Abstract: 17O

Muscle stem cell function is maintained in mouse models of type 1 diabetes

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Type 1 diabetes mellitus (T1DM) is a systemic metabolic disease characterised by an autoimmune response to insulin-secreting pancreatic beta cells. T1DM patients frequently suffer from diabetic myopathy, a complication that affects muscle health and function (Orlando *et al*., 2017). Diabetic myopathy also increases the susceptibility to muscle injury and impairs muscle regeneration after injury (Dial *et al*., 2021). Since muscle stem cells (MuSCs) are critical for muscle regeneration and maintenance of muscle health, we tested the hypothesis that MuSC function is compromised in T1DM.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). Two C57Bl/6 mouse models of T1DM were employed; the streptozotocin induced diabetic (STZ) and the Ins2 (Akita) mouse. STZ mice were injected with streptozotocin (40 mg/kg) or vehicle (citrate buffer) intraperitoneally for 5 consecutive days to induce diabetes at 8 weeks of age. Akita mice are heterozygous for a mutation in the *Ins2* gene, which causes spontaneous development of diabetes at 4 weeks of age. Akita mice were compared with wildtype (WT) littermate controls At 12-16 weeks of age (4-8 weeks duration of disease), mice were killed by rapid cervical dislocation and the hindlimb skeletal muscles excised for isolation of MuSCs or stored for later histological analyses. Experiments were conducted on MuSCs *in vitro* between passage 2 and 5 and cultured in low glucose media supplemented with foetal bovine serum and fibroblast growth factor.

Both models of T1DM had sustained hyperglycaemia after T1DM onset [HbA1c: Vehicle 4.5% (n=5) vs. STZ 10.8% (n=5), ($P<0.05$); and WT 4.5% (n=6) vs. Akita 10.9% (n=4) ($P<0.05$)]. Muscle atrophy was evident in the tibialis anterior muscles of both diabetic models, with STZ mice exhibiting a 31.6% and Akita mice a 16.3% loss in absolute muscle mass compared with control animals [Vehicle 56.4 mg [n=5] vs. STZ 38.8 mg [n=5] ($P<0.05$); and WT 50.6 mg (n=15) vs. Akita 42.4 mg (n=14) ($P<0.05$)]. Normalised muscle mass (to tibial length) was similarly reduced in both diabetic models compared with their respective controls. MuSC number was reduced in diabetic mice, but there was no difference in the intrinsic proliferative or differentiation capacity of MuSCs *in vitro* between diabetic and non-diabetic mice. The reduced population of MuSC in STZ diabetic mice was further evident based on 59% fewer myogenic cells on isolated myofibers after 72 hours in culture.

These data suggest that the remaining MuSCs in T1DM mouse models are fully functional when isolated from the diabetic environment. Thus, impaired muscle regeneration in T1DM is likely attributed to environmental factors and a reduced MuSC population. Further studies will assess whether maintenance of glucose control can prevent the loss of MuSCs and improve muscle regenerative capacity.

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