Abstract: 29O

Pericyte characterisation in healthy and type 2 diabetic skeletal muscle microvasculature

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Background: The skeletal muscle microvasculature is a key regulator of peripheral resistance and plays a major role in determining muscle function in health, exercise and type 2 diabetes (T2D). Despite being studied for over 100 years, we are yet to fully understand how capillary blood flow in muscles is controlled and how this changes with diseases such as T2D. Pericytes (PC) are capillary bound cells that have been recently rediscovered in muscle. Despite recent studies showing that pericytes are contractile and capable of regulating capillary diameter, little is known about PC distribution in the muscle microvasculature and how this is impacted by diseases such as T2D. In this study, we aimed to characterise PC in healthy and T2D skeletal muscle.

Methods: Male and female *Tg(Cspg4-DsRed.T1)1Akik/J* mice were allocated to either control diet (CD; 6% fat wt/wt, male n=9, female n=4) or high fat diet (HFD; 23% fat wt/wt, male n=17) for 17 weeks. To model T2D we induced moderate and severe hyperglycemia in 12 of the HFD-fed mice by treatment with streptozotocin (STZ; MOD - 200mg/kg n=5, SEV - 250-300mg/kg n=7, infused using osmotic mini pumps over 14 days). In week 17, mice underwent a 2 hour glucose tolerance test (GTT; 2.0g/kg ip.glucose) and were euthanised by cardiac perfusion with PBS, followed by 4% paraformaldehyde and PBS containing 1.25% gelatin and 0.1% FITC-dextran to mark the lumen of the vasculature. The tibialis anterior and gastrocnemius were excised and sectioned transversely and longitudinally. They were processed for immunohistochemistry and imaged using confocal microscopy.

Results: Capillary density was consistent in transverse sections of male and female gastrocnemius (614±167 vs 538±336 capillary/mm^2, p=0.697). There was no difference in pericyte coverage of these capillaries between males and females (96.0±2.4 vs 92.6±4.0%, p=0.192). HFD mice were obese (33.0±3.5 vs 38.7±4.1 g, p=0.011) and had elevated fasting blood glucose regardless of STZ dose (9.6±0.9 vs 14.7±5.1 mmol/L, p=0.036) compared to CD mice. Blood glucose concentration during 2hr GTT increased step wise with the addition of HFD and increasing dose of STZ (CD 11.0±0.9 vs HFD 15.9±3.4 vs MOD 23.5±3.7 vs SEV 31.2±2.6, p=<0.001). In the gastrocnemius, we saw no change in capillary density (CD 133±27 vs HFD 139±24 vs MOD 129±33 vs SEV 168±19 capillary/mm^2, p=0.059). However, analysis in longitudinal sections of the tibialis anterior found that the number of pericytes along the length of capillaries was reduced by ~30% in SEV compared to CD (9.0±2.7 vs 6.09±3.40 cells/mm, p=0.02). In addition, we identified changes in pericyte morphology including discovered swelling and fragmentation of pericyte cytoplasmic processes that usually encircle and traverse the length of skeletal muscle capillaries.

Conclusion: In summary, male and female skeletal muscle capillaries are similar in density and have a pericyte coverage of ~ 95%. In T2D, we saw no change in capillary density, however the number of pericyte cell bodies was reduced by ~30% and we saw morphological changes that suggest these cells may be damaged. Given pericytes are known regulators of capillary blood flow in other organs, our work suggests that muscle pericytes may have important physiological functions to control capillary blood flow and therefore may contribute to blood flow dysregulation contributing to T2D.