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

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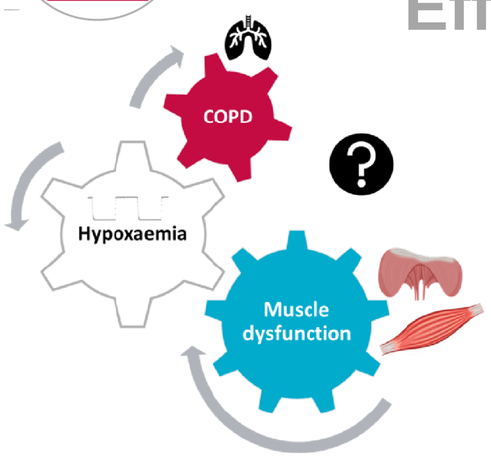
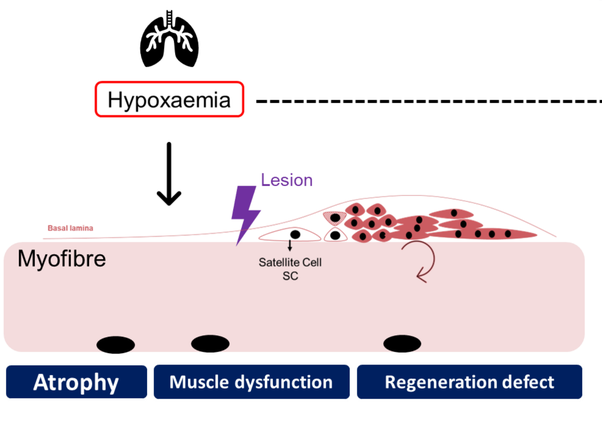


Introduction

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 favoring 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.

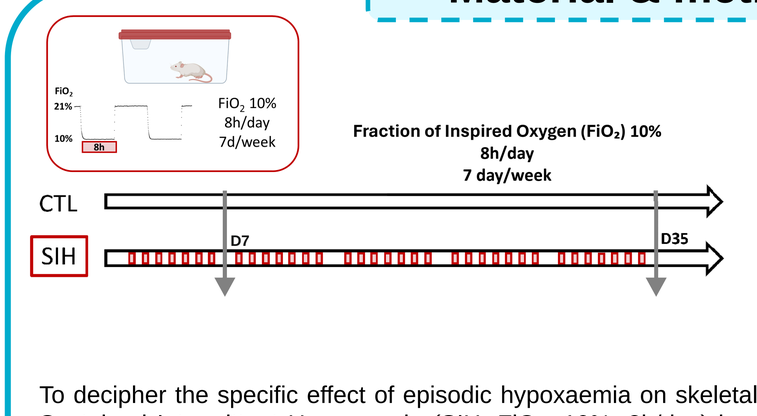
Aims

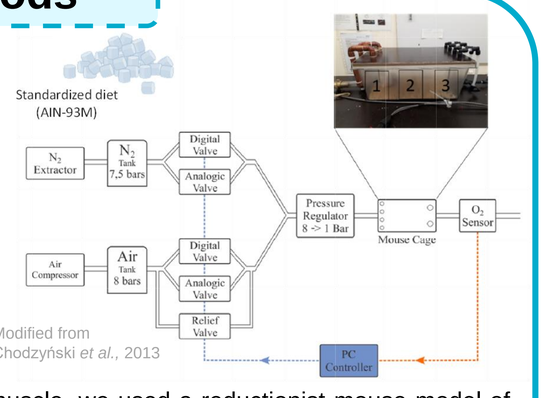
AIM #1. To determine specific effects of the hypoxemic component of COPD on muscle structure, mass, functions, and regeneration.



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

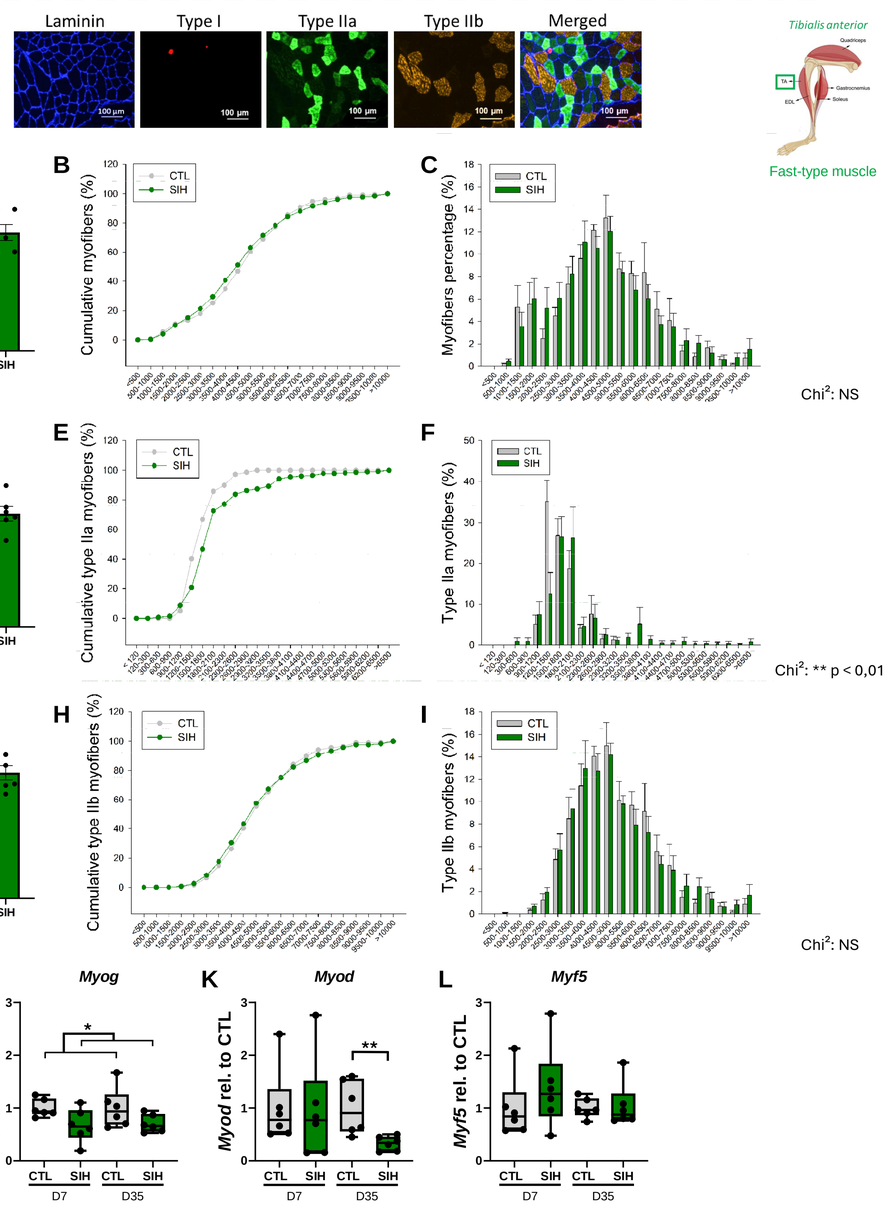
Material & methods





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 a homogeneous 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 (D7) and late (D35) timepoints.

Effect of SIH on myofibre size and myogenic factors in the fast TA muscle



Effet of SIH on myofibre size and myogenic factors in the slow *soleus* muscle

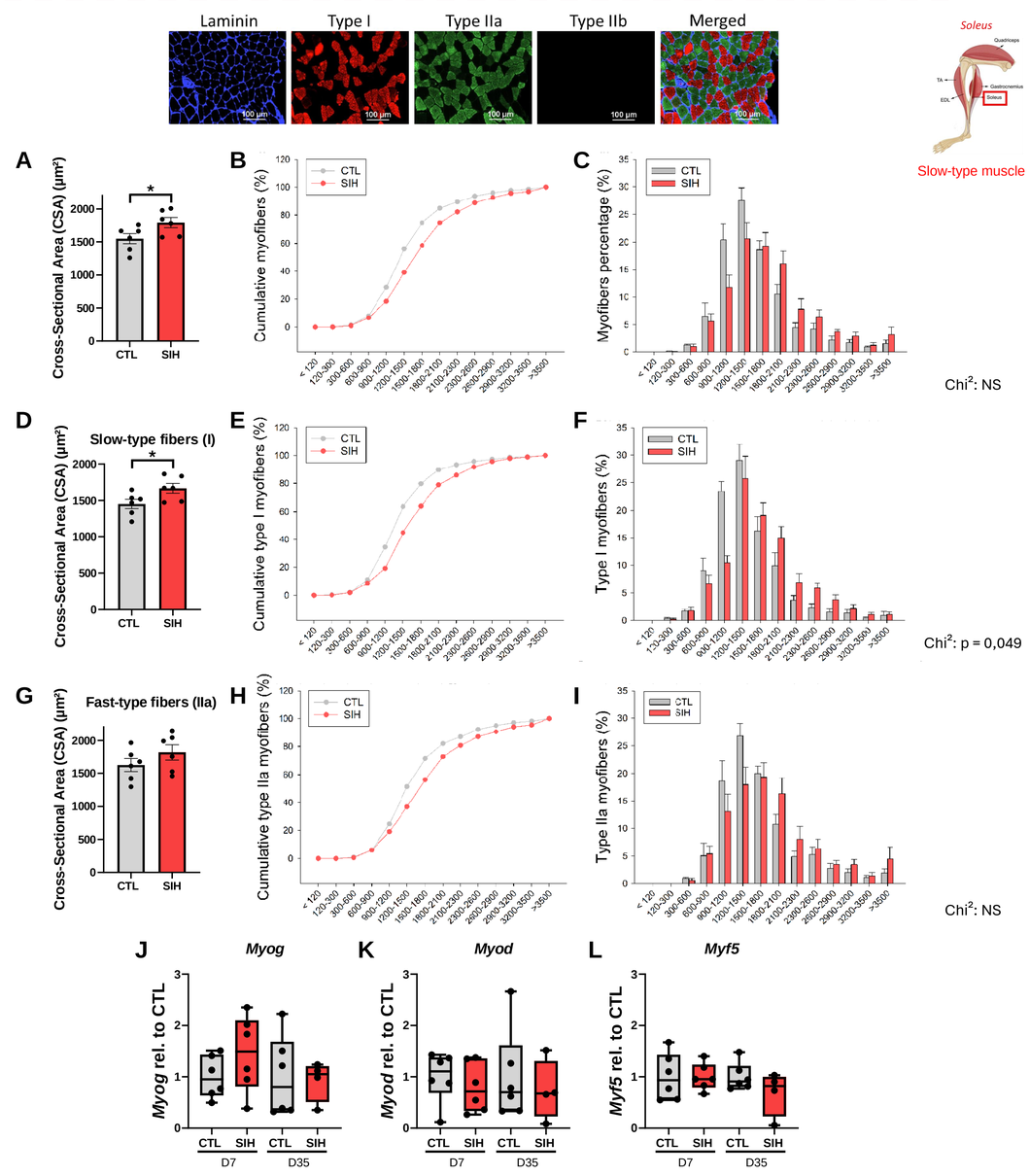


Figure 1. Effect of 7 and 35 days of Sustained Intermittent Hypoxaemia (SIH) on mouse *tibialis anterior* (TA) muscle Cross-Sectional Area (CSA), myofiber size distribution and myogenic marker expression. TA muscle cryosection of SIH and CTL mice were submitted to a co-immunofluorescence by using antibodies directed against MyHC7 (type I fibres), MyHC2 (type IIa fibres), MyHC4 (type IIb fibres) and laminin. **(A, D, G)** Each myofibre CSA was measured by using the Image J software. Data represented as mean ± SEM. **(C, F, I)** Myofibers were classified in clusters according to their area (µm²). **(B, E, H)** Cumulative percentage. **A.** CSA of the whole TA muscle section; t-test: NS (n = 6). **D.** TA type IIa myofiber CSA; t-test: NS (n=6). **G.** TA type IIb myofiber CSA, t-test: NS (n=6). **C.** Whole TA fibre size distribution; Chi²: NS (n=6). **F.** TA type IIa fibre size distribution; **: p < 0,01, Chi² (n=6). **I.** TA type IIb fibre size distribution; Chi²: NS (n=6). **(J-L)** RT-qPCR were performed on the TA muscle by using the ΔΔCt method (housekeeping gene: *Rplp0*; data normalised to CTL). **J.** *Myog* (encoding Myogenin) expression; *: p < 0,05, SIH vs CTL, Two Way ANOVA (n=6). **K.** *Myod1* (encoding Myoblast determination protein 1) expression; **: p < 0,01, SIH vs CTL, Mann-Whitney Rank Sum Test (n=6). **L.** *Myf5* (encoding Myogenic factor 5) expression; Mann-Whitney Rank Sum Test: NS (n=6). Data presented as box plots.

Figure 2. Effect of 7 and 35 days of Sustained Intermittent Hypoxaemia (SIH) on mouse *soleus* muscle (Cross-Sectional Area (CSA), myofiber size distribution and myogenic marker expression. *Soleus* 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)** Each myofibre CSA was measured by using the Image J software. Data represented as mean ± SEM. **(C, F, I)** Myofibers were classified in clusters according to their area (µm²). **(B, E, H)** Cumulative percentage. **A.** CSA on the whole *soleus* muscle section; *: p < 0,05, t-test (n=6). **D.** *Soleus* type I myofibre CSA; *: p < 0,05, t-test (n=6). **G.** *Soleus* type IIa myofiber CSA, t-test: NS (n=6). **C.** Whole *Soleus* fibre size distribution; Chi²: NS (n=6). **F.** *Soleus* type I fibre size distribution; Chi²: p = 0,059 (n=6). **I.** *Soleus* type IIa fiber size distribution; Chi²: NS (n=6). **(J-L)** RT-qPCR were performed on the *soleus* muscle by using the ΔΔCt method (housekeeping gene: *Rplp0*; data normalised to CTL). **J.** *Myog* expression in the *soleus* muscle; Two Way ANOVA: NS (CTL: n=6; SIH: n=5). **K.** *Myod1* expression in the *soleus* muscle; Two Way ANOVA: NS (C T L : n = 6 ; S I H : n = 5) . **L.** *Myf5* expression in the *soleus* muscle; Two Way ANOVA: NS (CTL: n=6, SIH: n=5). Data presented as box plots.

ApN pathway in the SIH model

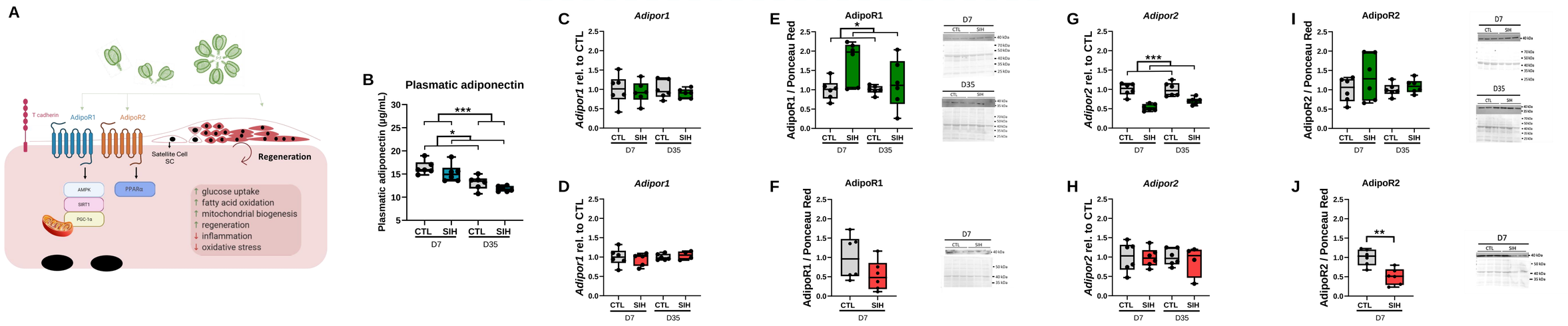


Figure 3. Effect of 7 and 35 days of SIH on Adiponectin (ApN) pathway at the muscle level in mice. A. Schematic representation of the ApN pathway. **B.** Plasmatic ApN was measured by using the Quantikine ELISA kit - Mouse Adiponectin/Acrp30 Immunoassay (R&D systems); ***: p <0,001, J7 vs J35, Two Way 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 by using 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 by using 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).

Conclusion & 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 using a model of cardiotoxin (CTX)-induced muscle lesion.

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