Increasing nuclear NAD+ biosynthesis induces muscle remodelling without alterations in myofibrillar Ca^{2+} sensitivity

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Nicotinamide adenine dinucleotide (NAD+) is a ubiquitous metabolite involved in a multitude of reactions throughout the cell. Recent studies have implicated NAD+ levels as influencing many of the pathways involved in obesity and ageing, which has has sparked a surge in interest in better understanding NAD biology.

Nicotinamide mononucleotide adenylyltransferase (NMNAT) is a key enzyme of the NAD+ salvage pathway, that catalyses the conversion of NAD+ precursors to NAD+, thus playing a major role in maintaining cellular NAD+ concentrations. However, little is known about the metabolic role of NMNATs or how changing NAD+ levels *via* NMNAT manipulation alters metabolism. Studies from our laboratory (presented elsewhere at this meeting) showed that increasing NAD+ biosynthesis in the nucleus causes a reduction (30-40%, $P<0.001$) in skeletal muscle mass and reduced muscle size in NMNAT1Tg mice, the latter primarily due to smaller individual muscle fibres.

In the current study, we used a recently engineered "MyoRobot" automated force transducer and length actuator platform designed for higher throughput assessment of muscle biomechanics or biopolymer material testing, to see if there were any actual changes in the contractile properties of the contractile proteins in these smaller fibres. We used transgenic mice globally overexpressing NMNAT1. Mice were killed (Ethics approval UNSW 15/48B) with an overdose of isoflurane and the EDL muscle dissected out and stored in cold relaxing solution. Single muscle fibre segments were dissected out of the EDL muscle, attached to the force transducer and voice coil actuator pins on the "MyoRobot" and chemically skinned before activating them in a series of EGTA buffered Ca^{2+} solutions of defined pCa to quantify their contractile properties.

In total, two mice each (WT, Tg) were analysed with a total of 24/23 single EDL fibre segments. Each protocol consisted of an initial maximum Ca^{2+} activation, followed by a defined graded Ca^{2+} force response and followed by a last maximum activation to quantify the run-down. There was no effect of NMNAT1 overexpression on the contractile proteins of fibres, with Ca^{2+} sensitivity (pCa50) and dynamic biosensor range (Hill coefficient, reflecting binding stoichiometry of Ca^{2+} ions to troponin C) being similar between WT 5.5 and overexpressing NMNAT1 fibres 5.6. There was a difference in absolute force in NMNAT1 TG fibres, which was likely due to their reduced size, as no significant difference in specific max. force was observed.

These results demonstrate that the reduction in whole muscle mass reported in NMNAT1Tg mice has not markedly altered the basic contractile properties of the contractile proteins on the single fibre level.

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