Fatty Infiltration Of Rotator Cuff Muscles After Tenotomy Is Associated With Desensitation Of Androgen Receptor Signalling

Martin Flück, PhD¹, Severin Ruoss, MSc¹, Céline Ferrié, Msc¹, Christoph Möhl, Bsc¹, Hans Hoppeler, MD², Mario Benn³, Brigitte von Rechenberg, MD vet ECVS³, Mazda T. Farshad, MD¹, Karl Wieser, MD¹, Dominik C. Meyer, MD⁴, Christian Gerber¹.

¹Department of Orthopaedics, Balgrist University Hospital, University of Zurich, Zurich, Switzerland, ²University of Berne, Berne, Switzerland, ³Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, ⁴Department of Orthopedics, Balgrist University Hospital, University of Zurich, Zurich, Switzerland.


Introduction: Muscle retraction after tenotomy is associated with the transition of skeletal muscle tissue into a catabolic state reflected by fatty atrophy. Application of steroids at the time of tendon release can prevent fatty atrophy. Conversely, application of anabolic steroids is not associated with any increase of muscle tissue once fatty atrophy is established (Gerber et al 2011). We hypothesised that resistance to anabolic steroids after muscle retraction secondary to tendon release is associated with a decreased expression of androgen responsive genes, and altered expression of gene transcripts which promote muscle protein synthesis and fat cell differentiation.

Methods: Effects of muscle retraction, lengthening and re-attachment were studied in an experimental model for release of infraspinatus muscle (ISP) in female Swiss Alpine sheep (Gerber et al 2012). Two groups of animals were studied: Group 1 was subjected to 16 weeks of release, 6 weeks of progressive re-lengthening, and 12 further weeks of attachment (Gerber et al 2012). Group 2 was subjected to 16 weeks of release and 6 weeks of attachment (n=12; Gerber et al 2014). In six animals of this group 150mg of nandrolone decanoate per week were injected in glutaeus maximus muscle immediately after tendon release or reattachment. Macroscopic changes in ISP were recorded at each surgical intervention with magnetic resonance spectroscopy and computer tomography and subjected to volumetric analysis (OsiriX © Pixmao Sarl). Biopsies were collected from discrete, non-overlapping muscle locations and subjected to the measure of muscle fibre composition by quantitative microscopy, androgen receptor (AR) protein levels by immunoblotting and ribonucleic acids with next generation sequencing.

Results: Sixteen weeks of tenotomy increased fat content from a mean of 10% to 50% and reduced muscle volume by a mean of 23.8%. AR protein concentration per actin protein in ISP was reduced by 27% with tenotomy and returned to normal levels after 6 weeks of progressive re-lengthening, but this was not maintained after 12 further weeks of attachment (Fig. 1).
Figure 1: Representative example of androgen receptor protein levels in immunoblots (top) and contractile protein as detected on the Ponceau S stained blot (bottom) in ISP samples of one animal at different time points. 20 mg total protein was loaded per lane in the following order: 1) Before tenotomy, 2) after 16 weeks of tenotomy, 3) after 6 weeks of re-lengthening, 4) after 12 weeks of re-attachment.
13821 gene transcripts were detected and 1397 of these were affected by tenotomy (p<0.01, Statistical analysis for microarrays). 945 of the altered transcripts, many of which concerned gene ontologies related to muscle contraction, metabolism and extracellular structure, were down-regulated. 452 transcripts were up-regulated and this set was enriched for genes regulating lipid transport and localization and mitochondrial membrane (Fig. 2). A number of the altered transcripts concerned genes which expression had been shown before to be subject to genomic regulation by the anabolic receptor (Wyce et al 2010). Nandrolone injections blunted the increase in interstitial fat content when given at
the onset of tendon release, but did not affect transcripts in infraspinatus muscle, except one, i.e. 3 beta-hydroxy steroid dehydrogenase, involved in steroid metabolism.

**Discussion:** The findings support the view that the desensitisation of rotator cuff muscle to anabolic steroids is driven by loss of functional attachment and loading through a down-regulation of androgen receptor levels per contractile protein. Intriguingly the action of anabolic steroids on the suppression of lipogenic processes does not involve altered transcript expression in the affected skeletal muscle.

**Significance:** Prevention of fatty atrophy by nandrolone decanoate does involve pathways outside the muscle tissue being primarily affected by tenotomy. Future studies may aim to preserve anabolic signalling in retracted muscle by stimuli that reflect functional attachment.

*ORS 2015 Annual Meeting*

*Poster No: 0528*