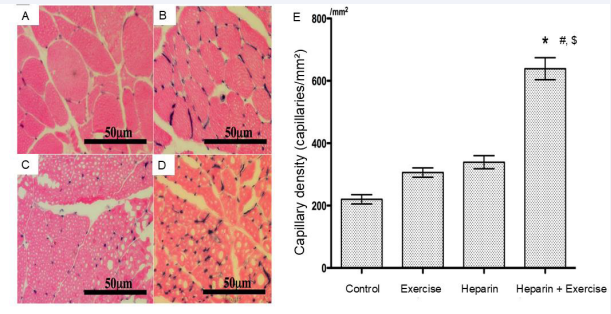


Figure 4 Effects of heparin and exercise on capillary formation in ischaemic skeletal muscle.

Left: Representative cross-sectional micrographs of gastrocnemius muscles from the ischaemic hindlimbs 14 days after surgery (original magnification, ×400). Capillaries were visualized by alkaline phosphatase staining and appear as dark spots or lines: (A) Control, (B) Exercise, (C) Heparin, and (D) Heparin + Exercise groups.

Right: Capillary density (capillaries/mm2) in the ischaemic gastrocnemius muscle after 14 days of treatment. Capillary density was significantly higher in the Heparin + Exercise group than in the Control group. Data are presented as the mean ± SEM. *P < 0.05 versus Control. # P<0.05 vs. Exercise group. $ P<0.05 vs. Heparin group.

within ischaemic muscle, these findings indicate enhanced angiogenesis.

L-NAME attenuated the beneficial effects of treatment (Figure 5). Exercise duration in the Heparin + Exercise group was reduced from 11.99 ± 4.64 to 3.58 ± 2.15 min, whereas that in the Exercise group decreased from 9.51 ± 5.18 to 5.40 ± 3.90 min (Figure 5A–C).

In addition, L-NAME significantly reduced hindlimb perfusion, particularly in the heparin and Heparin + Exercise groups (Figure 5D–F). In contrast, although L-NAME significantly reduced capillary density only in the Heparin + Exercise group (Figure 5G–I), the extent of this reduction was relatively small compared with that observed in perfusion recovery. These findings suggest that NO signalling may have a greater role in functional perfusion recovery, potentially through collateral vessel remodelling, than in capillary angiogenesis.

DISCUSSION

The present study demonstrated that exercise performed after subcutaneous heparin administration significantly enhanced perfusion recovery, exercise capacity, and capillary formation in a rat hindlimb ischaemia model. The principal findings were as follows: (1) heparin administration rapidly increased circulating HGF levels; (2) combined heparin and exercise therapy produced greater improvements in hindlimb perfusion, exercise capacity, and capillary density than either intervention alone; and (3) inhibition of NO synthesis by

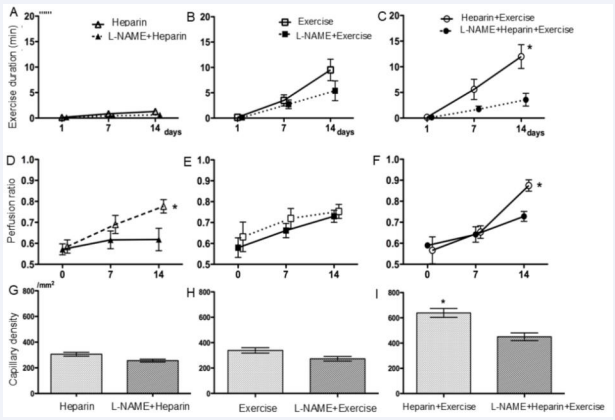


Figure 5 Effects of nitric oxide synthase inhibition on exercise capacity, hindlimb perfusion, and capillary density.

Serial changes in exercise capacity on days 1, 7, and 14 (top panels) and in the hindlimb perfusion ratio (ischaemic/non-ischaemic limb) on days 0, 7, and 14 (middle panels) after induction of hindlimb ischaemia are shown. Capillary density in the ischaemic gastrocnemius muscle was assessed on day 14 (bottom panels). Open symbols and bars represent groups without L-NAME treatment, whereas closed symbols and hatched bars represent the corresponding groups treated with L-NAME. Triangles indicate the Heparin groups; squares, the Exercise groups; and circles, the Heparin + Exercise groups. L-NAME significantly attenuated perfusion recovery in the Heparin group. In the Heparin + Exercise group, L-NAME significantly attenuated the improvements in exercise capacity, perfusion recovery, and capillary density. Data are presented as the mean ± SEM. *P < 0.05 versus the corresponding group without L-NAME treatment.

L-NAME markedly attenuated these beneficial effects, suggesting that NO-dependent vascular remodelling plays a central role in the therapeutic response.

PAD is increasingly recognized as a disorder characterized not only by large-vessel obstruction but also by endothelial dysfunction, impaired angiogenic responsiveness, microvascular dysfunction, and chronic inflammation [8,10,11]. Experimental hindlimb ischaemia models and clinical studies distinguish collateral artery remodelling from capillary angiogenesis and skeletal muscle adaptation [22-31]. Although supervised exercise therapy remains the cornerstone of PAD management, its efficacy is frequently limited by impaired vascular remodelling within ischaemic skeletal muscle [7,22,23]. Exercise induces multiple physiological stimuli, including increased shear stress and tissue hypoxia, which activate HIF-1α-, VEGF-, and NO-dependent vascular remodelling [8,12-16,24,26-30]. Among these, shear stress is a major physiological stimulus for angiogenesis. Milkiewicz et al. [13], demonstrated that increased blood flow directly upregulated VEGF expression and promoted capillary growth even in the absence of marked tissue hypoxia. These observations suggest that enhancing endogenous angiogenic signalling may augment the vascular benefits of exercise therapy.

A novel finding of the present study is that heparin administration increased circulating HGF levels, and combined heparin and exercise treatment enhanced perfusion recovery. These findings suggest that HGF mobilization may contribute to the vascular response to combined treatment, although a causal role for HGF was not directly tested. Heparin rapidly releases HGF bound to endothelial heparan sulfate proteoglycans, markedly increasing circulating HGF concentrations [17,18]. Furthermore, heparin may enhance NO bioavailability indirectly by mobilizing matrix-bound myeloperoxidase and attenuating oxidative endothelial injury [32-34]. HGF is a potent angiogenic and tissue-regenerative factor [32,35-37]. HGF promotes endothelial cell proliferation, migration, survival, and angiogenesis through activation of c-Met signalling and downstream PI3K/Akt/eNOS pathways, thereby enhancing NO bioavailability [38,39]. In this study, exercise likely provided complementary physiological stimuli, particularly increased endothelial shear stress, while heparin increased circulating HGF levels. These stimuli may have converged on NO-dependent signalling pathways to amplify vascular remodelling. The modest effect of heparin alone further suggests that HGF acts primarily as a biological primer that enhances exercise-induced vascular adaptation rather than directly inducing vascular growth.

An important observation of the present study was the differential effect of L-NAME on hindlimb perfusion and capillary density. Laser Doppler perfusion imaging reflects functional blood flow through the vascular network, including collateral arteries and the microcirculation. Its measurements are also influenced by vascular conductance and vasomotor tone. Although L-NAME significantly reduced both parameters, the observed inhibitory effect was greater on perfusion recovery than on capillary density. These findings suggest that NO signalling is particularly important for collateral artery remodelling (arteriogenesis), whereas angiogenesis is additionally regulated by NO-independent mechanisms. This interpretation is consistent with previous studies demonstrating that enlargement and remodelling of pre-existing collateral arteries are the principal determinants of post-occlusive blood flow recovery, whereas capillary angiogenesis primarily facilitates oxygen diffusion within ischaemic skeletal muscle [12,16,24]. Angiogenesis may additionally proceed through NO-independent pathways involving HIF-1α-, VEGF-, and PGC-1α-mediated signalling activated during exercise [8,9,12,13].

Previous studies have demonstrated that aging markedly impairs ischaemia-induced angiogenesis by reducing endothelial responsiveness to angiogenic stimuli [40]. Because PAD predominantly affects older individuals, therapeutic strategies targeting the endogenous HGF–NO axis may partially restore impaired vascular adaptation. Although chronic systemic administration of heparin is unlikely to be clinically feasible because of bleeding risk, the mechanistic insights provided by the present study suggest that modulation of the HGF–NO pathway may represent a promising strategy for future regenerative therapies designed to enhance the vascular benefits of exercise.

Several limitations of this study should be acknowledged. First, although the findings strongly suggest involvement of the HGF–NO signalling pathway, endothelial NO synthase activity, c-Met activation, VEGF expression, and other downstream molecular mechanisms were not directly assessed. Therefore, the precise signalling pathways responsible for the observed vascular effects remain to be elucidated. Second, the acute hindlimb ischaemia model used in this study does not fully replicate the complex pathophysiological features of human peripheral artery disease, including chronic atherosclerosis, inflammation, aging, and metabolic dysfunction. Third, the sample size was relatively small, and the long-term effects of combined therapy on vascular remodelling, collateral vessel maturation, and functional recovery were not evaluated. Further studies are warranted to clarify the molecular mechanisms involved and to determine the long-term therapeutic potential of this strategy in clinically relevant models of PAD.

In conclusion, exercise performed after heparin administration enhanced perfusion recovery and increased capillary density in ischaemic hindlimbs. Attenuation of perfusion recovery by L-NAME supports a contribution of NO signalling to the observed benefit. Heparin-induced HGF mobilization may be involved, but its causal contribution remains unconfirmed.

METHODS

Animal Preparation

All animal experiments were conducted at Jichi Medical University Omiya Medical Center between 2005 and 2008 in accordance with the institutional guidelines for the care and use of laboratory animals in effect at the time and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). An institutional animal ethics approval number could not be identified in the archived records available for this historical study.

Male Wistar rats (n = 59; age, 32–50 weeks; CLEA Japan, Inc., Tokyo, Japan) were used in this study.

Protocol 1: Dose–Response Effect of Heparin on Serum HGF Levels

To determine the temporal changes in serum hepatocyte growth factor (HGF) levels after heparin administration, 16 rats were randomly assigned to receive heparin sodium at 300, 600, or 1,200 U/kg or normal saline (n = 4 per group). All rats were anaesthetised with intraperitoneal pentobarbital sodium (40 mg/kg). Blood samples were collected from the tail vein before heparin administration (baseline). Rats received a single subcutaneous injection of heparin sodium (300, 600, or 1,200 U/kg; Shimizu Pharmaceutical Co., Ltd., Tokyo, Japan) or normal saline. Serum samples were collected before and 60, 120, and 180 min after injection.

Blood samples were collected into serum collection tubes, allowed to clot at room temperature, and centrifuged at 4°C. The separated serum was stored at −80°C until analysis. Serum HGF concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Institute of Immunology, Tokyo, Japan) according to the manufacturer's instructions. The assay sensitivity was 0.2 ng/mL.

Protocol 2: Hindlimb Ischaemia Model

Forty-three male Wistar rats (32–50 weeks old, body weight 460–668 g) were used for the hindlimb ischaemia study. Rats were anaesthetised with intraperitoneal pentobarbital sodium (40 mg/kg). Unilateral hindlimb ischaemia was induced as previously described [22,23]. Briefly, through a lower abdominal incision, the right external iliac artery and distal femoral artery were exposed and ligated. Unilateral hindlimb ischaemia was induced by ligation and excision of the right external iliac and distal femoral arteries, together with ligation of all visible side branches. The animals were randomly assigned to one of the following seven groups:

Control group (n = 7)

Heparin group (n = 6)

Exercise group (n = 6)

Heparin + Exercise group (n = 6)

L-NAME + Heparin group (n = 6)

L-NAME + Exercise group (n = 6)

L-NAME + Heparin + Exercise group (n = 6)

To minimize differences in spontaneous physical activity, four rats were housed together in standard cages (floor area: 836 cm^2). Systolic blood pressure and heart rate were measured using a tail-cuff system (BP-98A; Softron, Tokyo, Japan).

Heparin Administration

Based on the results of Protocol 1, a dose of 300 U/kg was selected for subsequent experiments. Rats assigned to heparin-treated groups received subcutaneous heparin (300 U/kg) twice daily (09:00 and 15:00) for 14 consecutive days beginning one day after surgery.

In the Heparin + Exercise groups, heparin was administered 10 min before each exercise session. Animals not receiving heparin were injected with an equivalent volume of saline.

Exercise Training

Exercise training was initiated one day after surgery.

Rats assigned to exercise groups underwent treadmill exercise using a motor-driven treadmill (Rotor Road Model SN-445; Shinano Manufacturing Co., Tokyo, Japan). Animals exercised for 30 min twice daily (09:10 and 15:10) for 14 days.

The treadmill speed was maintained at 15 rpm, corresponding to a surface speed of approximately 3.3 m/min.

L-NAME Administration

To evaluate the role of nitric oxide (NO), rats assigned to L-NAME groups received intraperitoneal administration of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg) twice daily for 14 days. L-NAME was administered immediately before heparin injection and/or exercise training. The dose was selected based on previous studies demonstrating effective inhibition of endothelial nitric oxide synthase activity. Rats not receiving L-NAME received an equivalent volume of vehicle intraperitoneally.

Assessment of Hindlimb Perfusion

Hindlimb perfusion was serially evaluated using laser Doppler perfusion imaging (LDPI) (moorLDI2™, Moor Instruments Ltd., Devon, UK), according to established methods for experimental hindlimb ischaemia [22,23]. Perfusion measurements were obtained immediately after surgery and on postoperative days 7 and 14. Before scanning, rats were anaesthetised with pentobarbital sodium and hindlimb hair was removed with an electric clipper. Animals were placed on a thermostatically controlled heating plate maintained at 37°C throughout

the procedure. Two consecutive scans were performed for each animal, and the average value was used for analysis. To minimize variability caused by environmental conditions, blood flow was expressed as the ratio of perfusion in the ischaemic (right) limb to that in the non-ischaemic (left) limb.

Assessment of Exercise Capacity

Exercise capacity was evaluated on postoperative days 7 and 14 using the treadmill test.

Rats walked at 15 rpm (approximately 200 m/h), and the duration of continuous walking was recorded. Two measurements were obtained at each time point, and the average value was used for analysis.

Capillary Density in Gastrocnemius Muscle

On day 14, rats were euthanised and the white portion of the gastrocnemius muscle was harvested. Muscle specimens were rapidly frozen in liquid nitrogen-cooled isopentane and stored at −80°C. Frozen sections (10 μm thick) were prepared using a cryostat. Capillaries were identified by alkaline phosphatase staining. Capillary density was quantified by counting the number of capillaries and expressed as capillaries/mm^2.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) or standard error of the mean (SEM), as indicated. Comparisons between two independent groups were performed using the unpaired Student's t-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Serial measurements of hindlimb perfusion and exercise capacity were analyzed using two-way repeated-measures ANOVA to evaluate the effects of treatment, time, and their interaction, followed by Tukey's post hoc test. All tests were two-sided, and a P value <0.05 was considered statistically significant. The statistical software and version used for the analyses were not documented in the archived study records.

Acknowledgements and disclosures

The authors thank Sachimi Jinbo for assistance with exercise training and drug administration in the rats and Keiko Yoshizawa for administrative support.

Funding

This work was supported by a Young Investigator Award from Jichi Medical University in 1999 (to Takanori Yasu) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant No. 21H04290 to Takanori Yasu).

Author contributions

Taishi Hirahara, Yasuhiro Maejima, and Takanori Yasu conceived and designed the study. Taishi Hirahara performed the experiments and drafted the manuscript. Yuma Tamura and Tomoki Tsurumi performed the statistical analyses. Yasuhiro Maejima, Masanobu Kawakami, and Takanori Yasu interpreted the data and critically revised the manuscript. All authors reviewed and approved the final manuscript and agree to be accountable for the work.

Data availability statement

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

Artificial intelligence disclosure

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, San Francisco, CA, USA) solely to improve the English language and readability. The authors reviewed the edited text and take full responsibility for the final content.

REFERENCES

1. Adou C, Magne J, Gazere N, Aouida M, Chastaingt L, Aboyans V. Global epidemiology of lower extremity artery disease in the 21st century (2000-21): a systematic review and meta-analysis. Eur J Prev Cardiol. 2024; 31: 803-811.
2. Gerhard-Herman MD, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, et al. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2017; 135: e726-e779.
3. Aboyans V, Ricco JB, Bartelink MEL, Björck M, Brodmann M, Cohnert T, et al. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries Endorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). Eur Heart J. 2018; 39: 763-816.
4. Makita S, Yasu T, Akashi YJ, Adachi H, Izawa H, Ishihara S, et al. JCS/JACR 2021 Guideline on Rehabilitation in Patients With Cardiovascular Disease. Circ J. 2022; 87: 155-235.
5. Azuma N, Iida O, Soga Y, Ohura N, Ohki T, Ozaki M, et al. JCS/JSVS 2022 Guideline on the management of peripheral arterial disease. 2022.
6. Stewart KJ, Hiatt WR, Regensteiner JG, Hirsch AT. Exercise training for claudication. N Engl J Med. 2002; 347: 1941-1951.

7. McDermott MM, Ades P, Guralnik JM, Dyer A, Ferrucci L, Liu K, et al. Treadmill exercise and resistance training in patients with peripheral arterial disease with and without intermittent claudication: A randomized controlled trial. JAMA. 2009; 301: 165-174.

8. Hamburg NM, Balady GJ. Exercise rehabilitation in peripheral artery disease: Functional impact and mechanisms of benefits. Circulation. 2011; 123: 87-97.

9. Handschin C, Spiegelman BM. The role of exercise and PGC-1α in inflammation and chronic disease. Nature. 2008; 454: 463-439.

10. Hamburg NM, Creager MA. Pathophysiology of intermittent claudication in peripheral artery disease. Circ J. 2017; 81: 281-289.

11. Kavurma MM, Bursill C, Stanley CP, Passam F, Cartland SP, Patel S, et al. Endothelial cell dysfunction: Implications for the pathogenesis of peripheral artery disease. Front Cardiovasc Med. 2022; 9: 1054576.

12. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? J Appl Physiol. 2004; 97: 1119-1128.

13. Milkiewicz M, Brown MD, Egginton S, Hudlicka O. Association between shear stress, angiogenesis, and VEGF in skeletal muscles in vivo. Circ Res. 2001; 8: 229-241.

14. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, et al. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest. 1998; 101: 2567-2578.

15. Yu J, deMuinck ED, Zhuang Z, Drinane M, Kauser K, Rubanyi GM, et al. Endothelial nitric oxide synthase is critical for ischemic remodeling, mural cell recruitment, and blood flow reserve. Proc Natl Acad Sci U S A. 2005; 102: 10999-11004.

16. Lloyd PG, Yang HT, Terjung RL. Arteriogenesis and angiogenesis in rat ischemic hindlimb: Role of nitric oxide. Am J Physiol Heart Circ Physiol. 2001; 281: H2528-H2538.

17. Okada M, Matsumori A, Ono K, Miyamoto T, Takahashi M, Sasayama S. Hepatocyte growth factor is a major mediator in heparin-induced angiogenesis. Biochem Biophys Res Commun. 1999; 255: 80-87.

18. Matsumori A, Ono K, Okada M, Miyamoto T, Sato Y, Sasayama S. Immediate increase in circulating hepatocyte growth factor/scatter factor by heparin. J Mol Cell Cardiol. 1998; 30: 2145-2149.

19. Yang HT, Ogilvie RW, Terjung RL. Heparin increases exercise-induced collateral blood flow in rats with femoral artery ligation. Circ Res. 1995; 76: 448-456.

20. Antonicelli R, Sardina M, Scotti A, Bonizzoni E, Paciaroni E. Randomized trial of the effects of low-dose calcium-heparin in patients with peripheral arterial disease and claudication. Am J Med. 1999; 107: 234-239.

21. Maejima Y, Yasu T, Ueba H, Kobayashi N, Hashimoto S, Kubo N, et al. Exercise after heparin administration: New therapeutic program for patients with non-option arteriosclerosis obliterans. Circ J. 2005; 69: 1099-1104.

22. Couffinhal T, Silver M, Zheng LP, Kearney M, Witzenbichler B, Isner JM. Mouse model of angiogenesis. Am J Pathol. 1998; 152: 1667-1679.

23. Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, et al. Therapeutic angiogenesis: A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hindlimb model. J Clin Invest. 1994; 93: 662-670.

24. Scholz D, Ziegelhoeffer T, Helisch A, Wagner S, Friedrich C, Podzuweit T, et al. Contribution of arteriogenesis and angiogenesis to postocclusive hindlimb perfusion in mice. J Mol Cell Cardiol. 2002; 34: 775-787.

25. Niiyama H, Huang NF, Rollins MD, Cooke JP. Murine model of hindlimb ischemia. J Vis Exp. 2009; 23: 1035.

26. Annex BH. Therapeutic angiogenesis for critical limb ischaemia. Nat Rev Cardiol. 2013; 10: 387-396.

27. Hiatt WR. Medical treatment of peripheral arterial disease and claudication. N Engl J Med. 2001; 344: 1608-1621.

28. McDermott MM. Exercise training for intermittent claudication. J Vasc Surg. 2017; 66: 1612-1620.

29. Treat-Jacobson D, McDermott MM, Bronas UG, Campia U, Collins TC, Criqui MH, et al. Optimal exercise programs for patients with peripheral artery disease: A scientific statement from the American Heart Association. Circulation. 2019; 139: e10-e33.

30. Yasu T. Comprehensive cardiac rehabilitation program for peripheral arterial disease. J Cardiol. 2022; 80: 303-305.

31. Pipinos II, Judge AR, Selsby JT, Zhu Z, Swanson SA, Nella AA, et al. The myopathy of peripheral arterial occlusive disease: part 1. Functional and histomorphological changes and evidence for mitochondrial dysfunction. Vasc Endovascular Surg. 2008; 41: 481-489.

32. Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol. 1992; 119: 629-641.

33. Baldus S, Rudolph V, Roiss M, Ito WD, Rudolph TK, Eiserich JP, et al. Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase. Circulation. 2006; 113: 1871-1878.

34. Azekoshi Y, Yasu T, Watanabe S, Tagawa T, Abe S, Yamakawa K, et al. Free fatty acid causes leukocyte activation and resultant endothelial dysfunction through enhanced angiotensin II production in mononuclear and polymorphonuclear cells. Hypertension. 2010; 56: 136-142.

35. Nakamura T, Sakai K, Nakamura T, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. J Gastroenterol Hepatol. 2011; 26: 188-202.

36. Boros P, Miller CM. Hepatocyte growth factor: A multifunctional cytokine. Lancet. 1995; 345: 293-295.

37. Morishita R, Nakamura S, Hayashi S, Taniyama Y, Moriguchi A, Nagano T, et al. Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hindlimb ischemia model as cytokine supplement therapy. Hypertension. 1999; 33: 1379-1384.

38. Van Belle E, Witzenbichler B, Chen D, Silver M, Chang L, Schwall R, et al. Potentiated angiogenic effect of scatter factor/hepatocyte growth factor via induction of nitric oxide synthase. Circulation. 1998; 97: 381-390.

39. Cooke JP, Losordo DW. Nitric oxide and angiogenesis. Circulation. 2002; 105: 2133-2135.

40. Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, et al. Age-dependent impairment of angiogenesis. Circulation. 1999; 99: 111-120.