# Regeneration of the rat tibialis anterior muscle is impaired despite induction of the SPARC-$\beta$-catenin pathway during post-immobilization recovery

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## Background & Aims

The immobilization-induced tibialis anterior (TA) muscle atrophy worsens after cast removal concomitantly with changes in the extracellular matrix composition[1,2].

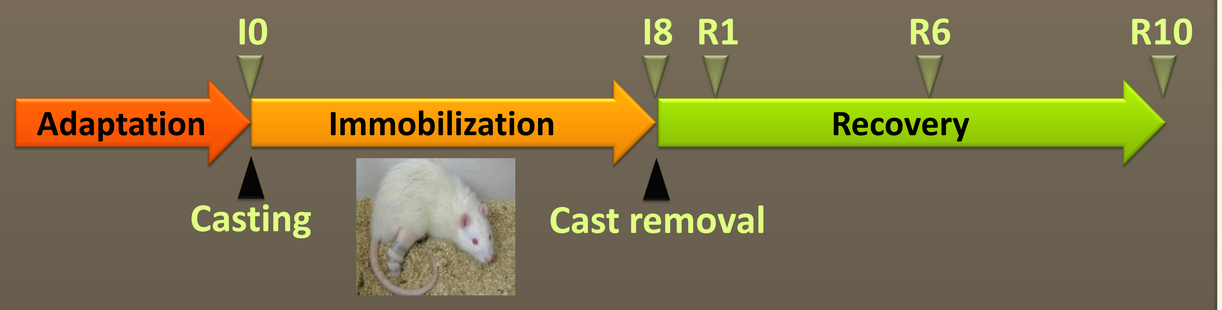
SPARC is a matricellular glycoprotein involved in tissue response to injury and in stabilization of β-catenin, which induces muscle regulatory factors (MRFs) controlling muscle regeneration.

We hypothesized that SPARC expression changed upon immobilization and could be involved in the worsening of TA muscle atrophy by altering muscle regeneration processes pending cast removal.

[1]Vazeille et al. Am J Physiol Endocrinol Metab 2008, [2]Slimani et al. Am J Physiol Endocrinol Metab 2012

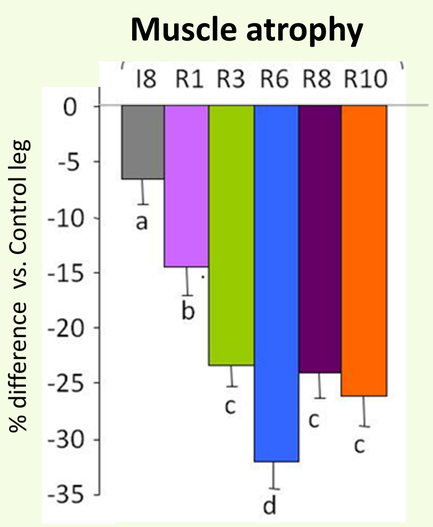
## Methods

Wistar rats were subjected to unilateral hindlimb immobilization for 8 days (I8) or not (I0), and allowed to recover for 1 to 10 days (R1-10).

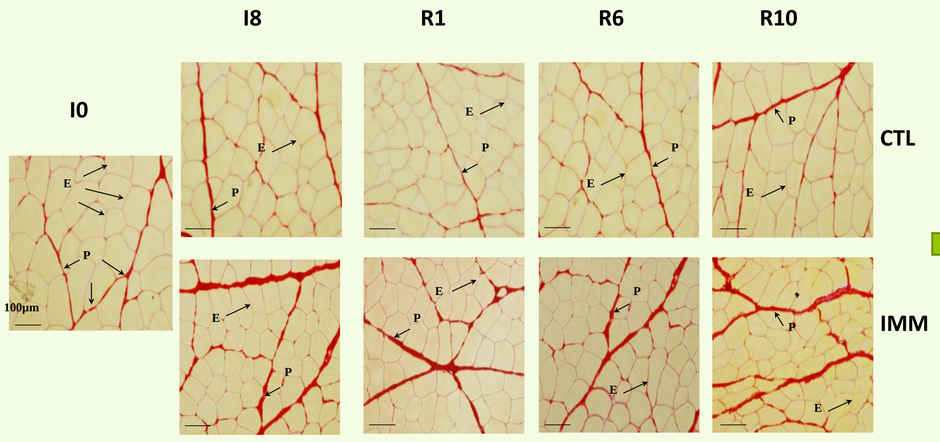



Expression of SPARC, β-catenin, and proliferative (i.e. MyoD and MyF5) or differentiation (i.e. myogenin) MRFs were assessed by Western blots and/or RT-qPCR in previously immobilized TA during recovery.

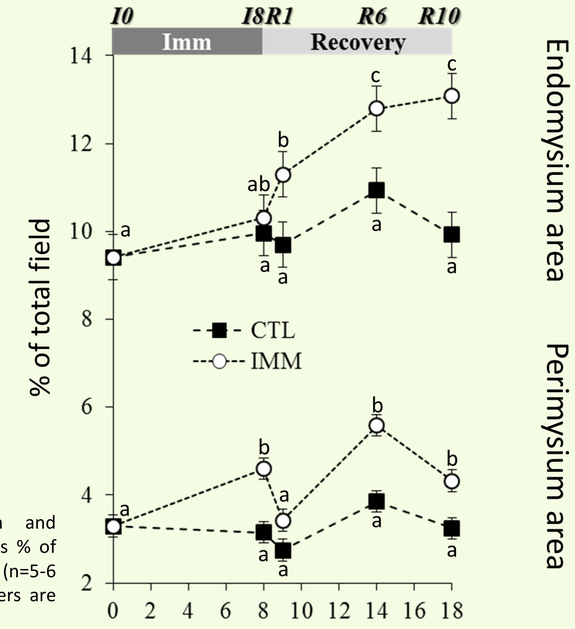
## I/ Tibialis anterior muscle atrophy worsened rapidly after cast removal concomitantly with endomysium thickening




Tibialis anterior muscle atrophy is expressed as % difference vs. controlateral control leg are means ± SEM (n=10-11 rats/group). Bars with different letters are significantly different.

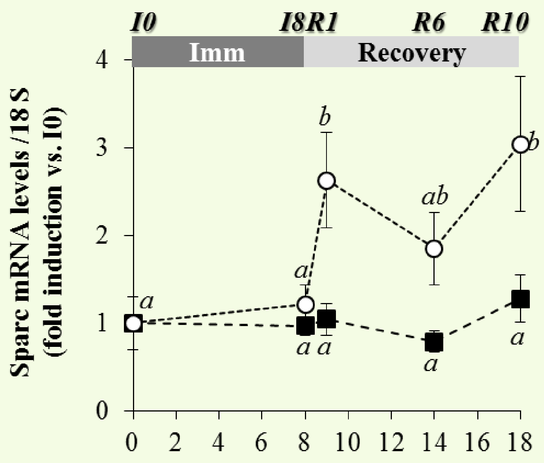



Ten-micrometer-thick cross-sections from control (CTL) and immobilized (IMM) tibialis anterior muscles were stained with Picro-Sirius red, which reveals intramuscular connective tissue Observations and image acquisitions were performed using a photonic microscope in bright-field mode (Olympus BX-51, Tokyo, Japan) coupled to a high-resolution cooled digital camera (Olympus DP72) and Cell-D software (Olympus Soft Imaging Solutions, Münster, Germany). E, endomyisum; P, perimysium.

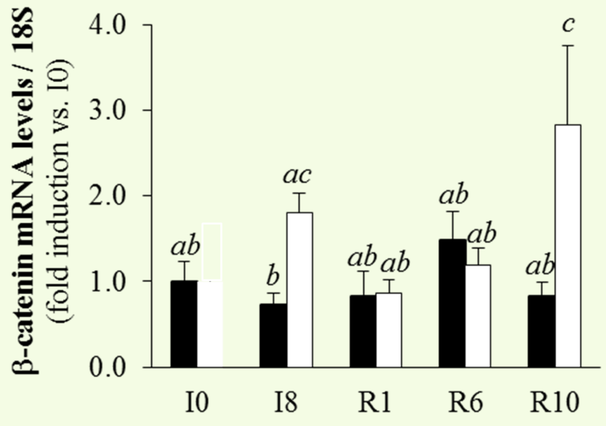


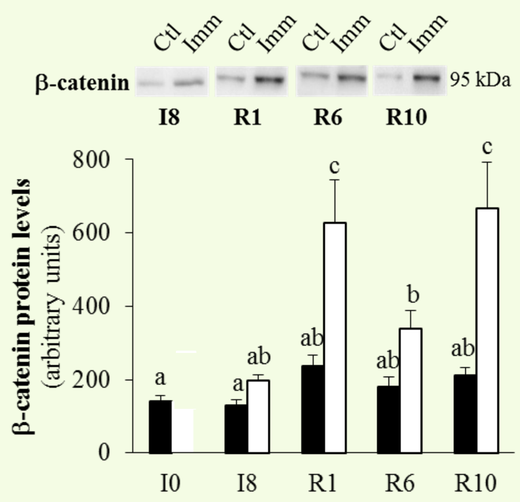

After quantification, endomysium and perimysium areas were expressed as % of total field and are means ± SEM (n=5-6 rats/group). Dots with different letters are significantly different.

## II/ Sparc mRNA levels also increased rapidly after cast removal




## III/ β-catenin mRNA and protein levels increased during immobilization and/or recovery in a time dependent manner

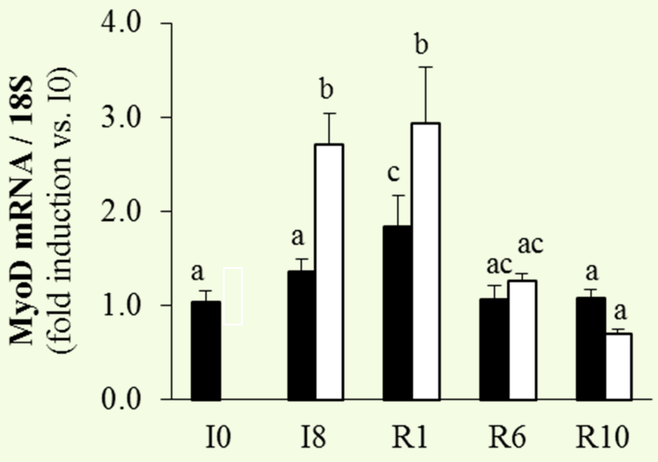


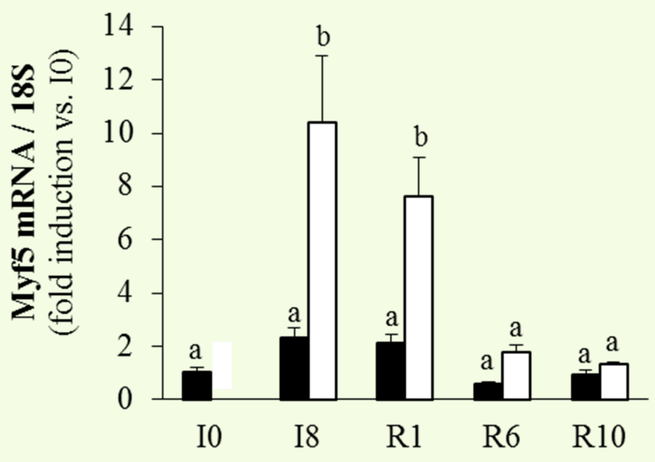



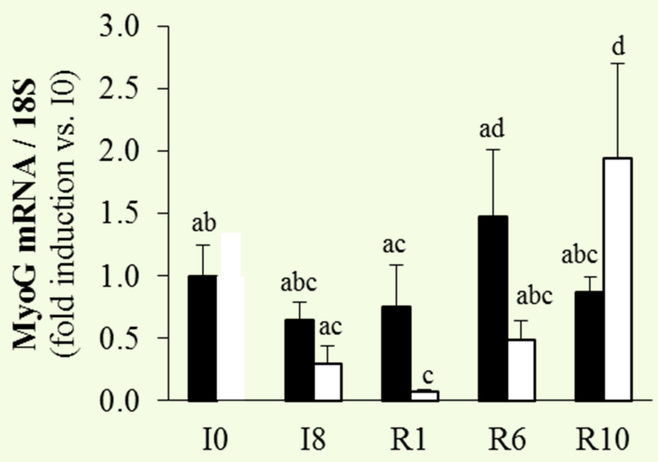

β-catenin protein levels from control (Ctl) or immobilized (Imm) tibialis anterior muscles were detected using a polyclonal antibody (Sigma-Aldrich). Signals were quantified after acquisition using a chemiluminescence imaging System (Syngene G:BOX XT4).
Data are arbitrary units, wre normalized against ponceau red staining for uneven unloading, and are means ± SEM (n=10-11 rats/group). Bars with different letters are significantly different.

mRNA levels in control and immobilized tibialis anterior muscles were assessed by RT-qPCR, expressed as fold induction vs. I0 group and are means ± SEM (n=10-11 rats/group). Bars with different letters are significantly different.

## IV/ MyoD and Myf5 mRNA levels increased concomitantly to increased b-catenin expression at I8 and R1, whilst myogenin mRNA levels first decreased at I8 and R1, and increased thereafter concomitantly to increased b-catenin expression at R10.




## Conclusions

We report an induction of the SPARC-β-catenin pathway associated with increased mRNAs of the proliferative MRFs (Myf5 and MyoD) in the recovering TA early after cast removal. The differentiation MRF myogenin was first largely repressed, but increased later on, when TA started to recover. Altogether, the data suggest that the TA tended to preserve muscle regeneration potential through induction of proliferative MRFs. However this process was poorly efficient presumably because of an alteration myogenic differentiation.