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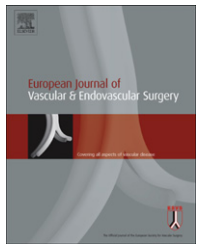

Exercise Linked to Transient Increase in Expression and Activity of Cation Channels in Newly Formed Hind-limb Collaterals

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Abstract *Objective:* This study aimed to compare arteriogenesis after femoral artery occlusion as influenced by exercise or arteriovenous shunt and follow changes in collateral transient receptor potential cation channel, subfamily V, member 4 (Trpv4).
Design: A prospective, controlled study wherein rats were subjected to femoral artery ligation (FAL), or FAL + arteriovenous shunt. Collateral Trpv4 was determined 0.5 and 6 h post exercise.
Methods: Rats were subjected to exercise for 15 min, twice daily. The number and diameter of collaterals were assessed after 7 days. Collateral Trpv4 expression was quantified by reverse transcription-polymerase chain reaction.
Results: Collateral number and diameter per limb were significantly higher in the shunt group (number: 16.0 ± 2.4 and diameter: 216.0 ± 34 μm) compared to the ligature (number: 9.4 ± 2 and diameter: 144 ± 21 μm) and exercise groups (number: 9.9 ± 2.5 and diameter: 151 ± 15 μm). Trpv4 expression in collaterals harvested 0.5 h post exercise was not significantly different from expression in shunted rats. It was significantly lower in collaterals harvested 6 h post exercise (comparable to that in ligated rats).
Conclusion: Collateral formation was greater in the shunt group than in the exercise group. Exercise-induced Trpv4 up-regulation, not significantly different from that achieved with shunt, returned to control values when evaluated 6 h post exercise. More frequent exercise to chronically increase fluid shear stress, as with a shunt model, may be required for sufficient arteriogenesis to compensate for peripheral occlusion.

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Introduction

Occlusive vascular disease remains the most important cause of worldwide death and morbidity. Treatment aims towards limb salvage, improvement of the patient's quality of life and mortality reduction and includes re-vascularisation (open or endovascular), drug therapy and exercise training.[1] In some cases, re-vascularisation is not feasible or fails, and other palliative measures, including limb amputation, may be required. Although collateral vessels bypassing an occlusion spontaneously develop in patients with ischaemic vascular disease,[2] this collateral growth insufficiently compensates for the deteriorated blood flow. Great efforts have been made in the last years in developing therapeutic approaches to stimulate pre-existing compensatory blood vessels, a process called arteriogenesis.[3] The most important triggers are physical forces, such as altered shear force, caused by the large pressure difference in the pre-existing arterioles connecting upstream with downstream branches (relative to the occlusion point) as the result of arterial occlusion.[4–6]

Redirecting the blood flow — after leaving the collateral network — directly to the venous system by creating an arteriovenous (AV) anastomosis between the distal stump of the occluded femoral artery and the accompanying vein increases flow in the collateral network with a subsequent increase in fluid shear stress (FSS).[7] In a pig and rabbit hind-limb arterial occlusion model where the collateral blood flow was directly diverted into the venous system, leading to chronically elevated FSS in the collateral system, collateral growth was markedly stimulated.[8] This new shear stress model was transferred to the rat in which genome-wide analysis is possible, whereas in the rabbit it is not.

Transient receptor potential cation channel, subfamily V, member 4 (Trpv4) has been identified as a 'shear stress regulated' channel, the activation of which triggers collateral growth and remodelling. mRNA abundance of Trpv4 is constantly up-regulated in FSS-stimulated collaterals and an elevated protein expression during collateral growth has been localised to the FSS-sensing endothelium.[9]

Exercise clearly plays a role in the treatment of patients with claudication, improving symptoms and walking ability.[10] The capability of exercise to alter FSS with the subsequent effect on Trpv4, an initial mediator of arteriogenesis, is of particular interest. This study aimed to investigate collateral development and gene expression of the FSS-regulated channel Trpv4 after exercise training or AV shunt in rats.

Materials and methods

This study was performed according to Section 8 of the German Law for the Protection of Animals, which conforms to the US National Institutes of Health (NIH) guidelines.

Adult male Sprague-Dawley rats were subjected to femoral artery ligation (FAL). FAL was followed either by AV shunt formation between the distal femoral artery stump and accompanying vein (Fig. 1) or by exercise training. Trpv4 was determined 0.5 h and 6 h post exercise, after 7 days of training. Rats with FAL, with no shunt or exercise training, were termed the ligature group. In six rats, an AV shunt was re-occluded 1 day prior to tissue harvesting and used for quantification of gene expression (shunt re-occlusion group). The number of values for each group for collateral number and size were ligature-14, shunt-10, and exercise-12. The number of values for each group for Trpv4 expression were shunt-11, exercise early (harvested 0.5 h post exercise)-6, exercise late (harvested 6 h post exercise)-6, shunt re-occlusion-6 and ligature-7.

Femoral artery ligature

Anaesthesia was induced by administering ketamine hydrochloride (100 mg per kg of body weight) and xylazine

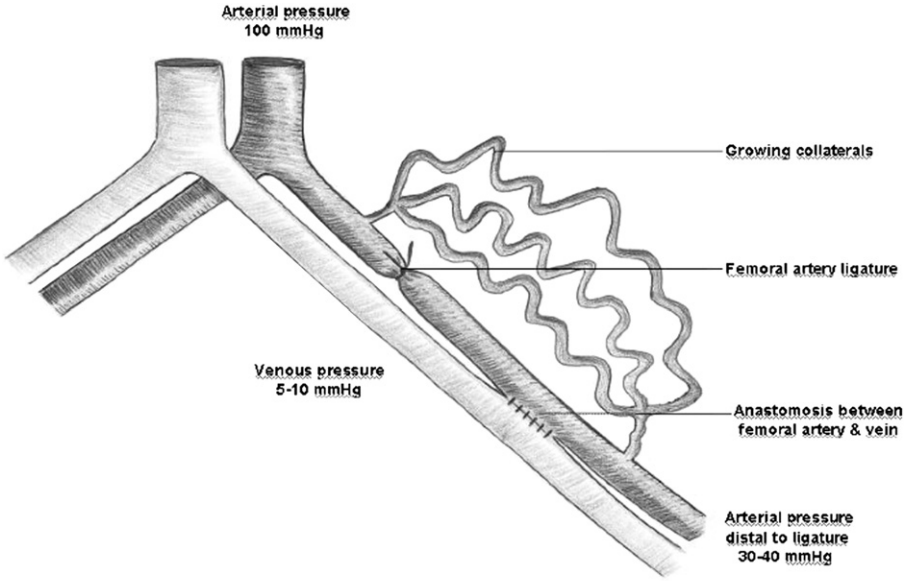

Figure 1 Shunt model. An anastomosis is made between the femoral artery and vein and the femoral artery is ligated proximal to the anastomosis.