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

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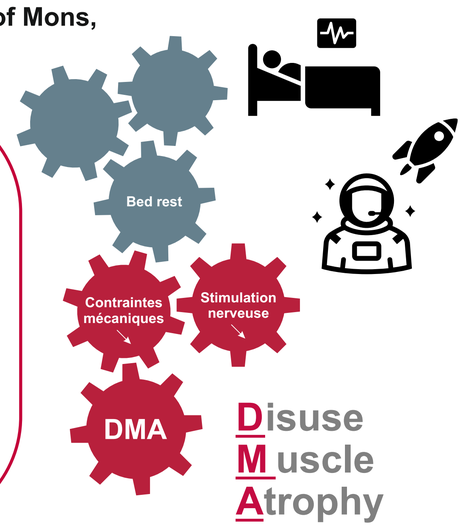
Introduction

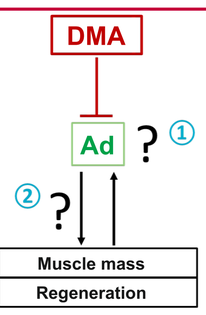
Skeletal muscle deconditioning is an important issue for patients managed in Intensive-Care Unit (ICU) where prolonged bedrest results in the development of a Disuse-mediated Muscle Atrophy (**DMA**) associated to an impaired regeneration potential compromising muscle recovery (Matsuba Y et al., Acta 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.



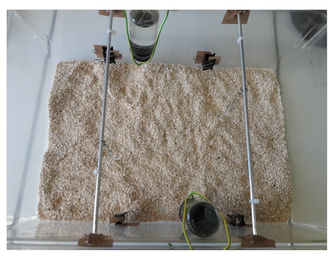
Aims

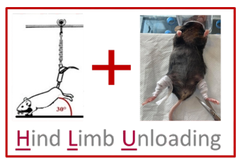


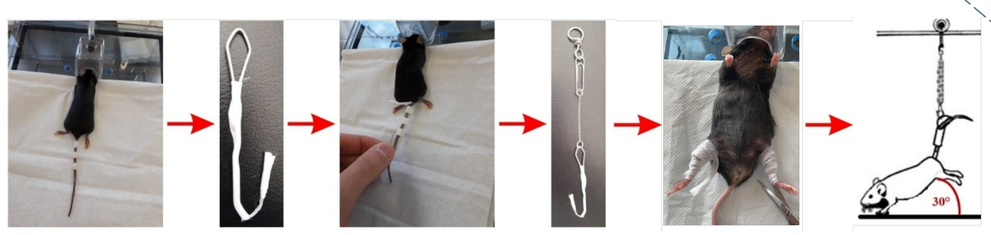
① Ad protective role against DMA ?
② Ad and exerkines pro-myogenic effect ?

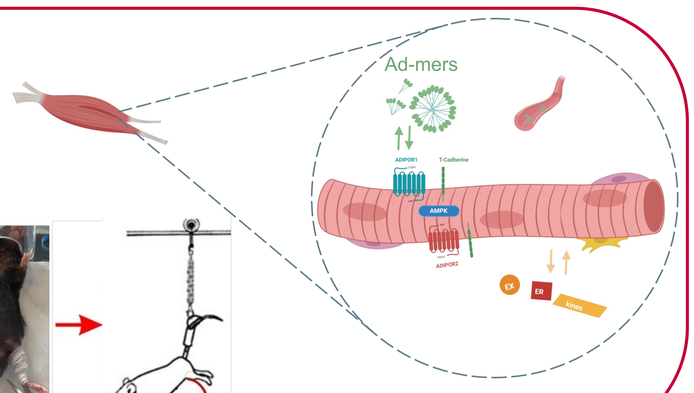
Methods

① Ad protective role against DMA ?
In vivo DMA murine model









Atrophy evaluation in the DMA model

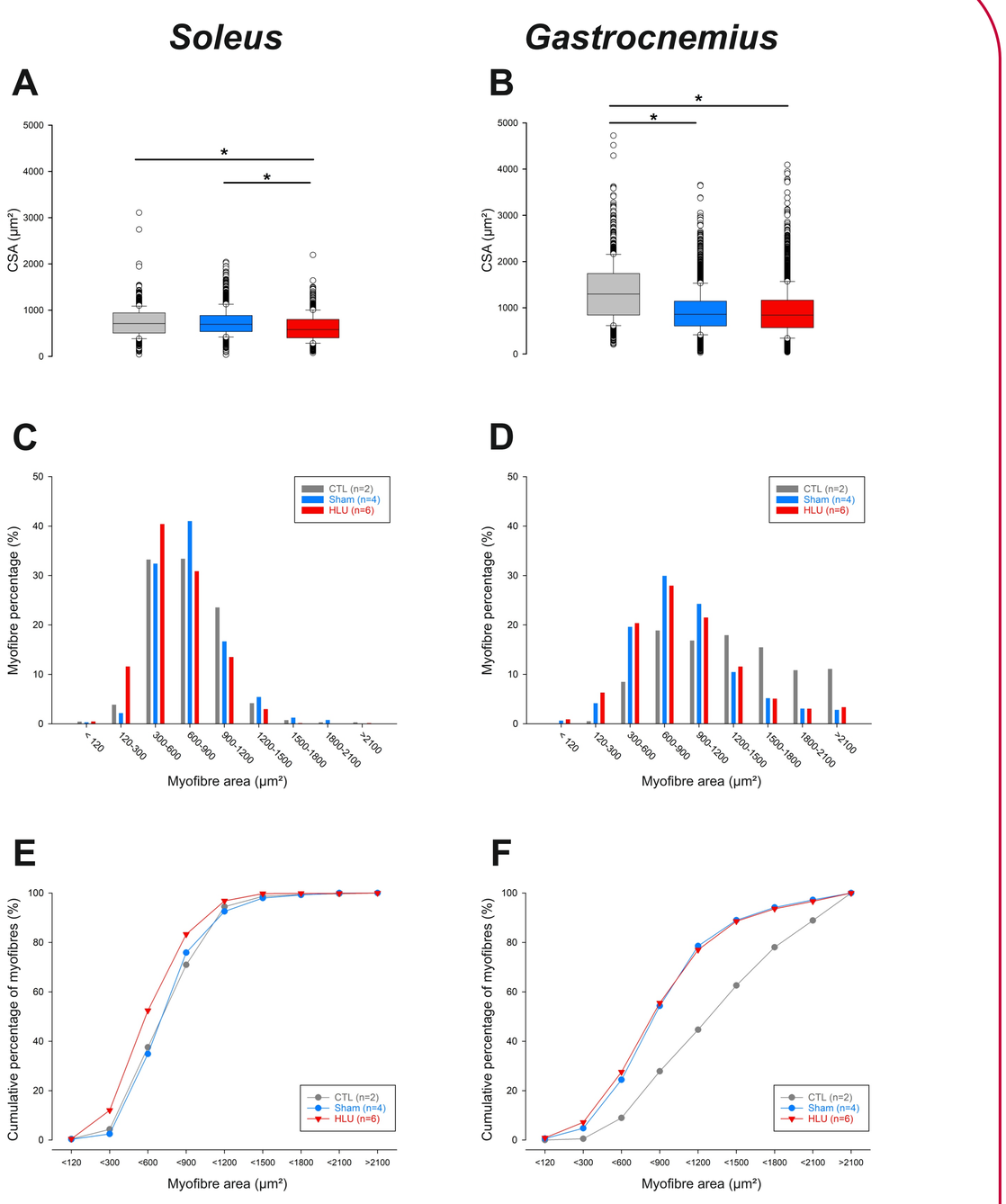


Figure 1. Effect of 14 days Hindlimb Unloading coupled with immobilization (HLUI) on mouse *Soleus* and *Gastrocnemius* muscles: Cross-Sectional Area (CSA) and fibre size distribution. Myofibre area were determined on muscle cross sections by using the *Image J* software. (**A-B**) The CSA was determined in *Soleus* (A) and *Gastrocnemius* (B) muscles. Data represented as boxplot. *: p<0,05, ANOVA on ranks (Dunn's Method) (**C-D**) Myofibres were classified in clusters according to their area. Chi-square: p<0,001 (**E-F**). Cumulative percentage of myofibres in clusters (**C**) and (**D**), respectively.

Ad pathway evaluation in the DMA model

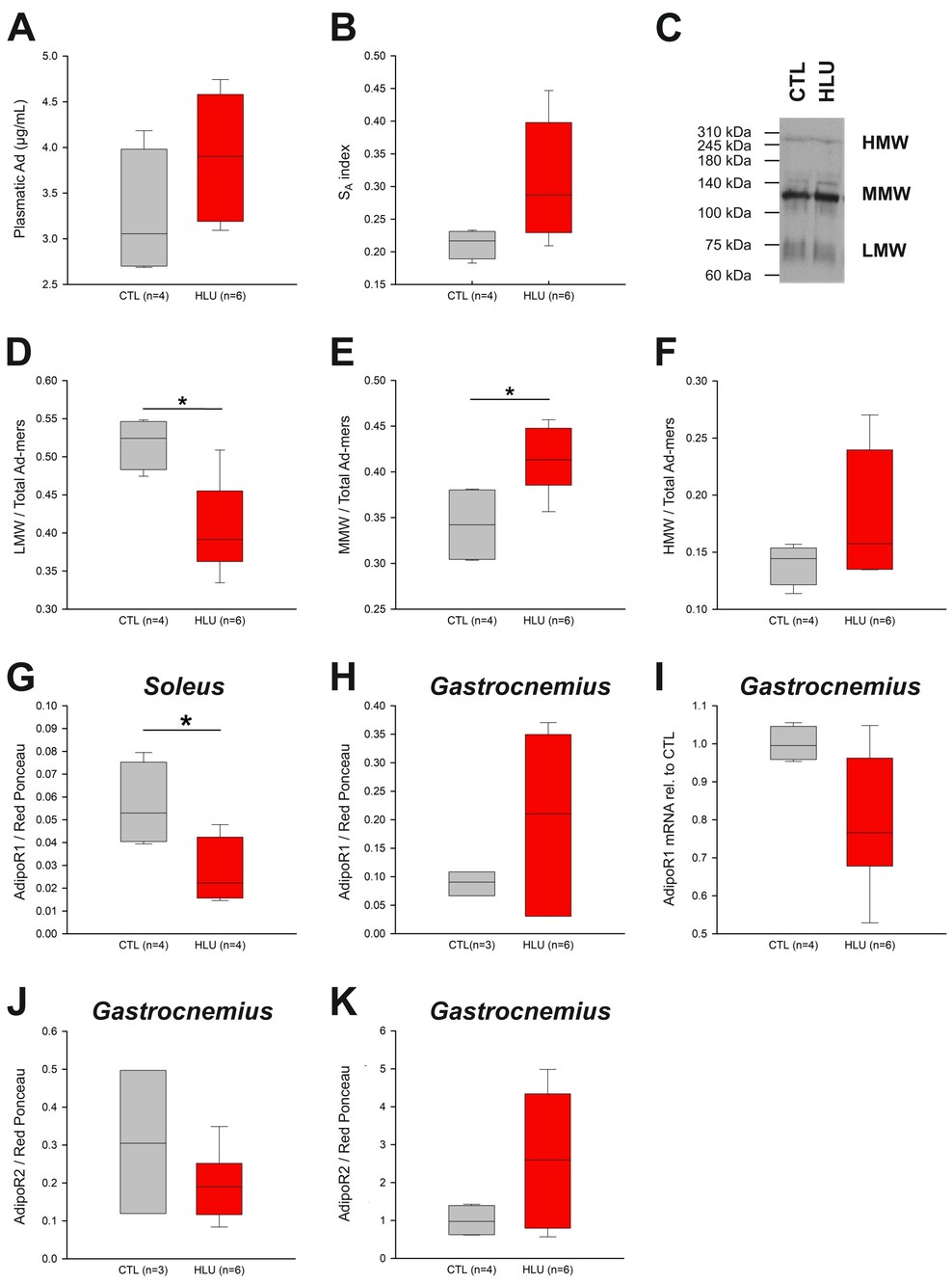


Figure 2. Effect of 14 days Hindlimb Unloading coupled with Immobilization (HLUI) on Adiponectin (Ad) pathway in mice. (**A**) Plasmatic Ad was measured in plasma by using the Quantikine® ELISA kit – Mouse Adiponectin/Acrp30 Immunoassay. One way ANOVA: NS. (**B-F**) Ad-mers distribution determined by 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. (**G-H**) AdipoR1 protein level in *Soleus* (**G**) and *Gastrocnemius* (**H**) muscles were determined by Western blot analyses. *: p<0.05; t-test. (**I**) AdipoR1 mRNA level was determined in the *Gastrocnemius* muscle by RTqPCR analyses with ΔΔCt method (RPLP0) and normalised to CTL. t-test: NS. (**J**) AdipoR2 protein level in the *Gastrocnemius* muscle was determined by Western blot analyses. t-test: NS.(**K**) AdipoR2 mRNA level was determined in the *Gastrocnemius* muscle by RTqPCR analyses with ΔΔCt method RPLP0) and normalised to CTL. t-test: NS.
Data represented as boxplot.

Conclusion

In conclusion, we optimized a device allowing to mimic DMA in mouse hindlimb muscles. Indeed, the decrease in muscle CSA and modifications in fibre size distribution indicate an atrophic phenotype in *Soleus* and *Gastrocnemius* muscles in HLUI mice.

Regarding Adiponectin pathway in our murine model of DMA, proportion of Low Molecular Weight (MW) circulating forms is decreased in favour of Medium MW multimers in HLUI mice although total Ad plasmatic level is unchanged. Importantly, AdipoR1(Ad receptor 1) protein level is reduced in the slow-twitch muscle *Soleus*. Our data also suggest that disuse-mediated AdipoR1 downregulation is fibre type-dependant.

Ongoing studies aim to further determine whether Ad may constitute a good therapeutic target to improve muscle mass and regeneration potential in DMA.

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