Arteriogenesis and ischemia impair functional vasodilation in resistance arteries

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Introduction

Patients with peripheral arterial occlusive (PAOD) disease have impaired vasodilation. Identifying the mechanism of this impaired could lead to improved therapies. Co-morbidities common to PAOD, e.g. hypercholesterolemia, are known to impair vasodilation, but arterial occlusion alone also impairs vasodilation. Although impaired vasodilation in lower-leg arterioles is well described in animal models of PAOD, the impact of arterial occlusion on collateral and resistance arteries is unknown. Collateral resistance is the largest determinant of downstream flow and resistance arteries contribute up to half of the resistance to skeletal muscle blood flow, so identifying the impact of arterial occlusion on these vessels will likely provide the greatest potential therapeutic benefit. Therefore, the goal of this project is determine the impact of arteriogenesis and ischemia on collateral artery and resistance artery vasodilation, respectively. The long-term goal of this work is identify potential therapeutic targets for impaired vasodilation in patients with PAOD.

Experimental Model – Intravital Microscopy

We measured feed artery diameter using Side-stream Dark Field (SDF) intravital microscopy. SDF collects the reflected light from oblique 530nm pulses, which is the isosbestic point for hemoglobin, so the vasculature appears dark and the parenchymal and stromal tissue appears light. Our intravital imaging station is shown in **Figure 2**. Example images of the muscular branch artery (at rest and following functional vasodilation) are shown in **Figure 3**.

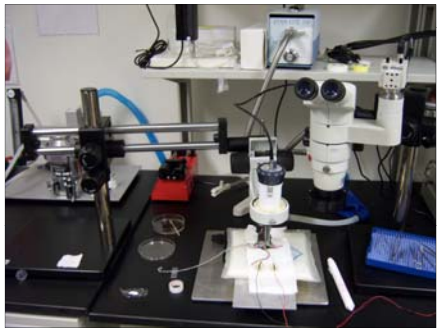

Figure 1. Intravital microscopy imaging station for functional vasodilation measurements.

Intravital Microscopy- Functional Vasodilation

We electrically stimulate muscle contraction in the gracilis muscles to assess the endogenous vasodilation pathways in the profunda femoris artery. The (-) stimulating electrode is placed in the superficial surface of the gracilis muscles and the (+) ground electrode is placed in the skin. The motor end plate is stimulated with to 1mA pulses of 200-500µs duration at 8Hz for 90sec. Example resting and dilated profunda femoris arteries are shown in **Figure 2**.

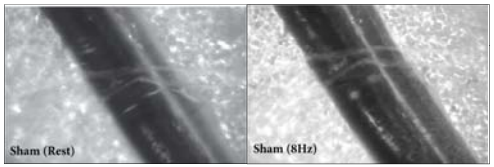

Figure 2. Still images of the muscular branch artery at rest (left) and following 90 seconds of 8Hz muscle contraction (right).

Surgical Model I_- Arteriogenesis

To determine the impact of elevated flow and arteriogenesis on collateral artery vasodilation, we utilized an experimental model that involved ligation of the femoral artery between the profunda femoris and popliteal branches (proximal ligation, **Figure 3a**) or just distal to the popliteal (distal ligation, **Figure 3b**). These surgeries lead to arteriogenesis in the profunda femoris and its development as the stem region of a collateral circuit.

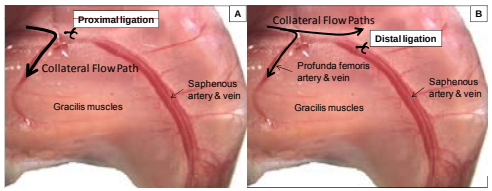

Figure 3. Medial aspect of the mouse hindlimb showing site of femoral artery ligation and primary collateral flow paths.

Arteriogenesis Impairs Functional Vasodilation

To test the hypothesis that arteriogenesis impairs endogenous vasodilation pathways, we measured profunda femoris artery diameter before and after electrical stimulation of the gracilis muscles with tungsten microelectrodes with 1mA pulses of 200µs duration at 8Hz for 90sec, **Figures 4 & 5**.

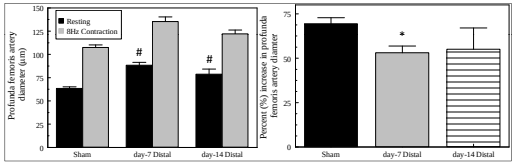

Figure 4. Left panel, profunda femoris artery diameter at rest and following muscle contraction in sham-operated animals and at day-7 and day-14 following distal femoral artery ligation. **Right panel**, percent increase in profunda femoris artery diameter following muscle contraction, same groups as left panel. * p < 0.05 vs sham vasodilation, # p <0.05 vs sham resting.

As a corollary to the overal hypothesis, we tested the hypothesis that a greater arteriogenesis stimulus would result in a greater impairment in vasodilation. The primary stimulus for arteriogenesis is elevated endothelial shear, so to test this corollary, we performed a two ligation surgeries. The distal femoral artery ligation (**Figure 4**) presumably results in a moderate increase in profunda femoris shear stress, because the popliteal artery can also serve as a collateral circuit in this model and the proximal femoral ligation (**Figure 5**) presumably results in a large increase in profunda femoris shear stress because the ligation is placed upstream of the popliteal.

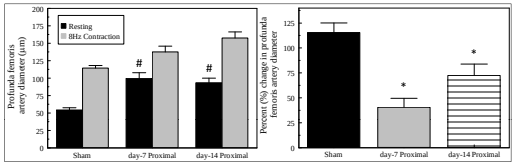

Figure 5. Left panel, profunda femoris artery diameter at rest and following muscle contraction in sham-operated animals and at day-7 and day-14 following proximal femoral artery ligation. **Right panel**, percent increase in profunda femoris artery diameter following muscle contraction, same groups as left panel. * p < 0.05 vs sham vasodilation, # p <0.05 vs sham resting.

Surgical Model II_- Ischemia

To determine the impact of ischemia on feed artery vascular reactivity, we utilized an experimental model that involved resection of the femoral artery-vein pair from just upstream of the profunda femoris to the distal saphenous, **Figure 6**. This surgery presumably produces ischemia in the profunda femoris artery and the downstream gracilis muscle microcirculation.

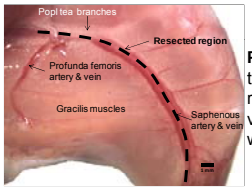

Figure 6. Medial aspect of the mouse hindlimb. The region of femoral artery-vein resection is indicated with a dashed line.

Ischemia Eliminates Functional Vasodilation in Response to Moderate Muscle Contraction

To test the hypothesis that ischemia impairs endogenous vasodilation pathways, measured profunda femoris artery diameter at day-14 following resection, before and after electrical stimulation of the gracilis muscles with tungsten microelectrodes with 1mA pulses of 200µs duration at 8Hz for 90sec, **Figures 7 & 8**.

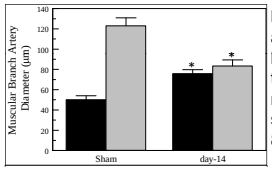

Figure 7. Profunda femoris artery diameter at rest (black bar) and immediately following the cessation of moderate muscle contraction (grey bar), stimulated at 1mA, 200µs, 8Hz, 2min. * p < 0.05 vs sham.

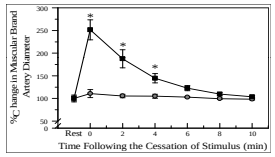

Figure 8. Functional vasodilation (% change from resting or 100%) of the ischemic profunda femoris artery with moderate intensity muscle contraction for sham (black) and day-14 ischemic (grey).

Ischemia does not Affect Functional Vasodilation during Intense Muscle Contraction

To further examine the impact of ischemia on profunda femoris artery functional vasodilation, we measured functional vasodilation at day-14 following resection, before and after electrical stimulation of the gracilis muscles with tungsten microelectrodes with 1mA pulses of 200µs duration at 8Hz for 90sec, **Figure 9**. The increased stimulus duration produces a more robust muscle contraction and presumably a greater stimulus for vasodilation

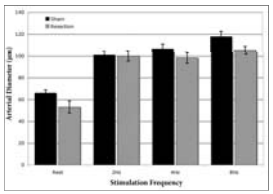

Figure 9. Muscular branch artery diameter at d14 following femoral artery resection in response to increasing stimulus frequency- 2, 4, or 8Hz and 1mA, 500µs, 90sec.

Ischemia Delays Return to Baseline Diam
Following Intense Muscle Contractio

As seen in Figure 9, a more intense muscle contraction can
normal vasodilation in the ischemic profunda femoris artery.
restoration of resting diameter seemed to be delayed in these
Therefore, we examined the time course of vessel diameter be
after the electrical stimulation of the gracilis muscles with
microelectrodes with 1mA pulses of 500µs duration at 8Hz f
Figure 10.

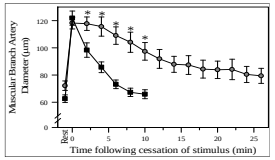

Figure 10. Functiona
vasodilation recovery
ischemic profunda fe
artery following high i
contraction. Sham op
(black circles) and da
ischemic arteries (gre
circles). * p < 0.05 vs

Ischemia Impairs Endothelial & Smoo
Muscle-dependent Vasodilation

To test the hypothesis that impaired vasodilation following isch
due to impaired endothelial-dependent vasodilation,
profunda femoris artery diameter at day-14 following resectio
and application of acetylcholine, sodium nitroprussi
norepinephrine (10^{-4}M) to the physiological salt soluti
physiological salt solution was bubbled with 95%N_2-5%CO_2 and
to ~35°C.

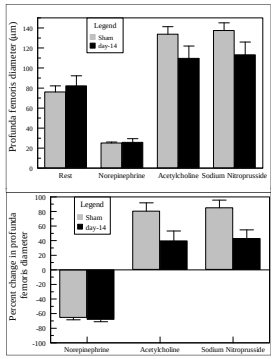

Figure 11. Profunda
artery diameter at res
following vasodilatio
acetylcholine and so
nitroprusside, and fo
vasoconstriction to
norepinephrine. **Top**
absolute diameter; **B**
Panel- percent chan
baseline.

Summary

• Arteriogenesis transiently reduces functional vasodilation in c
resistance arteries. The degree of reduction appears to be impa
the arteriogenesis stimulus, **Figures 4 & 5**.

• Ischemia reduces functional vasodilation in resistance arteries
7 & 8. The extent of this reduction is impacted by the muscle
contraction intensity and presumably vasodilation stimulus, **Fig**
This reduction appears to be due to reduced smooth muscle re
Figure 11.

• Ischemia delays the restoration of resting diameter in resistan
arteries following intense muscle contraction, **Figure 10**. This de
not due to impaired responsiveness to norepinephrine, **Figure 1**

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