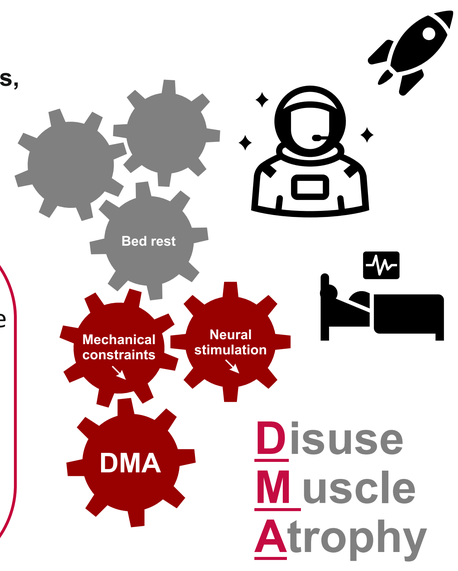
Adiponectin pathway in a murine model of disuse muscle atrophy: an *in vivo* study

S.Szczepanski[1], M.Limpens[1], AE.Declèves[2], A.Legrand[1], A.Tassin[1]

1. Lab. Respiratory Physiology, Pathophysiology and Rehabilitation, Research Institute for Health Sciences and Technology, University of Mons,
2. Lab.Metabolic and Molecular Biochemistry, Research Institute for Health Sciences and Technology, University of Mons



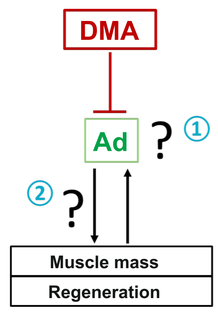
Introduction

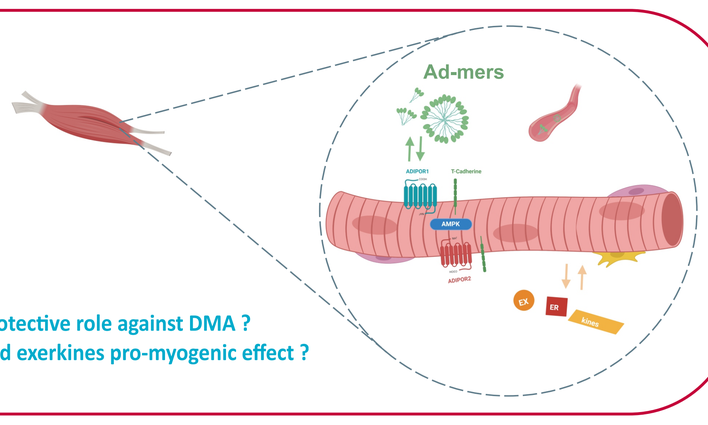
Skeletal muscle deconditioning is an important issue for patients managed in Intensive-Care Unit (ICU) where prolonged bedrest results in the development of a Disuse-mediated Muscle Atrophy (**DMA**) associated to an impaired regeneration potential compromising muscle recovery (Matsuba Y et al., Acta Physiologica, 2009). Despite exercise training (ET) is the only effective treatment against DMA, ET intolerance limits rehabilitation. As well, mechanisms implicated in its beneficial effects must be clarified.

Skeletal muscle is an endocrine organ that secretes myokines and **exerkines** are those that results from ET. Among those myokines, adiponectin (**Ad**) is an adipo/myokine with anti-inflammatory, antioxidant, and pro-myogenic properties. While increasing evidence highlights its positive role in skeletal muscle, Ad pathway was found altered in a DMA murine model (Goto et al., PloS ONE, 2013).

We hypothesize that muscle deconditioning is associated to Ad pathway alterations which could reinforce (i) the loss of muscle mass and (ii) the impairment of regeneration potential in a vicious circle. A murine model of Hindlimb Unloading and Immobilisation (**HLUI**) was therefore developed in our laboratory to investigate Ad pathway in a context of DMA.

Aims



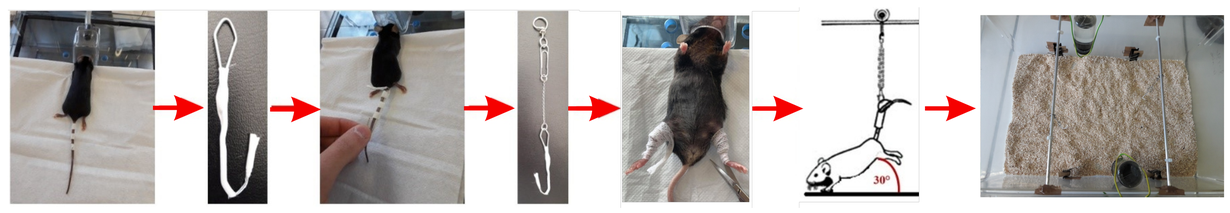


① Ad protective role against DMA ?
② Ad and exerkines pro-myogenic effect ?

Methods

① Ad protective role against DMA ?

In vivo DMA murine model



Atrophy evaluation in the DMA model

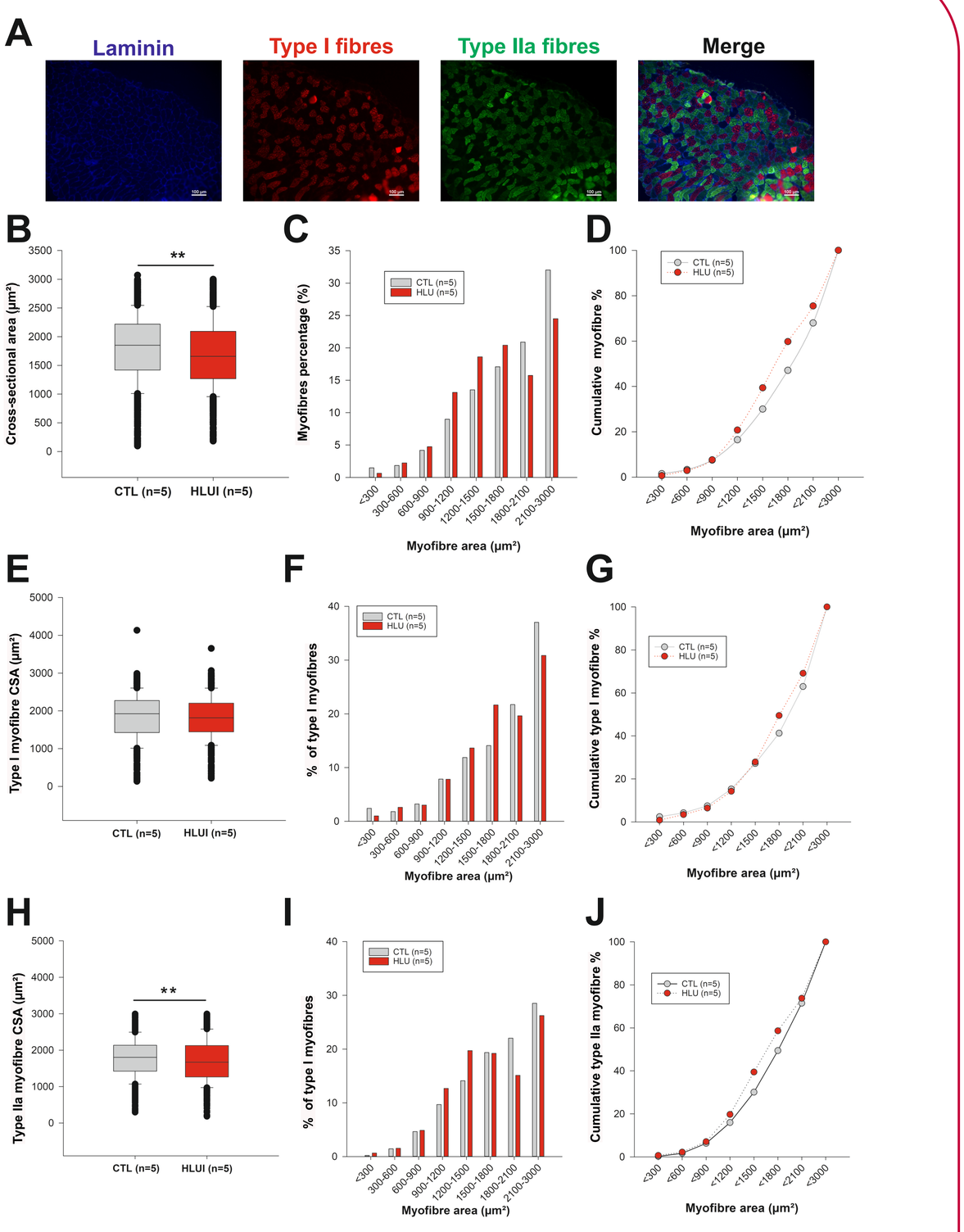


Figure 1. **Effect of 14 days of Hindlimb Unloading coupled with Immobilization (HLUI) on mouse *Soleus* muscle: Cross-sectional Area (CSA) and type I and IIa fibre size distribution.** Each myofibre CSA was measured on *Soleus* muscle cryosections following type I, IIa and IIb immunofluorescence detection and morphometrical analyses with the *Image J* software. **(A)** Representative field. ***(left)*** CSA was determined in all fibres **(B),** in type I **(E)** and in type IIa **(H)** fibres. Data represented as boxplot. **p<0.001, Mann-Whitney Rank Sum Test. ***(center)*** Myofibres were classified in clusters according to their area (µm²). All fibres **(C)**, type I fibres **(F)** and type IIa fibres **(I)**. Chi-square: p<0.001 in C, F and I. ***(right)*** Cumulative percentages of myofibres in clusters in all fibres **(D)**, in type I fibres **(G)** and in type IIa fibres **(J)**.

Plasmatic components of Ad pathway in the DMA model

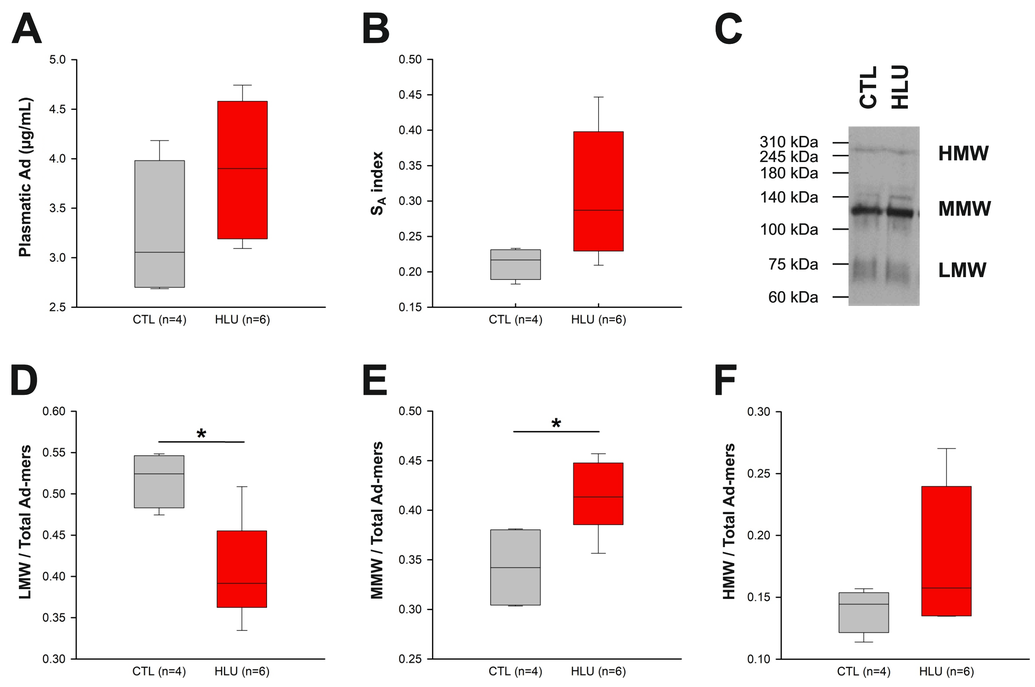


Figure 2. Effect of 14 days Hindlimb Unloading coupled with Immobilization (HLUI) on Adiponectin (Ad) pathway in mice. (**A**) Plasmatic Ad was measured in plasma by using the Quantikine® ELISA kit – Mouse Adiponectin/Acrp30 Immunoassay. One way ANOVA: NS. (**B-F**) Ad-mers distribution determined by Western blot analysis. (**B**) S_AIndex corresponds to HMW on total Ad-mers (Ad circulating forms) ratio (HMW/(LMW+MMW+HMW). One Way ANOVA: NS. (**C**) Representative blot. (**D-F**) Western blot densitometric analyses were performed with the *Image J* software and each circulating form signal was normalised on total Ad-mers signal. *: p<0.05, t-test. Data represented as boxplot.

Muscle components of Ad pathway in the DMA model

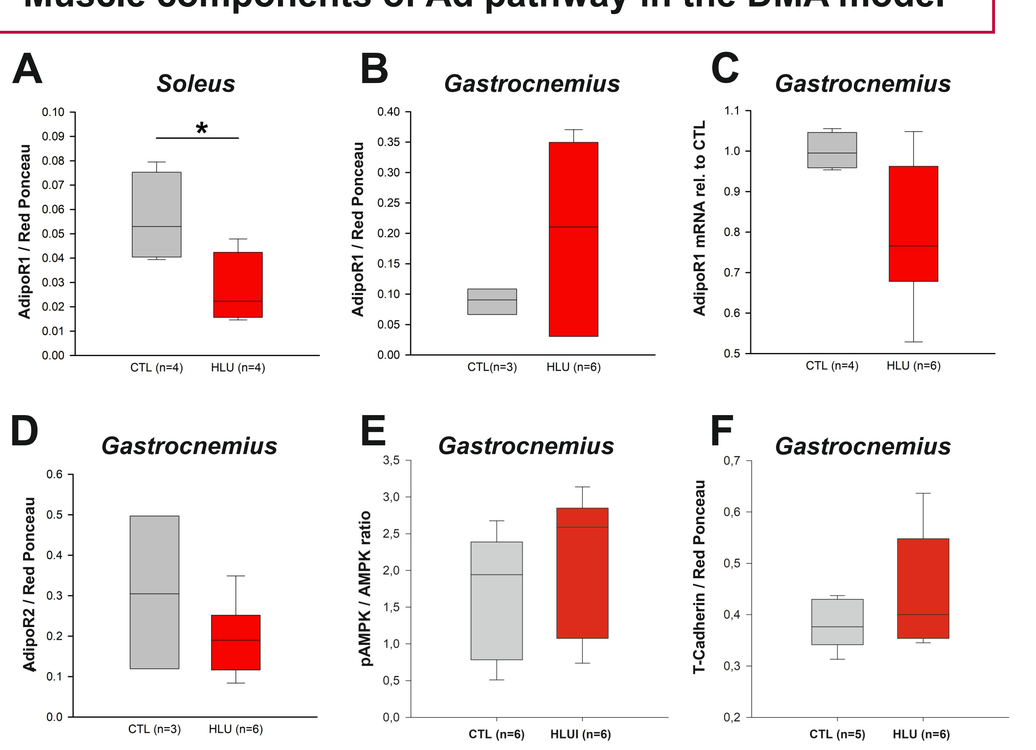


Figure 3. Effect of 14 days Hindlimb Unloading coupled with Immobilization (HLUI) on Adiponectin (Ad) pathway in mice *Soleus* and *Gastrocnemius* muscles. (A-B) AdipoR1 protein level in **(A)** *Soleus* and **(B)** *Gastrocnemius* muscles was determined by Western blot (WB). Data represented as boxplot; *: p<0.05; t-test. **(C)** AdipoR1 mRNA level was assessed in the *Gastrocnemius* muscle by RTqPCR with ΔΔCt method (housekeeping gene: RPLP0; data normalised to CTL). Data represented as boxplot; t-test: NS. **(D)** AdipoR2 protein level was determined in the *Gastrocnemius* muscle by Western blot. Data represented as boxplot; t-test: NS. **(E)** AMPK activity was determined by WB in the *Gastrocnemius* muscle from CTL and HLUI mice. Total AMPK (AMPKtot) and its phosphorylated form (pAMPK) were immunodetected with antibodies directed against the AMPK α subunit and AMPK-Thr172 phosphorylation, respectively. Signals were normalised on Red Ponceau. AMPK activity was defined as the ratio pAMPK/AMPKtot. Data represented as boxplot; t-test: NS.**(F)** T-cadherin Ad coreceptor protein level was determined by (WB) in the *Gastrocnemius* muscle from CTL and HLUI mice. Signals corresponding to T-cadherin (at 100 and 130kDa) were quantified by densitometric analyses with the Image J Software and normalised on Red Ponceau. Data represented as boxplot; t-test: NS.

Conclusions

In conclusion, we optimized **a model allowing to mimic DMA in mouse hindlimb muscles** (**HLUI**). Indeed, the decrease in muscle CSA and modifications in fibre size distribution indicate an atrophic phenotype in the slow-twitch *Soleus* and the fast-twitch *Gastrocnemius* muscles. Specific measurement of type IIa fibre area in the *Soleus* muscle revealed a reduction in type IIa fibre CSA associated to a modified myofibre size distribution towards fibres with medium area. Type I myofiber size distribution was also modified in favour of fibres with medium area. Fibre type analyses are ongoing in the *Gastrocnemius* muscle.

Regarding **plasmatic components of Adiponectin (Ad) pathway** in our murine model of DMA, proportion of Low Molecular Weight (MW) circulating forms is decreased in favour of Medium MW multimers although total Ad plasmatic level is unchanged.

Ad pathway was also found altered in disused muscles. Ad receptors and T-Cadherin co-receptor were unchanged at the mRNA and protein level in the *Gastrocnemius* muscle, as well as AMPK activity. However, the *Soleus* muscle showed a reduction in AdipoR1 protein level suggesting that disuse-mediated AdipoR1 downregulation might be fibre type-dependant. Further experiments are planned to assess AdipoR1 downstream pathway in this muscle.

Prospects

Ongoing studies aim to further determine whether Ad may constitute a good therapeutic target to improve muscle mass and regeneration potential in DMA through gain and loss of function experiments.

Acknowledgements :
Sébastien Szczepanski benefits from a FRIA grant (F.R.S - F.N.R.S.)
To B. Blairon, V. Jenart for technical help
To L. Paprzycki, L. Fievet and V. Blommaert (MSc students) who contributed to the study.

Fundings:

University of Mons **Contact : sebastien.szczepanski@umons.ac.be / alexandra.tassin@umons.ac.be**