Online Supplement

Methods:

**X-Ray Angiography**

Seven or 28 days after right femoral artery occlusion the left femoral artery was ligated. Via a cannula (0.8 mm outer diameter) in the descending thoracic aorta, at a pressure of 100 mmHg, distal vessels were rinsed for 2 minutes with phosphate buffered saline (PBS) containing 1 g/l adenosine (Fluka, Steinheim, Germany) and 4 mg/l papaverine (Paveron, Linden Arzneimittel, Germany) followed by 2% formaldehyde in PBS for 3 min, buffer for 2 minutes, and finally a two minute infusion of a contrast agent containing 5% gelatin and about 40% bismuth oxychloride\textsuperscript{17} at a temperature of 30°C and a pressure of 200 mmHg. Mice were chilled in ice water. X-rays were taken with a Machlett-Balteau x-ray source (20 kV, 8 mA 2.5 minutes). Films were scanned at a resolution of 3600 × 1800 DPI, interpolated to 3600 DPI, (Microtek ArtixScan 1800f) and analyzed independently by two blinded observers. Numbers of collateral arterial connections bridging the ligation site were counted, their diameters measured at the level of the ligation site with a customized version of ImageJ permitting automatic border detection, and relative conductance scores were calculated using the formula:

\[
\text{Relative Collateral Conductance score} = \frac{\sum R_{CA}^4}{R_{FA}^4}
\]

where \(n\) is the number of collaterals, \(R_{CA}\) is the radius of a collateral arterial vessel, \(R_{FA}\) the radius of the ipsilateral femoral artery proximal to the ligation site.

To assess preexistent collaterals, the degree of distal hindlimb filling on the acutely ligated left side was scored independently by two blinded observers. A modification of the Rentrop Score\textsuperscript{18} was used, which classified collateral-dependent filling as 0 = no filling of collaterals and hindlimb vessels distal to the level of the femoral artery occlusion site, 1 = some filling of proximal thigh collaterals distal to level of arterial occlusion but no or only very faint filling of distal hindlimb vessels, 2 = markedly reduced filling of distal vessels, 3 = complete filling of distal hindlimb vessels.

**Histology**

5-bromo-2’-desoxyuridine (BrdU) (Sigma, Taukirchen, Germany) was added to drinking water (80 mg/100ml) of BALB/c and C57 BL/6 mice (n=5 per group) one day before surgery and changed every three days. Seven days after surgery, mice underwent perfusion fixation and angiography. Complete cross-sections of medial and caudal thigh muscles at the level of arterial occlusion were frozen in liquid nitrogen after cryoprotection in 20% sucrose/PBS. Five µm sections were stained with a fluorescein-conjugated anti-BrdU monoclonal antibody (In Situ Cell Proliferation Kit, FLUOS, Roche Diagnostics, Mannheim, Germany), after pretreatment with ethanol, trypsin and hydrochloric acid according to instructions. Subsequently, staining with DAPI (Sigma) and TRITC-labeled BS-I lectin (0.01 mg/ml, Sigma) or a Cy3 conjugated monoclonal anti α smooth muscle actin antibody (Sigma) was performed. Uptake of BrdU by arterioles/arteries was quantified on 2 complete cross-sections of the medial and caudal thigh muscles at the level of the ligation.
Statistical Analysis
Data were analyzed with one way ANOVA or, if not normally distributed or variances unequal with Kruskall-Wallis one way ANOVA on ranks with the appropriate confirmation tests for the comparison of three strains, and student T-test or Mann-Whitney U rank-sum test for comparison of two groups (SigmaStat for Windows, version 3.0.1). Statistical significance was defined as p<0.05.

Results:

**Figure 1.** Representative laser Doppler images of distal hindlimbs at different time points.

![Laser Doppler Images](image-url)
**Figure II.** Individual laser Doppler values. For means and SEM, please see Fig 1a.

![Graph showing individual laser Doppler values for different genotypes (C57BL/6, 129S2/Sv, BALB/c) over time (0-28 days).](image)

**Figure III.** MR-flow image at calf level of a BALB/c mouse, three days after surgery. Vessels with flow (high-signal intensity) are visible in the tail (T) and the left non-ligated side (L). Only minimal flow on the ligated side (R). The right calf is swollen. (See figure 1B for quantification of flow and figure 1F for quantification of cross-sectional area ratios).

![MR-flow image showing vessels with flow in the tail and left side, and minimal flow in the right side.](image)
Figure IV. Reactive hyperemia in feet quantified by LDI three weeks after surgery. After transient cuff inflation, six successive LDI scans were performed, each lasting one minute.

A, Relative flux values (compared to baseline before cuff inflation) non-ligated side.

B, Relative flux values in ligated side.

C, Right-to-left ratio of flux values before cuff inflation (R pre / L pre), and ratio of maximal flux during hyperemia phase in ligated and non-ligated side (R max / L max). Values are mean±SEM, n=5 per strain.
Figure V. Individual EPR oximetry data on right medial thigh muscles. For means and SEM of right and left hindlimbs, please see Fig 3, upper panel.
Figure VI. X-ray angiogram of a BALB/c mouse 28 days after ligation of the right femoral artery (*). The left side is not ligated which allows for better filling of preexistent collateral vessels by the contrast agent (however collateral-dependent filling cannot be graded). The stem (origin) and target vessels of collateral arteries were identical for BALB/c, C57BL/6, and 129S2 mice. Subcutaneous collateral vessels are not visible on this image of a BALB/c mouse, as the skin was removed prior to immersion of these mice in Boine’s solution for fixation and bone demineralization. Collateral arterial connections in the cranial thigh muscles are fed by two arteries supplying the quadriceps muscle: a proximal one (arrowhead with open circle, ○) originating from the very proximal internal iliac artery, and the a. circumflexa femoris lateralis (arrowhead with triangle, ▲), a branch of the external iliac artery. Collaterals from these arteries anastomose distally with vessels related to the a. genus descendens (empty white arrowhead) and a more lateral artery (arrowhead with asterisk, *) that connects to the popliteal artery (arrowhead with pound sign, #). Collateral arteries in the medial and caudal thigh muscles originate from the internal iliac artery (arrowhead with closed circle, ●) and the deep (caudal) femoral artery (arrowhead with square, ■). These collateral vessels anastomose distally with the saphenous artery (arrowhead with empty square, □) and frequently are somewhat tortuous. On the non-ligated side preexistent collateral arteries can be identified in the same pattern as on the ligated side, even though not as well filled with contrast agent; they are smaller than on the ligated side and not tortuous.