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**Immediate Effects of Arterial Ischemia and Venous Occlusion on the Function of Vascularized Groin Lymph Nodes in a Rodent Model**

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**BACKGROUND:** Lymphedema can be successfully addressed by physiological surgical procedures, like vascularized lymph node transfer. Previously, our research team defined the critical ischemia time of lymph nodes to be 5 hours. This consecutive study investigated the distinctive effects of arterial ischemia and venous occlusion on lymph nodes.

**METHODS:** Bilateral pedicled groin lymph node flaps were raised in 27 male Lewis rats. The vascular pedicles were dissected and artery and vein separated. Arteries were clamped on left, veins were clamped on right sides for either 1, 3, 4 or 5 hours. After clamp release, reperfusion was allowed for 2 hours. Lymph node flap perfusion and drainage function were assessed using laser doppler flowmetry and indocyanine green lymphography (ICG). Morphological changes were detected by histologic assessments, using HE staining, TUNEL- and glutathione assays.

**RESULTS:** Laser doppler at arterial ischemia times of 1, 3, 4 and 5 hours, as measured after 2 hours of reperfusion, sensed perfusion units (PU) of  $126.62 \pm 23.18$ ,  $92.78 \pm 24.71$ ,  $93.80 \pm 12.60$  and  $37.48 \pm 15.56$ , while PU of venous occlusion times were  $42.02 \pm 10.93$ ,  $48.37 \pm 28.57$ ,  $44.96 \pm 9.40$  and  $7.76 \pm 10.27$ , respectively. Lymphatic drainage of ICG showed mean latency times of  $5.33 \pm 0.88$ ,  $9.00 \pm 3.21$ ,  $10.00 \pm 2.08$  and  $24.50 \pm 11.50$  seconds post arterial clamping and  $25.00 \pm 3.61$ ,  $26.00 \pm 3.06$ ,  $23.33 \pm 4.41$  and  $152.00 \pm 0$  seconds after venous clamping at 1, 3, 4 and 5 hours, respectively. Histologically, arterial ischemia showed slight medullary congestion at all time points, whereas venous occlusion depicted severe medullary and cortical congestion/hemorrhage in groups exposed to  $\geq 3$  hours of clamping. Cell damage as detected by glutathione and TUNEL assay was found to appear earlier in venous occlusion, showing a glutathione peak of  $20.70 \pm 11.08$  nM/ml after 4 hours of venous clamping.

**CONCLUSION:** Effects of arterial ischemia and venous occlusion can be clearly differentiated from the first hour onwards. The critical venous occlusion time is 4 hours, which is in contrast to our previously detected critical ischemia time of 5 hours.