Single fibre responses of MARPs to an acute bout of resistance exercise in untrained individuals

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Muscle Ankyrin Repeat proteins (MARPs) have been postulated to be mechanical stress sensing proteins that play a role in adaptive responses of skeletal muscle. MARPs are muscle specific transcription factors that possess the ability to shuttle to the nucleus and influence gene expression. Two members of the MARP family include Ankrd 2/ARPP and DARP/Ankrd 23. Cell biology experiments show that Ankrd 2 normally exists in the nucleus of proliferating myoblasts but translocates to the cytosol upon induction of differentiation (Bean *et al.*, 2011). In resting human skeletal muscle, ~50% of Ankrd 2 was freely diffusible and therefore localised to cytosol in skinned single fibres (Wette *et al.*, 2013). Also, when differentiating C_2C_{12} myoblasts are exposed to oxidative stress, Ankrd 2 redistributes from the cytosol to the nucleus (Cenni *et al.*, 2011). However, it is uncertain if movement of Ankrd 2 between subcellular compartments would be evident in mature skeletal muscle cells (fibres) as there are no data that have quantitatively assessed MARPs translocation ability in skeletal muscle. This study measured the diffusibility of Ankrd 2 and DARP in single muscle fibres in response to a high intensity resistance exercise (RE) session consisting of strength and power-based exercises.

Five, healthy, recreationally active but non-resistance trained men were recruited. Percutaneous needle biopsy samples were obtained from the *vastus lateralis* muscle under local anaesthetic (2% Xylocaine) before and 3 h following a bout of RE. Single muscle fibres were isolated from a portion of the biopsy under paraffin oil and then mechanically skinned. Fibres were placed in physiological solution for 10 min (Wash) to allow cytosolic proteins to diffuse out, and then subjected to triton treatment for a further 10 min which extracted the membrane bound/associated proteins, before collecting nuclear and cytoskeletal proteins in the skinned fibre. Fibres and washes/treatments were later analysed for Ankrd 2 and DARP protein levels using Stain-Free Western blot technology. Preliminary data from two participant's shows that the mean relative percentage of Ankrd 2 in the wash is ~17% lower 3 h post RE (Table). This indicates movement of Ankrd 2 from the cytosol to another subcellular compartment such as the nucleus or cytoskeleton has occurred. In comparison, DARP was not detected in the cytosol, and there was no change its distribution following RE. We cannot rule out the possibility that DARP may be moving between nuclear and cytoskeletal components following RE.

Table: Mean percentage (SD) of Ankrd 2 and DARP diffusing out of skinned human muscle fibres in 10 min from fibres collected before and 3 h after a RE session. Data obtained are expressed as the amount of Ankrd 2/DARP present in the wash as a percentage of the total present in the wash/fibre pair. N=2 subjects, n=4 fibres per time (pre/3 h post RE). Type I fibres (n=4) were positive for MHCI and negative for MHCIIa detection while Type II fibres (n=4) showed the opposite detection pattern.

Protein	Pre	3h
Ankrd 2 % present in wash	68% (4%)	51% (11%)*
DARP % present in wash	0%	0%

*significantly different from pre (P = 0.028, unpaired t-test)

These findings provide novel knowledge of the *in vivo* regulation of MARP protein in skeletal muscle, specifically the translocation of Ankrd 2 from the cytosol to the nucleus or cytoskeletal components in response to RE.

Bean C, Facchinello N, Faulkner G & Lanfranchi G. (2008). The effects of Ankrd2 alteration indicate its involvement in cell cycle regulation during muscle differentiation. *Biochim Biophys Acta* **1783,** 1023-1035.

Cenni V, Bavelloni A, Beretti F, Tagliavini F, Manzoli L, Lattanzi G, Maraldi NM, Cocco L & Marmiroli S. (2011). Ankrd2/ARPP is a novel Akt2 specific substrate and regulates myogenic differentiation upon cellular exposure to H_2O_2. *Mol Biol Cell* **22,** 2946-2956.

Wette SG, Smith HK, Lamb GD, Murphy RM. (2013). Effects of high intensity power resistance exercise and feeding on mechanosensing and stress-related gene expression in human skeletal muscle. *Proc Aust Physiol Soc* **43**, 17P.