Visualisation of intramuscular lipid droplet and perilipin 2 association in isolated human muscle fibres

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Lipid droplets (LD) are recognised as functional organelles that form physical connections with the mitochondria, endoplasmic reticulum, peroxisomes and lysosomes. The LD contains an array of proteins on its surface which appear to regulate the storage, mobilisation and trafficking of intracellular fatty acids between these various organelles (Gao and Goodman 2015). In skeletal muscle, LDs are highly abundant in type I muscle fibres and provide an important substrate for mitochondrial β-oxidation during moderate intensity exercise. In response to exercise training, remodelling of the intramuscular LD pool is demonstrated by increased LD content, greater expression of the perilipin (PLIN) family of LD proteins and increased LD-mitochondria connections (Shepherd *et al.* 2013). Such adaptations contribute to the higher rates of intramuscular-derived fatty acid oxidation observed following endurance training. In contrast, the accumulation of intramuscular LDs in obesity and ageing are linked to dysfunctional lipid metabolism and lowered insulin sensitivity in skeletal muscle.

In the present study, we aimed to develop and implement an immunofluorescence microscopy approach to study the localisation of intramuscular LDs, and their association with the LD protein PLIN2, in single fibres isolated from human muscle biopsies.

Recreationally active (n=6) and endurance trained cyclists (n=4) were recruited (aged 27±2 years, BMI 23.7±0.5 $kg \cdot m^{-2}$, mean±SEM). Maximal aerobic capacity and peak fat oxidation rates were determined through an incremental exercise test on a cycle ergometer. On a separate visit, a single percutaneous muscle biopsy was obtained from the *vastus lateralis*. Fibre type specific LD and PLIN2 content was assessed on muscle cryosections as previously described (Shaw *et al.* 2009). On a separate portion of the biopsy, small bundles of fibres (~10 fibres) were immediately isolated, pinned at resting length, fixed and permeabilized. Individual fibres were teased apart and immunostained using an antibody against PLIN2 (AP125, Progen) and LDs were detected using the lipid dye bodipy. Imaging was performed using confocal microscopy.

The trained group had a higher $VO_{2\ max}$ (62.7±2.8 *versus* 44.8±2.2 $ml \cdot kg \cdot min^{-1}$) and higher peak fat oxidation rates (0.51±0.03 *versus* 0.20±0.03 $g \cdot min^{-1}$) compared to the untrained group ($P<0.05$). Muscle fibre type specific analysis revealed significantly greater LD content ($P<0.05$) and PLIN2 abundance ($P=0.05$) in the trained group. Z stack imaging of single fibres revealed LDs distributed through the entire portion of the muscle fibre. LDs were frequently observed in pairs aligned with the I-band region of the sarcomere. LDs were also commonly grouped in series aligned with the longitudinal axis of the muscle fibre. PLIN2 was identified as punctate structures distributed throughout the muscle fibres in a similar manner to the LD. Colocalisation analysis demonstrated frequent colocalisation of PLIN2 with the LDs. Higher magnification images demonstrated the appearance of rings of PLIN2 which encircled many of the LDs, although LDs without PLIN2 were also clearly visible.

This study demonstrates that immunofluorescence imaging of LDs in isolated single muscle fibres offers a viable approach to investigate LD content, localisation and association with related proteins and/or organelles. The methodology presented can be used to generate novel information on the regulation of fatty acid trafficking and metabolism in human skeletal muscle with relevance for adaptation to chronic exercise training and in the development of lipid-related metabolic dysfunction.

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