Acute Hypoxia Rapidly Alters Myotube Size *in vitro* & Myostatin Signaling *in vivo*

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Introduction

Skeletal muscle is the largest tissue in humans and is key in thermoregulation, locomotion, glycolysis and homeostasis. Muscle atrophy increases mortality in a variety of disorders. Myostatin is a powerful regulator of muscle mass[1] and acts via multiple pathways to induce atrophy[2].

Figure 1: Healthy individuals at altitude lose muscle mass.

Hypoxia is a poorly understood inducer of muscle atrophy. Atrophy is seen in mountaineering humans[3] (figure 1). Rats exposed to hypoxia for 5 weeks show increased myostatin expression[4], as do COPD patients who are chronically (at least 6 months) hypoxemic. Both COPD patients and mountaineering individuals are difficult models to study, as they present with several confounding factors.

We therefore aimed to investigate the effect of hypoxia upon myostatin expression *in vitro* and *in vivo* in healthy humans, hypothesizing that hypoxia alone would be sufficient to induce increased myostatin expression and therefore be causative of muscle atrophy.

In vitro – Does hypoxia directly increase myostatin & induce atrophy?

C2C12 myoblasts were either scratched and exposed to hypoxia or differentiated into myotubes and exposed to hypoxia.

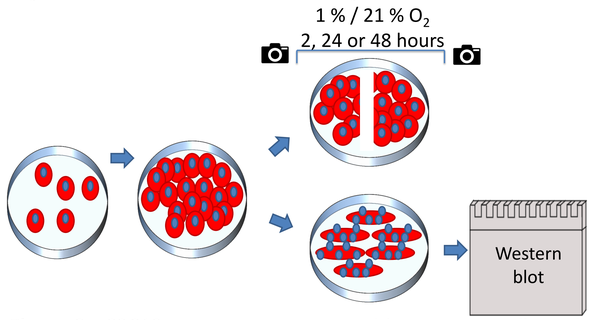

Figure 2:. C2C12 myoblasts were plated in growth media and grown to confluence. Cells were then either scratched for chemotaxis (top) or differentiated into myotubes (bottom) before being exposed to 1 % or 21 % O_2 for 2, 24 or 48 hours.

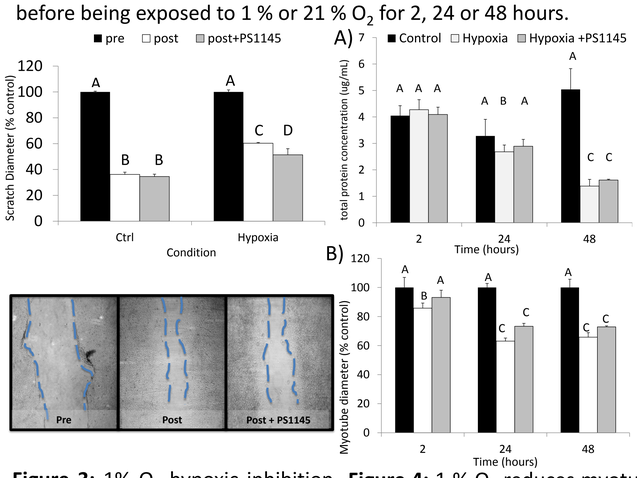

Figure 3: 1% O_2 hypoxic inhibition reduces chemotaxis in a NF-κB dependent manner. Representative images shown.

Figure 4: 1 % O_2 reduces myotube **A)** protein content & **B)** diameter in a time-dependent manner which is offset by PS1145.

Despite alterations in total protein content and cell size, no change in cellular myostatin expression was measured (data not shown).

In vivo – Does hypoxia increase myostatin?

Participants (N = 8, ♂) were exposed to hypoxic (12 % O_2) or control (21 % O_2) conditions in a counter-balanced design.

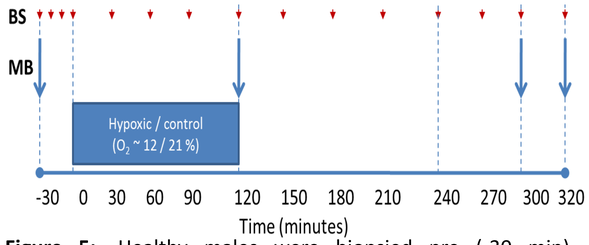

Figure 5:. Healthy males were biopsied pre (-30 min), immediately post (120 min), 300 min and 320 minutes post 2 hours of 12 % or 21 % O_2. N = 8. MB = MB, BS = blood sample.

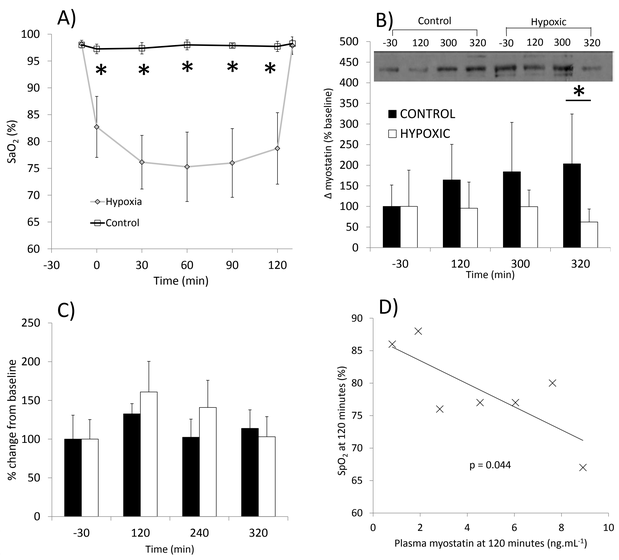

Figure 6: A) SpO_2 is reduced during 12 % O_2 exposure. **B)** Muscle myostatin is reduced 320 minutes after hypoxic exposure relative to control time point. Representative Western blot shown. **C)** Plasma myostatin is not altered by 2 hours hypoxia. **D)** Drop in SpO_2 correlates with increase in plasma myostatin immediately following hypoxic exposure.

Discussion

Hypoxia rapidly induces atrophy of myotubes *in vitro*, with decreased protein content and decreased size, in a time-dependent manner. Hypoxia decreased muscle myostatin content *in vivo* relative to resting control, suggesting myostatin was either degraded or left the muscle tissue to act in its endocrine role.

These results suggest hypoxia alone is sufficient to induce atrophy. This may in part explain hypoxic conditions or disorders where muscle atrophy is seen. Current work is examining the dose- and time-dependent effect of hypoxia *in vivo* (figures 7 & 8), before attempts to inhibit the effect of hypoxia upon muscle mass *in vivo* can be attempted in a similar manner to our *in vitro* work.

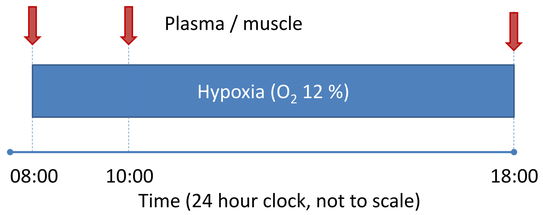

Figure 7: Healthy males (N = 8) biopsied pre- and post-exposure to 10 hours of 12 % O_2. Plasma collected pre- and post-exposure.

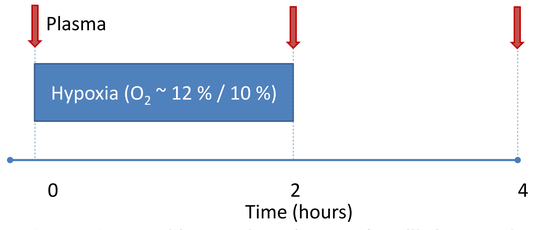

Figure 8: Healthy males (N = 8) will have plasma collected pre-, post- and 2 hours post-exposure to 2 hours of 12 % or 10 % O_2 in a counter-balanced design, with a 7 day washout between exposures.

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4. Hayot, M. et al. Myostatin up-regulation is associated with the skeletal muscle response to hypoxic stimuli. Molecular and cellular endocrinology (2010).

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