Effect of Episodic Hypoxaemia on skeletal muscle: which association with ApN pathway modifications?

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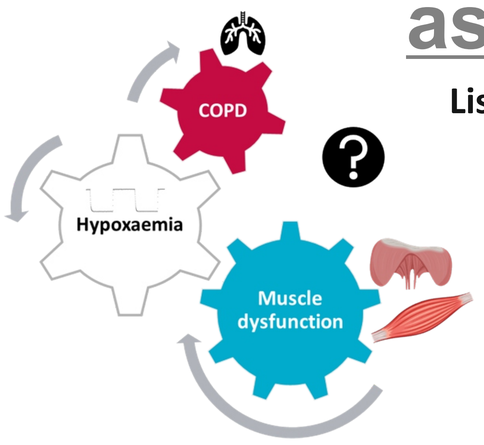
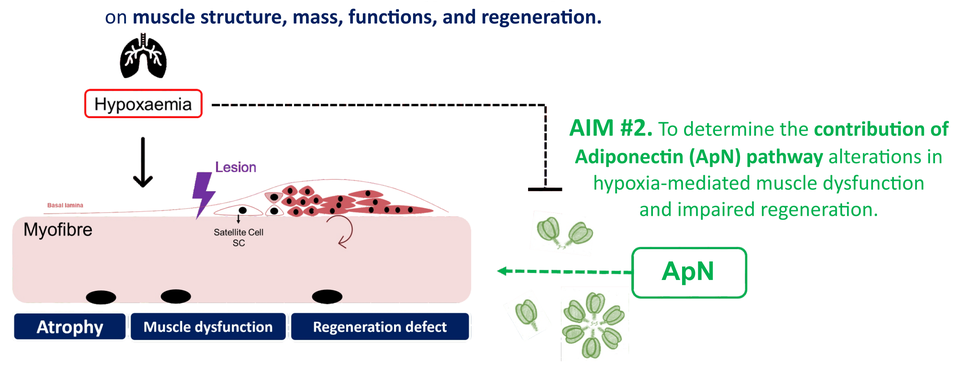


Introduction

Episodic hypoxaemia, a major pathological component of progressive respiratory insufficiencies, is associated with systemic comorbidities including skeletal muscle dysfunction. Underlying mechanisms need to be clarified but interestingly, an impaired adult myogenesis impacting muscle regeneration is suggested. Adiponectin (ApN) is an adipo/myokine favouring oxidative metabolism in skeletal muscle and exerting anti-inflammatory, anti-ageing, pro-myogenic and antioxidant effects. Given those properties and converging evidence of its alteration upon hypoxia, ApN pathway constitutes an attractive therapeutic target to counteract the effects of episodic hypoxaemia at the muscle level.

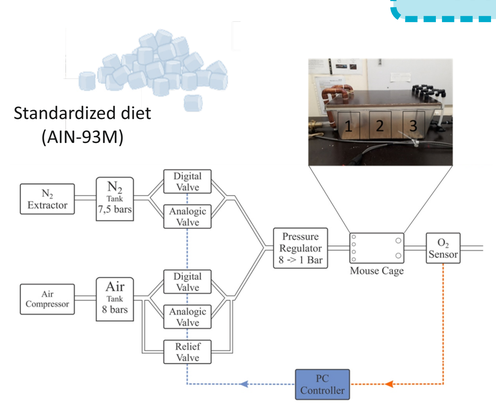
Aims

AIM #1. To determine specific effects of the **hypoxemic component** of COPD on **muscle structure, mass, functions, and regeneration.**

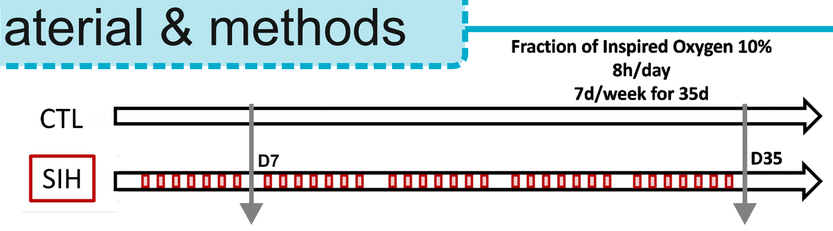


AIM #2. To determine the **contribution of Adiponectin (ApN) pathway** alterations in hypoxia-mediated muscle dysfunction and impaired regeneration.

Material & methods



Modified from Chodzyński et al., 2013.



To decipher the specific effect of episodic hypoxaemia on skeletal muscle, we used a reductionist mouse model of Sustained Intermittent Hypoxaemia (SIH; FiO_2: 10%, 8h/day) in a device optimized to avoid movement restriction and to ensure an even distribution of gaseous flow. Moreover, we limited antioxidant excess by using a specific diet. Muscle structural changes, expression of myogenic markers and ApN pathway were investigated in fast and slow-twitch muscle fibers at early and late timepoints.

Morphometric analysis in the SIH model

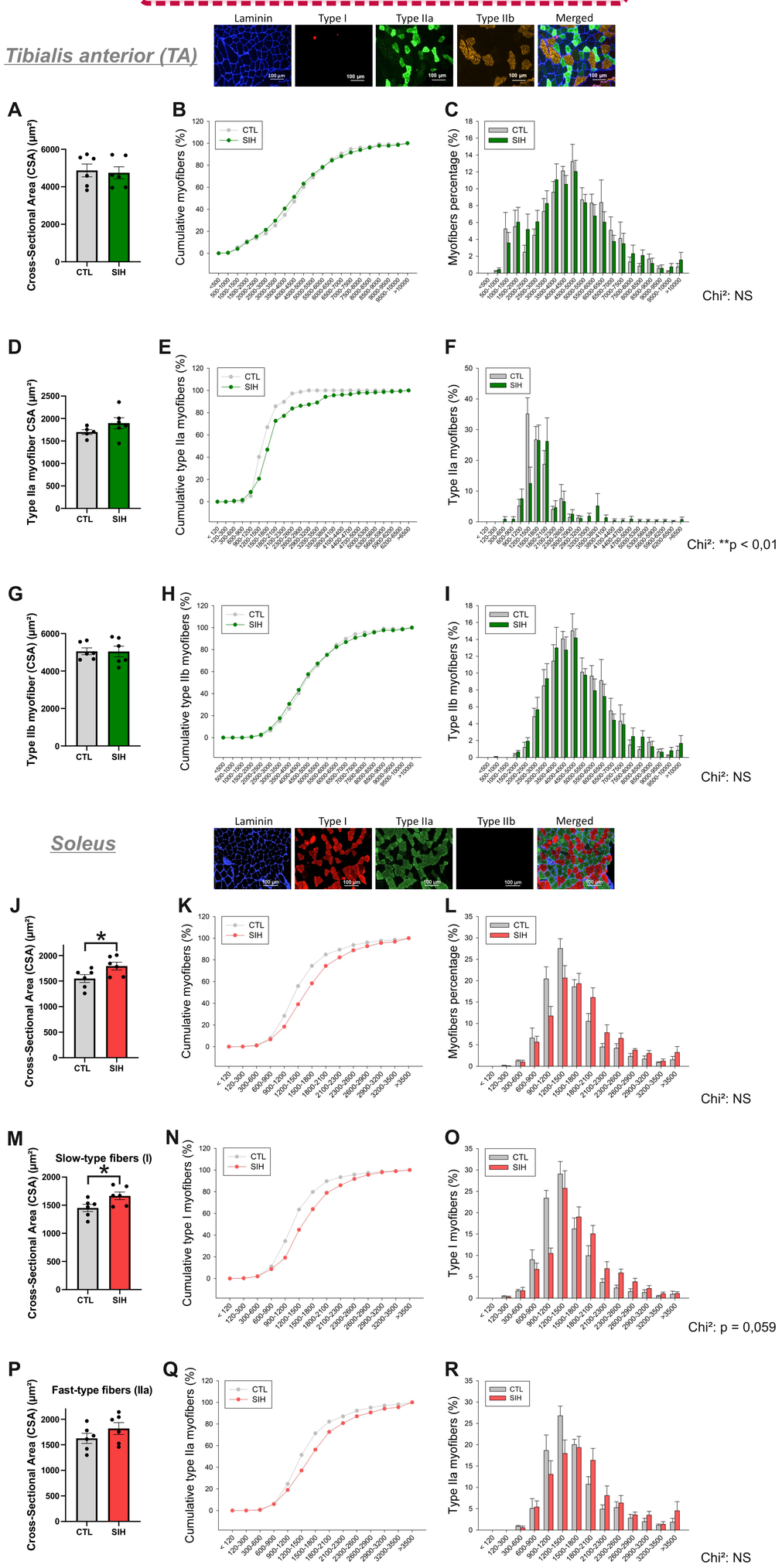


Figure 1. Effect of 35 days of Sustained Intermittent Hypoxaemia (SIH) on mouse *soleus* and *tibialis anterior* (TA) muscles: Cross-Sectional Area (CSA) and type I, IIa and IIb fiber size. TA **(A-I)** and *Soleus* **(J-R)** muscle cryosection of SIH and CTL mice were submitted to a co-immunofluorescence by using antibodies directed against MyHC7 (type I fibres), MyHC2 (type IIa fibres), MyHC4 (type IIb fibres) and laminin. **(A, D, G, J, M, P)** Each myofibre CSA was measured by using the Image J software. Data represented as mean ± SEM. **(C, F, I, L, O, R)** Myofibers were classified in clusters according to their area (µm²). **(B, E, H, K, N, Q)** Cumulative percentage. **A.** CSA of the whole TA muscle section; t-test: NS. **D.** TA type IIa myofiber CSA; t-test: NS. **G.** TA type IIb myofiber CSA, t-test: NS. **C.** TA type II fibre size distribution; Chi²: NS. **F.** TA type IIa fibre size distribution; **: p < 0,01, Chi². **I.** TA type IIb fiber size distribution; Chi²: NS. **J.** CSA on the whole *Soleus* muscle section; *: p < 0,05, t-test. **M.** *Soleus* type I myofibre CSA; *: p < 0,05, t-test. **P.** *Soleus* type IIa myofibre CSA, t-test: NS. **L.** *Soleus* type II fibre size distribution; Chi²: NS. **O.** *Soleus* type I fibre size distribution; Chi²: p = 0,059. **R.** *Soleus* type IIb fiber size distribution; Chi²: NS. n=6 mice per group.

Myogenic markers in the SIH model

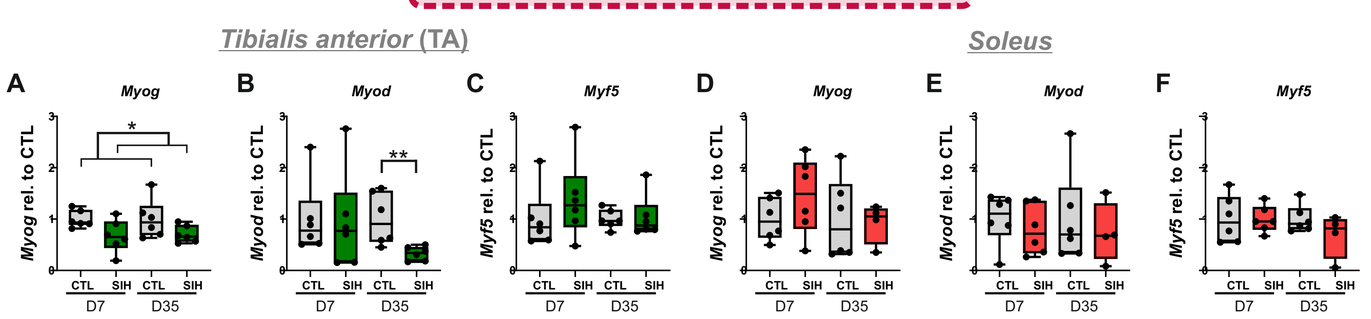


Figure 2. Analysis of myogenic marker expression in the SIH model at 7 and 35 days. RT-qPCR were performed on fast *tibialis anterior* (TA) **(A-C)** and slow *soleus* **(D-F)** muscles with the ΔΔCt method (housekeeping gene: *Rplp0*; data normalised to CTL). **A.** *Myog* (encoding Myogenin) expression in the TA muscle; *: p < 0,05, SIH vs CTL, Two Way ANOVA (n = 6). **B.** *Myod1* (encoding Myoblast determination protein 1) expression in the TA muscle; **: p < 0,01, SIH vs CTL, Mann-Whitney Rank Sum Test (n=6). **C.** *Myf5* (encoding Myogenic factor 5) expression in the TA muscle; Mann-Whitney Rank Sum Test, NS (n=6). **D.** *Myog* expression in the *soleus* muscle; Two Way ANOVA, NS (CTL: n=6; SIH: n=5). **E.** *Myod1* expression in the *soleus* muscle; Two Way ANOVA, NS (CTL:n=6; SIH: n=5). **F.** *Myf5* expression in the *soleus* muscle; Two Way ANOVA, NS (CTL:n=6; SIH: n=5).

ApN pathway in the SIH model

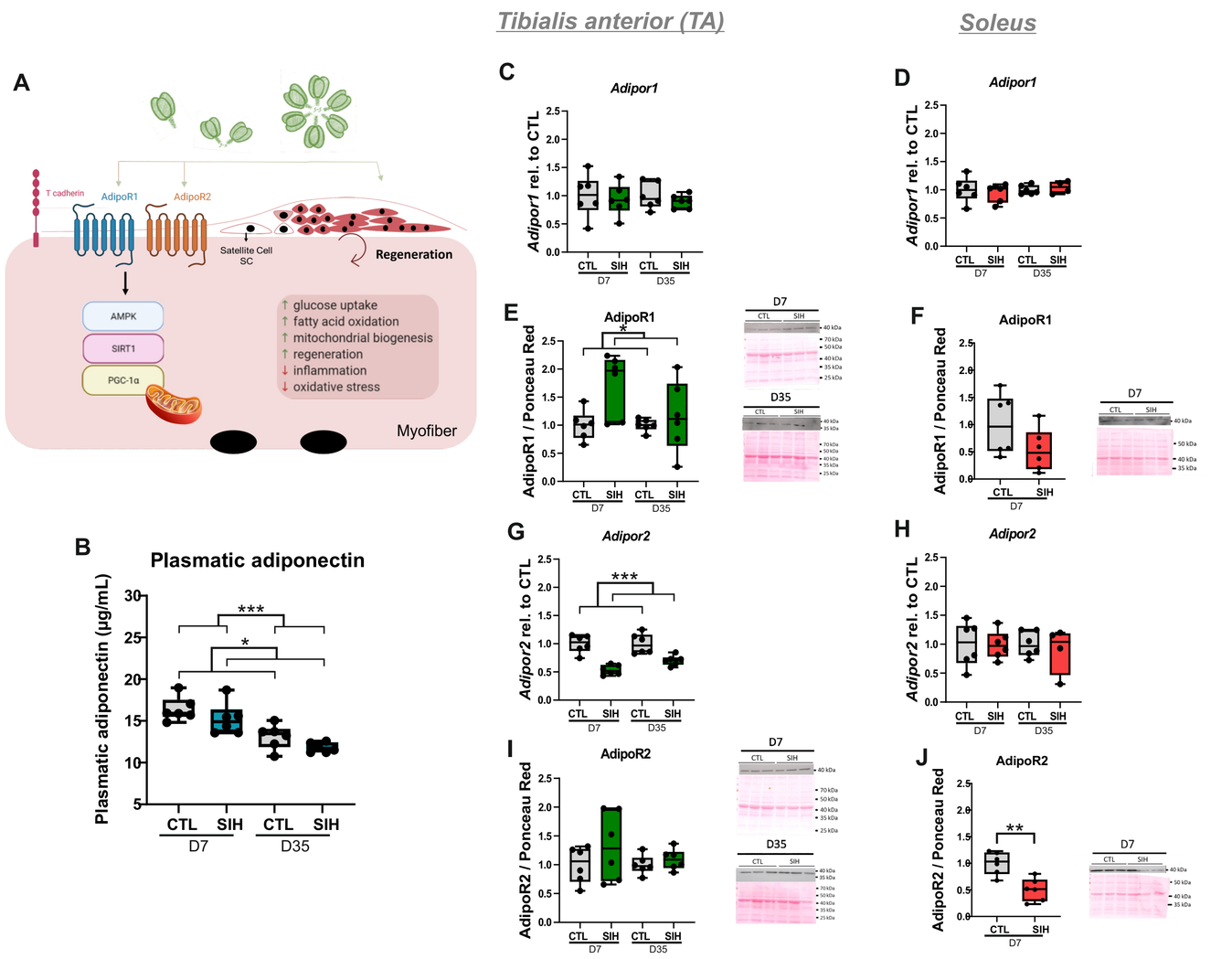
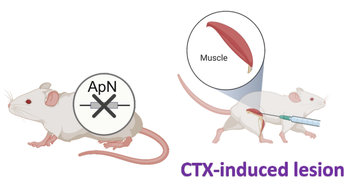


Figure 3. Effect of 7 and 35 days of SIH on Adiponectin (ApN) pathway at the muscle level in mice. A. Schematic representation of the ApN pathway. **B.** Plasmatic ApN was measured in plasma using the Quantikine ELISA kit - Mouse Adiponectin/Acrp30 Immunoassay; ***: p = <0,001, J7 vs J35, Two Way ANOVA; *: p < 0,05, SIH vs CTL, Two Way ANOVA (n=6). **C-D.** *AdipoR1* mRNA level was assessed in the TA **(C)** and the *soleus* **(D)** muscles by RT-qPCR with the ΔΔCt method (housekeeping gene: *Rplp0*; data normalised to CTL). **C.** Two Way ANOVA, NS (n=6). **D.** Two Way ANOVA; NS (CTL:n=6; SIH: n=5). **E-F.** AdipoR1 protein level in the TA **(E)** and the *soleus* **(F)** muscles was determined by a SDS-PAGE followed by a Western Blot (WB). Signals were quantified by densitometric analysis with the ImageJ software and normalised on Ponceau Red. **E.** *: p < 0,05, SIH vs CTL, Two Way ANOVA (n=6). **F.** t-test, NS (n=6).**G-H.** *AdipoR2* mRNA level was assessed in the TA **(G)** and the *soleus* **(H)** muscles by RT-qPCR with the ΔΔCt method (housekeeping gene: *Rplp0*; data normalised to CTL). **G.** ***; p < 0,001, SIH vs CTL, Two Way ANOVA (n=6). **H.** Two Way ANOVA; NS (CTL:n=6; SIH: n=5). **I-J.** AdipoR2 protein level in the TA (I) and the *soleus* **(J)** muscles was determined as in E-F. **I.** Mann-Whitney Rank Sum test; NS (n=6). **J.** t-test, **: p < 0,01 (n=6).

Conclusions & prospects

Episodic hypoxaemia in mice induces early alterations in the expression of myogenic markers as well as a muscle hypertrophy taking place over time, mainly in slow-type myofibers. Muscles from the SIH murine model also exhibit perturbations of AdipoR2 at the mRNA and/or protein levels at early and late time points. Further studies are ongoing to assess the contribution of ApN to hypoxaemia-mediated muscle dysfunction and regeneration defect.



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