# SEX DIFFERENCES IN MURINE STERNOHYOID MUSCLE TOLERANCE OF ACUTE SEVERE HYPOXIC STRESS

*Philip Lewis & Ken D. O'Halloran*

*Department of Physiology, School of Medicine, University College Cork, Ireland*


**Correspondence:**

Philip Lewis

Department of Physiology, School of Medicine,

University College Cork,

Western Gateway Building,

Western Road,

Cork, Ireland.


Tel: +49 15733749502

Email: philip.lewis@umail.ucc.ie


**Running head:** Sex differences in sternohyoid hypoxia tolerance

**Summary**

Given that sex differences inherent to muscle might at least contribute to male risk for obstructive sleep apnoea syndrome (OSAS), our objective was to test the hypothesis that male sternohyoid muscle exhibits greater susceptibility to severe hypoxic stress compared with female muscle. Adult male and female C57Bl6/J mouse sternohyoid isometric and isotonic functional properties were examined *ex vivo* at $35^{0}$C in tissue baths under control and severe hypoxic conditions. Hypoxia was detrimental to peak force (F*max*), work (W*max*) and power (P*max*), but not shortening velocity (V*max*). Two-way analysis of variance revealed a significant sex *x* gas interaction for F*max* ($p<0.05$), revealing inferior hypoxic tolerance in male sternohyoid muscle. However, increases in male shortening velocity in severe hypoxia preserved power-generating capacity which was equivalent to values determined in female muscle. F*max* decline in hypoxic female sternohyoid was considerably less than in male muscle, illustrating an inherent tolerance of force-generating capacity mechanisms to hypoxic stress in female airway dilator muscle. We speculate that this could confer a distinct advantage *in vivo* in terms of the defence of upper airway calibre.

**Keywords:** hypoxia, sex differences, respiratory muscle, contraction

**INTRODUCTION**

Sex is a risk factor for many diseases (Check Hayden, 2010) including respiratory diseases such as obstructive sleep apnoea syndrome (OSAS) with higher susceptibility in adult males (Partinen & Telakivi, 1992; Young *et al.*, 1993). The reasons for the disproportionate prevalence between the sexes are not well understood. Several studies have considered sex differences in upper airway anatomy and neural drive as important to predisposition and progression of OSAS, but their relevance remains unclear due to inconsistencies in the literature (Brown *et al.*, 1986; Popovic & White, 1995; Martin *et al.*, 1997; Pillar *et al.*, 2000; Jordan *et al.*, 2002; Edge *et al.*, 2012).

The transition from wakefulness to sleep state is associated with decrements in upper airway muscle tone (Remmers *et al.*, 1978; Worsnop *et al.*, 1998) predisposing to collapse of the upper airway. Recurrent obstructive apnoea – the hallmark feature of OSAS – occurs as a result of a failure of the upper airway dilator muscles to adequately defend airway calibre. Recurrent airway collapse is associated with exposure to hypoxia (Young *et al.*, 1993) which can cause respiratory muscle dysfunction and is considered progressive to OSAS (Bradford *et al.*, 2005). Respiratory muscle remodelling and dysfunction is observed both in patients with OSAS and in animal models of the disorder (Stauffer *et al.*, 1989; Sériès *et al.*, 1996; Carrera *et al.*, 1999; McGuire *et al.*, 2002; Bradford *et al.*, 2005; Skelly *et al.*, 2012*a*). Whilst assessment of rat upper airway muscle performance *ex vivo* shows no sex difference (Cantillon & Bradford, 1998), episodic hypoxia causes weakness in male, but not female, upper airway dilator muscle (Skelly *et al*, 2012a) and, although not statistically compared, the data suggests that female rat sternohyoid muscle better tolerates acute hypoxic stress (Skelly *et al.*, 2012*a*). Sex differences at the level of the pharyngeal dilator muscles could potentially contribute to the greater male predisposition for OSAS.

We sought to comprehensively compare male and female sternohyoid muscle performance *ex vivo* in control and severe hypoxic conditions. The sternohyoid is a fast fibre type, multi-functional muscle with a role in the control of pharyngeal airway calibre (van Lunteren *et al.*, 1987; Skelly *et al.*, 2012*b*). We tested the hypothesis that male sternohyoid muscle shows greater susceptibility to severe hypoxic stress compared with female muscle.

## METHODS

### Animals and muscle preparation

All protocols described in this study were approved by local ethics committee and performed under licence from the Irish Government Department of Health and Children in accordance with EU legislation. Thirty-four age- and weight- matched adult male and female C57Bl6/J mice (four groups: male control n=8; male hypoxia n=9; female control n=8; female hypoxia n=9) were anaesthetised by 5% isoflurane inhalation in air and euthanized by cervical dislocation. Sternohyoid whole muscles were excised for study and arranged longitudinally between plate electrodes, anchored at one end to a fixed base and connected at the other end to a dual-mode lever force transducer (Aurora Scientific, Canada) for assessment of contractile and endurance properties. Bundles were incubated at $35^{o}$C in Krebs solution (NaCl 120mM, KCl 5mM, $Ca^{2+}$ gluconate 2.5mM, $MgSO_4$ 1.2mM, $NaH_2PO_4$ 1.2mM, $NaHCO_3$ 25mM, glucose 11.5mM, and 25mM d-tubocurarine) gassed with either control (95% $O_2$/5% $CO_2$) or anoxic (95% $N_2$/5% $CO_2$) mixtures. Sternohyoid muscle performance has previously been shown to be optimal under hyperoxic conditions (95% $O_2$) compared with normoxic conditions (21% $O_2$), thus making hyperoxia the optimal control condition for this study (Skelly *et al*, 2010). We have previously demonstrated that ambient $PO_2$ is

~40mmHg in preparations gassed with anoxic gas (McDonald *et al.*, 2014). This generates severe hypoxic conditions in muscle preparations but it should be noted that preparations are viable and are responsive, for example, to pharmacological intervention (McDonald *et al.*, 2014). The experimental groups are designated 'hypoxia' due to the hypoxic nature of the tissue bath medium.

**Protocol**

*Twitch contractile kinetics:* Bundles were set to optimum length ($L_o$ – length at which peak twitch force occurs) by adjusting the position of the force transducer with a micro-positioner and stimulating with a single pulse at a supramaximal voltage until peak twitch force was achieved. Twitch kinetics (time to peak (TTP) and half-relaxation time (T50)) were measured from the peak twitch force recording. *Peak isometric tetanic force:* After five minutes equilibration time, the force transducer was set to maximum rigidity (~500mN; >maximum force the muscle can produce; >100% load) and a tetanic contraction was elicited by stimulating the bundle with supra-maximal voltage at 100Hz for 300ms. *Shortening length and velocity:* Following a five minute rest period, the transducer-lever system was set such that contractions were elicited ranging from 0-40% load in incremental steps (0, 1, 5, 10, 15, 20, 25, 30, 35, 40%) with one minute rest between each step. Shortening was determined as the maximum distance shortened over the whole contraction and shortening velocity was determined as distance shortened during the initial 30ms of shortening, as this is when velocity is greatest (Watchko & Daood, 1997; van Lunteren *et al.*, 2007; van Lunteren & Pollarine, 2010; Lewis *et al.*, 2015). Peak shortening velocity was measured at 0% load. *Work and Power:* Mechanical work and power were determined at each step of the incremental load test as the product of force *x* shortening and force *x* shortening velocity, respectively. Peak mechanical work and power are determined from the work-load and power-load relationships. *Fatigue Tolerance*: Five minutes after completion of the

incremental load step test, muscle endurance was assessed under isotonic conditions by repeated stimulation of the muscle at 100 Hz with 300ms trains every 2 seconds for a period of 2 minutes at 33% load, a load which has previously been shown elicits maximum power (Sieck & Prakash, 1997). Dynamic Muscle Control software (Aurora Scientific) was used to control isometric and isotonic contractions.

**Data Analysis**

Peak specific force (F$max$) was calculated in N/cm$^2$ of muscle cross-sectional area. Cross-sectional area was calculated as the blotted dry muscle bundle weight divided by the product of L$_o$ and the specific density, assumed to be 1.056g/cm$^3$. Specific shortening was calculated as length shortened per optimal length (L/L$_o$). Peak specific shortening velocity (V$max$) was expressed as L$_o$/s. Peak specific mechanical work (W$max$) was expressed as Joules/cm$^3$ and peak specific power (P$max$) as Watts/cm$^2$. For the assessment of isotonic fatigue, P$max$ was determined at the beginning of the trial and data determined after 2 min of repeated fatiguing contractions were expressed as a percentage of initial power at time zero. All values are expressed as mean ± SEM. After testing for normality and equal variance in the data sets, statistical comparisons were performed between groups using Student's t-test, one-way and two-way analysis of variance with Bonferroni multiple comparison post-hoc tests as appropriate using Graph-Pad Prism (USA). $P<0.05$ was the criterion for statistical significance.

**RESULTS**

**Twitch force and contractile kinetics**

Hypoxia significantly (p<0.05) decreased sternohyoid twitch force (Table 1). T50 (p<0.05), but not TTP, was significantly decreased under hypoxic conditions. Two-way analysis of variance revealed that there were no independent sex effects on isometric twitch force or contractile kinetics, and there was no sex $x$ gas interaction (Table 1).

**Peak isometric tetanic force, isotonic shortening velocity, work, and power**

Under control conditions, there was no difference between male and female sternohyoid contractile properties e.g. peak tetanic force (F$max$, $9.9 \pm 0.6$ vs $11.0 \pm 0.9$ N/cm$^2$), peak mechanical work (W$max$, $6.4 \pm 1.6$ vs $8.1 \pm 1.9$ J/cm$^2$), peak mechanical power (P$max$, $6.6 \pm 0.6$ vs $7.8 \pm 0.7$ W/cm$^2$) and maximum shortening velocity (V$max$, $5.3 \pm 0.8$ vs $4.9 \pm 0.5$ Lo/s) (Fig. 1A-D). Hypoxia resulted in significant reductions in F$max$ (p<0.001, two-way ANOVA), W$max$ (p<0.001), P$max$ (p<0.001), but not V$max$ (Fig. 1 A-D). There was no independent sex effect on contractile parameters. However, there was a sex $x$ gas interaction for F$max$ (p<0.05), indicative of superior hypoxic tolerance in female compared with male muscle. There was no independent sex effect or sex $x$ gas interaction for any other peak parameter (Fig. 1 A-D).

**Isotonic incremental load step test**

No independent sex differences were observed for sternohyoid shortening velocity-load relationship in control conditions (Fig. 2). Hypoxia (Fig. 2) significantly increased male sternohyoid shortening velocity at lower loads but not at higher loads (p<0.05, two-way ANOVA followed by Bonferroni post-hoc test). Conversely, female sternohyoid shortening velocity was significantly (p<0.05) depressed across the 20-35% load range. Sex differences (p<0.05) were observed in shortening velocity across 1-10% load in hypoxic conditions (Fig.

2), with lower values noted in female muscles. No significant differences were observed in shortening-load relationships across groups (Fig. 3). No independent sex differences were observed for sternohyoid work-load relationship or power-load relationship in either control or hypoxic conditions (Fig. 4-5). The effect of hypoxia on work-load and power-load relationships in male and female muscle preparations were equivalent — hypoxia significantly depressed these relationships across the 15-40% load range ($p<0.05$, $p<0.01$). The sternohyoid work-load relationship was left-shifted in female compared with male sternohyoid in hypoxic conditions (Fig. 4). A similar effect was observed for the sternohyoid power-load relationship in hypoxia (Fig. 5).

**Fatigue Tolerance**

Hypoxia significantly ($p<0.001$) decreased male and female sternohyoid isotonic fatigue tolerance (Fig. 6). No sex differences were observed in fatigue tolerance in control or hypoxic conditions.

**DISCUSSION**

This is the first study to explore the effects of acute severe hypoxia on pharyngeal dilator muscle isotonic contractile and endurance properties. We have established that there are sex differences in performance inherent to sternohyoid muscle. The main findings of this study are: 1) male and female sternohyoid muscle contractile and endurance properties are equivalent under control conditions; 2) acute severe hypoxic stress significantly decreases F*max*, W*max* and P*max*, but not V*max;* 3) hypoxia increases the curvature of the shortening velocity-load relationship in male and female sternohyoid muscle which contributes to depression of power generation; and 4) the magnitude of peak force decline is considerably

less in female compared to male sternohyoid muscle, illustrating an inherent tolerance to hypoxic stress in female muscle compared with male muscle. We speculate that the latter might confer a distinct advantage *in vivo* in terms of the defence of upper airway calibre.

**Isometric contractile differences in male and female sternohyoid muscle**

Force generation by the sternohyoid muscle is necessary *in vivo* as an airway defence mechanism against the sub-atmospheric pressures generated by the thoracic muscles that would otherwise cause airway collapse upon inspiration. Moreover, the sternohyoid and other upper airway dilator muscles are critically important in re-opening a collapsed pharyngeal airway, which is common in OSAS. Hypoxia significantly depresses sternohyoid muscle function, highlighting the potential for this stimulus — which can present as a consequence of obstructed breathing — to further exacerbate respiratory control through a depressant action on airway dilator muscles. It is plausible to suggest that the duration of an obstructive apnoeic event *in vivo* could be prolonged by the depressant action of hypoxia on airway dilator muscles such as the sternohyoid. It is also interesting to consider that upper airway patency might be challenged *in vivo* in circumstances of hypoxaemia such as that occurring in chronic respiratory diseases such as COPD, or perhaps at altitude in otherwise healthy individuals. Of note, sleep-disordered breathing can present in both of these populations (Anholm *et al.*, 1992; McNicholas, 2009). In particular, the overlap syndrome, namely sleep apnoea in COPD, might well relate, at least in part, to hypoxic depression of upper airway dilator muscles and redox remodelling of the upper airway dilator muscles in response to chronic exposures (Deegan & McNicholas, 1995; Bradford *et al.*, 2005; Owens & Malhotra, 2010; Lewis *et al.*, 2015).

Hypoxia adversely affected a number of contractile parameters with a prominent depressant effect on force-generating capacity (especially in males). Hypoxia also greatly enhanced isotonic fatigue. Our comprehensive assessment of function provides data on all aspects of muscle contractile behaviour. Arguably, peak isometric force is most relevant for the sternohyoid muscle, given that isometric contractions of the muscle most likely subserve its physiological function to stabilize the hyoid bone, working in concert with suprahyoid muscles to displace the hyoid bone anteriorly. Our study revealed a significant sex difference in the effect of severe hypoxia on sternohyoid muscle force-generating capacity. The apparent hypoxic tolerance of the female sternohyoid may be especially relevant to respiratory-related diseases characterised by hypoxia. If this inherent sex difference in the sternohyoid muscle (and potentially other skeletal muscles involved in regulating upper airway patency) response to hypoxia applies in humans, it may contribute to the male risk for the development of OSAS. The superior force-generating capacity of the female sternohyoid muscle in hypoxia compared with male counterparts could serve to protect against airway collapse, the risk of which is increased by augmented inspiratory effort in hypoxia. A greater susceptibility to hypoxic depression of force in males could increase the frequency and especially the duration of apnoeas.

**Isotonic contractile differences in male and female sternohyoid muscle**

It appears that the mechanism of hypoxic depression of work and power differs in male and female sternohyoid muscle. Whilst female sternohyoid muscle shows inherent hypoxic tolerance in terms of force-generating capacity, isotonic contractile properties were adversely affected by severe hypoxia to a greater extent than in male muscles. As such, on balance, no independent sex difference was noted for work-load and power-load relationships in hypoxia, owing to sex differences in the effects of hypoxia on shortening velocity, opposing the

differential effects observed on force. The increase in curvature between control and hypoxic shortening velocities, resulting in decreased velocity for both sexes at the load where peak power approximately occurs contributed to the observed shift in power between control and hypoxic conditions. The maximal velocity of shortening was more reduced by hypoxia in female than in male muscles, as observed in Fig. 2, but not significantly in Fig. 1. This statistical discrepancy is explained by differences in curvature of the response between male and female muscles. In short, hypoxia decreased mechanical work and power in male and female muscles but through different mechanisms. Though detrimental to mechanical function, the hypoxic impairment could be considered protective given that it prevents ATP consumption perhaps beyond critical levels, similar to the role of acidosis in fatigue. Owing to the paucity of information in respect of male and female respiratory muscle, the potential mechanisms underpinning sex differences in response to hypoxia are not clear.

**Molecular mechanisms that drive contractile differences**

Wüst et al., (2008) posit that sex-related differences in fatigue performance of human quadriceps femoris muscle are likely intrinsic to muscle on the basis of supporting evidence against differences in blood flow and ATP production (Wüst *et al.*, 2008). Fulco et al. (2001) hypothesize that sex differences in muscle performance in hypoxia may depend on differences in metabolic activity within fibre types (Fulco *et al.*, 2001) that are revealed with hypoxic exposure. Welle et al. (2008) found hundreds of genes that are differentially expressed in male and female vastus lateralis muscle (Welle *et al.*, 2008) including several genes encoding metabolic proteins. Interestingly, sex differences in myosin heavy chain gene expression are reported for human vastus lateralis (Welle *et al.*, 2008). Therefore, it is plausible that sex differences exist in the inherent contractile machinery of upper airway (and other respiratory) muscles that could give rise to differential outcomes in muscle performance

and muscle susceptibility to various stressors including hypoxia. To the best of our knowledge however, there are no reported structural differences in the contractile machinery of male vs. female sternohyoid muscle that are hypoxia sensitive. Sex differences in $Ca^{2+}$ release from the sarcoplasmic reticulum may also play role, as observed in cardiac muscle and vascular smooth muscle (Giachini *et al.*, 2012; Parks & Howlett, 2013). If this were the case in the model presented in the current manuscript, we would expect to see sex differences in other $Ca^{2+}$ dependent contractile parameters. However, V*max* is equivalent in male and female sternohyoid under control conditions, so neither myosin heavy chain differences nor $Ca^{2+}$ release/sensitivity differences are likely to underpin the observed sex differences. Of note, contractile and endurance properties are equivalent in male and female sternohyoid muscle under control conditions. Given the association of hypoxia with oxidative/nitrosative stress and muscle functional adaptation in patients and animal models (Lavie, 2003; Zhu *et al.*, 2003, 2005; Koechlin *et al.*, 2005; Ottenheijm *et al.*, 2006; Marin-Corral *et al.*, 2009; Sadasivam *et al.*, 2011; Skelly *et al.*, 2012*a*, 2013; Shortt *et al.*, 2014), the sex differences reported herein may relate to differences in the cellular response to redox stress. One study highlights stronger defences against oxidative stress in female rat liver, heart, brain, and kidney tissues compared with males (Katalinic *et al.*, 2005). Another study notes higher glutathione peroxidase enzyme (antioxidant) concentration in female rat gastrocnemius muscle compared with males (Colom *et al.*, 2007). However, more research is required to determine the extent of sexual dimorphism in skeletal muscle antioxidant defences and how this may affect muscle performance particularly in response to severe oxidative stress. Whilst the differences we observed are inherent to muscle, since they were revealed in an *ex vivo* preparation, we are not discounting a putative role for sex hormones which might also conceivably contribute to sex differences in muscle performance *in vivo* and which may have influenced the muscle response to hypoxic stress. For example, sex hormones can increase

respiratory muscle strength *in vivo* (da Silva *et al.*, 2006) and affect translational signalling in muscle (Welle *et al.*, 2008). Skeletal muscle has an important endocrine role (Pedersen, 2011) and potential sex differences in myokine production or autocrine signalling *per se* and in response to hypoxaemia could further contribute to sex differences in muscle performance. It could be suggested that the study design biased results obtained in the hypoxic muscle groups, exaggerating decline in function owing to reduced viability of the preparation. Whilst this is possible, we suggest that nevertheless the sex differences relate to intrinsic sex differences in the muscles that comes to the fore under severe hypoxic conditions. The sex difference in maximal force in hypoxia is observed very close to the beginning of the sequence of stimulations after 5 mins of equilibration, yet the muscle preparations are still viable 10 mins and several stimulations later. Thus we are confident that the results relate to intrinsic sex differences revealed in viable preparations.

Despite the clinical relevance, few studies characterise phenotypic responses to stressors such as hypoxia in male *and* female animals. The results presented here highlight the need to consider the possibility of differential sex-related effects which may ultimately have relevance to the characterisation of disease progression and also the response to therapeutic intervention in human patients.

**Conclusion**

We conclude that there are sex differences in hypoxic tolerance of murine upper airway dilator muscle. These differences potentially contribute to the differential development, progression, and outcomes of several respiratory-related diseases observed in male and female patients.

# REFERENCES

ANHOLM, JD, POWLES, AC, DOWNEY, R, HOUSTON, CS, SUTTON, JR, BONNET, MH, CYMERMAN, A: Operation Everest II: arterial oxygen saturation and sleep at extreme simulated altitude. *Am Rev Respir Dis* **145:** 817–826,. 1992.

BRADFORD, A, MCGUIRE, M, O'HALLORAN, KD: Does episodic hypoxia affect upper airway dilator muscle function? Implications for the pathophysiology of obstructive sleep apnoea. *Respir Physiol Neurobiol* **147:** 223–234,. 2005.

BROWN, IG, ZAMEL, N, HOFFSTEIN, V: Pharyngeal cross-sectional area in normal men and women. *J Appl Physiol* **61:** 890–895,. 1986.

CANTILLON, D, BRADFORD, A: Effect of gender on rat upper airway muscle contractile properties. *Respir Physiol* **113:** 147–156,. 1998.

CARRERA, M, BARBÉ, F, SAULEDA, J, TOMÁS, M, GÓMEZ, C, AGUSTÍ, AG: Patients with obstructive sleep apnea exhibit genioglossus dysfunction that is normalized after treatment with continuous positive airway pressure. *Am J Respir Crit Care Med* **159:** 1960–1966,. 1999.

CHECK HAYDEN, E: Sex bias blights drug studies. *Nature* **464:** 332–333,. 2010.

COLOM, B, ALCOLEA, MP, VALLE, A, OLIVER, J, ROCA, P, GARCÍA-PALMER, FJ: Skeletal muscle of female rats exhibit higher mitochondrial mass and oxidative-phosphorylative capacities compared to males. *Cell Physiol Biochem* **19:** 205–212,. 2007.

DEEGAN, P, MCNICHOLAS, W: Pathophysiology of obstructive sleep apnoea. *Eur Respir J* **8:** 1161–1178,. 1995.

EDGE, D, BRADFORD, A, O'HALLORAN, KD: Chronic intermittent hypoxia increases apnoea index in sleeping rats. *Adv Exp Med Biol* **758:** 359–363,. 2012.

FULCO, CS, ROCK, PB, MUZA, SR, LAMMI, E, BRAUN, B, CYMERMAN, A, MOORE, LG, LEWIS, SF: Gender alters impact of hypobaric hypoxia on adductor pollicis muscle performance. *J Appl Physiol* **91:** 100–108,. 2001.

GIACHINI, FRC, LIMA, V V, FILGUEIRA, FP, DORRANCE, AM, CARVALHO, MHC, FORTES, ZB, WEBB, RC, TOSTES, RC: STIM1/Orai1 contributes to sex differences in vascular responses to calcium in spontaneously hypertensive rats. *Clin Sci (Lond)* **122:** 215–226,. 2012.

JORDAN, AS, CATCHESIDE, PG, O'DONOGHUE, FJ, SAUNDERS, NA, MCEVOY, RD: Genioglossus muscle activity at rest and in response to brief hypoxia in healthy men and women. *J Appl Physiol* **92:** 410–417,. 2002.

KATALINIC, V, MODUN, D, MUSIC, I, BOBAN, M: Gender differences in antioxidant capacity of rat tissues determined by 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. *Comp Biochem Physiol C Toxicol Pharmacol* **140:** 47–52,. 2005.

KOECHLIN, C, MALTAIS, F, SAEY, D, MICHAUD, A, LEBLANC, P, HAYOT, M, PRÉFAUT, C: Hypoxaemia enhances peripheral muscle oxidative stress in chronic obstructive pulmonary disease. *Thorax* **60:** 834–841,. 2005.

LAVIE, L: Obstructive sleep apnoea syndrome--an oxidative stress disorder. *Sleep Med Rev* **7:** 35–51,. 2003.

LEWIS, P, SHEEHAN, D, SOARES, R, VARELA COELHO, A, O'HALLORAN, KD: Chronic sustained hypoxia-induced redox remodeling causes contractile dysfunction in mouse sternohyoid muscle. *Front Physiol*; DOI: 10.3389/fphys.2015.00122,. 2015.

VAN LUNTEREN, E, HAXHIU, MA, CHERNIACK, NS: Relation between upper airway volume and hyoid muscle length. *J Appl Physiol* **63:** 1443–1449,. 1987.

VAN LUNTEREN, E, POLLARINE, J, MOYER, M: Isotonic contractile impairment due to genetic CLC-1 chloride channel deficiency in myotonic mouse diaphragm muscle. *Exp Physiol* **92:** 717–729,. 2007.

VAN LUNTEREN, E, POLLARINE, J: Improvement of diaphragm and limb muscle isotonic contractile performance by K+ channel blockade. *J Neuroeng Rehabil* **7:** 1,. 2010.

MARIN-CORRAL, J, MINGUELLA, J, RAMÍREZ-SARMIENTO, AL, HUSSAIN, SNA, GEA, J, BARREIRO, E: Oxidised proteins and superoxide anion production in the diaphragm of severe COPD patients. *Eur Respir J* **33:** 1309–1319,. 2009.

MARTIN, SE, MATHUR, R, MARSHALL, I, DOUGLAS, NJ: The effect of age, sex, obesity and posture on upper airway size. *Eur Respir J* **10:** 2087–2090,. 1997.

MCDONALD, F, SKELLY, JR, O'HALLORAN, KD: The β2 adrenoceptor agonist terbutaline recovers rat pharyngeal dilator muscle force decline during severe hypoxia. *Oral Dis*; DOI: 10.1111/odi.12247,. 2014.

MCGUIRE, M, MACDERMOTT, M, BRADFORD, A: The effects of chronic episodic hypercapnic hypoxia on rat upper airway muscle contractile properties and fiber-type distribution. *Chest* **122:** 1400–1406,. 2002.

MCNICHOLAS, WT: Chronic obstructive pulmonary disease and obstructive sleep apnea: overlaps in pathophysiology, systemic inflammation, and cardiovascular disease. *Am J Respir Crit Care Med* **180:** 692–700,. 2009.

OTTENHEIJM, CAC, HEUNKS, LMA, GERAEDTS, MCP, DEKHUIJZEN, PNR: Hypoxia-induced skeletal muscle fiber dysfunction: role for reactive nitrogen species. *Am J Physiol Lung Cell Mol Physiol* **290:** l127–l135,. 2006.

OWENS, RL, MALHOTRA, A: Sleep-disordered breathing and COPD: the overlap syndrome. *Respir Care* **55:** 1333–1344; discussion 1344–1346,. 2010.

PARKS, RJ, HOWLETT, SE: Sex differences in mechanisms of cardiac excitation-contraction coupling. *Pflügers Arch  Eur J Physiol* **465:** 747–763,. 2013.

PARTINEN, M, TELAKIVI, T: Epidemiology of obstructive sleep apnea syndrome. *Sleep* **15:** s1–s4,. 1992.

PEDERSEN, BK: Muscles and their myokines. *J Exp Biol* **214:** 337–346,. 2011.

PILLAR, G, MALHOTRA, A, FOGEL, R, BEAUREGARD, J, SCHNALL, R, WHITE, DP: Airway mechanics and ventilation in response to resistive loading during sleep: influence of gender. *Am J Respir Crit Care Med* **162:** 1627–1632,. 2000.

POPOVIC, RM, WHITE, DP: Influence of gender on waking genioglossal electromyogram and upper airway resistance. *Am J Respir Crit Care Med* **152:** 725–731,. 1995.

REMMERS, JE, DEGROOT, WJ, SAUERLAND, EK, ANCH, AM: Pathogenesis of upper airway occlusion during sleep. *J Appl Physiol* **44:** 931–938,. 1978.

SADASIVAM, K, PATIAL, K, VIJAYAN, VK, RAVI, K: Anti-oxidant treatment in obstructive sleep apnoea syndrome. *Indian J Chest Dis Allied Sci* **53:** 153–162,. 2011.

SÉRIÈS, FJ, SIMONEAU, SA, ST PIERRE, S, MARC, I: Characteristics of the genioglossus and musculus uvulae in sleep apnea hypopnea syndrome and in snorers. *Am J Respir Crit Care Med* **153:** 1870–1874,. 1996.

SHORTT, CM, FREDSTED, A, CHOW, HB, WILLIAMS, R, SKELLY, JR, EDGE, D, BRADFORD, A, O'HALLORAN, KD: Reactive oxygen species mediated diaphragm fatigue in a rat model of chronic intermittent hypoxia. *Exp Physiol* **99:** 688–700,. 2014.

SIECK, G, PRAKASH, Y: Cross-bridge kinetics in respiratory muscles. *Eur Respir J* **10:** 2147–2158,. 1997.

DA SILVA, SB, DE SOUSA RAMALHO VIANA, E, DE SOUSA, MBC: Changes in peak expiratory flow and respiratory strength during the menstrual cycle. *Respir Physiol Neurobiol* **150:** 211–219,. 2006.

SKELLY, JR, BRADFORD, A, JONES, JF, O'HALLORAN, KD: Superoxide scavengers improve rat pharyngeal dilator muscle performance. *Am J Respir Cell Mol Biol* **42:** 725–731,. 2010*a*.

SKELLY, JR, BRADFORD, A, O'HALLORAN, KD: Intermittent hypoxia impairs pharyngeal dilator muscle function in male but not female rats. *Adv Exp Med Biol* **669:** 285–287,. 2010*b*.

SKELLY, JR, EDGE, D, SHORTT, CM, JONES, JFX, BRADFORD, A, O'HALLORAN, KD: Tempol ameliorates pharyngeal dilator muscle dysfunction in a rodent model of chronic intermittent hypoxia. *Am J Respir Cell Mol Biol* **46:** 139–148,. 2012*a*.

SKELLY, JR, EDGE, D, SHORTT, CM, JONES, JFX, BRADFORD, A, O'HALLORAN, KD: Respiratory control and sternohyoid muscle structure and function in aged male rats: decreased susceptibility to chronic intermittent hypoxia. *Respir Physiol Neurobiol* **180:** 175–182,. 2012*b*.

SKELLY, JR, ROWAN, SC, JONES, JFX, O'HALLORAN, KD: Upper airway dilator muscle weakness following intermittent and sustained hypoxia in the rat: effects of a superoxide scavenger. *Physiol Res* **62:** 187–196,. 2013.

STAUFFER, JL, BUICK, MK, BIXLER, EO, SHARKEY, FE, ABT, AB, MANDERS, EK, KALES, A, CADIEUX, RJ, BARRY, JD, ZWILLICH, CW: Morphology of the uvula in obstructive sleep apnea. *Am Rev Respir Dis* **140:** 724–728,. 1989.

WATCHKO, J, DAOOD, M: Combined myofibrillar and mitochondrial creatine kinase deficiency impairs mouse diaphragm isotonic function. *J Appl …*1416–1423,. 1997.

WELLE, S, TAWIL, R, THORNTON, CA: Sex-related differences in gene expression in human skeletal muscle. ed. Fairhead C. *PLoS One* **3:** e1385,. 2008.

WORSNOP, C, KAY, A, PIERCE, R, KIM, Y, TRINDER, J: Activity of respiratory pump and upper airway muscles during sleep onset. *J Appl Physiol* **85:** 908–920,. 1998.

WÜST, RCI, MORSE, CI, DE HAAN, A, JONES, DA, DEGENS, H: Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Exp Physiol* **93:** 843–850,. 2008.

YOUNG, T, PALTA, M, DEMPSEY, J, SKATRUD, J, WEBER, S, BADR, S: The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* **328:** 1230–1235,. 1993.

ZHU, X, HEUNKS, LM, VERSTEEG, EMM, VAN DER HEIJDEN, HFM, ENNEN, L, VAN KUPPEVELT, TH, VINA, J, DEKHUIJZEN, PNR: Hypoxia-induced dysfunction of rat diaphragm: role of peroxynitrite. *Am J Physiol Lung Cell Mol Physiol* **288:** 116–l26,. 2005.

ZHU, X, HEUNKS, LMA, MACHIELS, HA, ENNEN, L, DEKHUIJZEN, PNR: Effects of modulation of nitric oxide on rat diaphragm isotonic contractility during hypoxia. *J Appl Physiol* **94:** 612–620,. 2003.

**TABLES**

**Table 1: Male and female sternohyoid muscle contractile properties in control and hypoxia**

| | Control | | Hypoxia | | Two-way ANOVA |
| --- | --- | --- | --- | --- | --- |
| | Male | Female | Male | Female | |
| TTP (ms) | $10 \pm 0$ | $12 \pm 2$ | $11 \pm 1$ | $9 \pm 0$ | *gas effect |
| T50 (ms) | $12 \pm 1$ | $15 \pm 4$ | $9 \pm 1$ | $9 \pm 0.4$ | ns |
| Pt (N/cm$^2$) | $1.9 \pm 0.1$ | $2.0 \pm 0.1$ | $1.0 \pm 0.1$ | $1.3 \pm 0.1$ | *gas effect |

Values are mean $\pm$ SEM. Pt = peak twitch tension expressed as Force/CSA (N/cm$^2$); TTP = time to peak twitch tension expressed in milliseconds; T50 = peak twitch half relaxation time expressed in milliseconds. * $p<0.05$ gas effect, ns = not statistically significant; two-way ANOVA; n=8-9 per group

# FIGURE LEGENDS

**Figure 1: Male and female sternohyoid F*max*, V*max*, W*max* and P*max*.** (a) Sternohyoid peak specific force (F*max*, mean ± SEM) expressed as force/CSA (N/cm$^2$); (b) Sternohyoid peak specific shortening velocity (V*max*, mean ± SEM) expressed as optimal lengths/time (L$_o$/s); (c) Sternohyoid peak specific work (W*max*, mean ± SEM) expressed as Joules/cm$^3$; (d) Sternohyoid peak specific power (P*max*, mean ± SEM) expressed as Watts/CSA (cm$^2$); n=8-9 per group; *p<0.05, **p<0.01, ***p<0.001, two-way ANOVA.

**Figure 2: Male and female sternohyoid muscle shortening velocity-load relationship in control and hypoxia**. Sternohyoid specific shortening velocity (mean ± SEM) expressed as Lo/s as a function of load expressed as a percentage of peak force (force/peak force*100); n=8-9 per group.

**Figure 3: Male and female sternohyoid muscle shortening -load relationship in control and hypoxia**. Sternohyoid specific shortening (mean ± SEM) expressed as L/Lo as a function of load expressed as a percentage of peak force (force/peak force*100); n=8-9 per group.

**Figure 4: Male and female sternohyoid muscle work-load relationship in control and hypoxia**. Sternohyoid specific work (mean ± SEM) expressed as Joules/cm$^3$ as a function of load expressed as a percentage of peak force (force/peak force*100); n=8-9 per group.

**Figure 5: Male and female sternohyoid muscle power-load relationship in control and hypoxia**. Sternohyoid specific power (mean ± SEM) expressed as Watts/CSA (cm$^2$) as a function of load expressed as a percentage of peak force (force/peak force*100); n=8-9 per group.

**Figure 6: Sternohyoid isotonic fatigue index: male and female sternohyoid muscle power in control and hypoxia after 2mins of repeated stimulation**. Sternohyoid power after fatiguing stimulation (mean ± SEM) expressed as % of initial power; n=8-9 per group; ***p<0.005; two-way ANOVA: gas effect.

# FIGURES

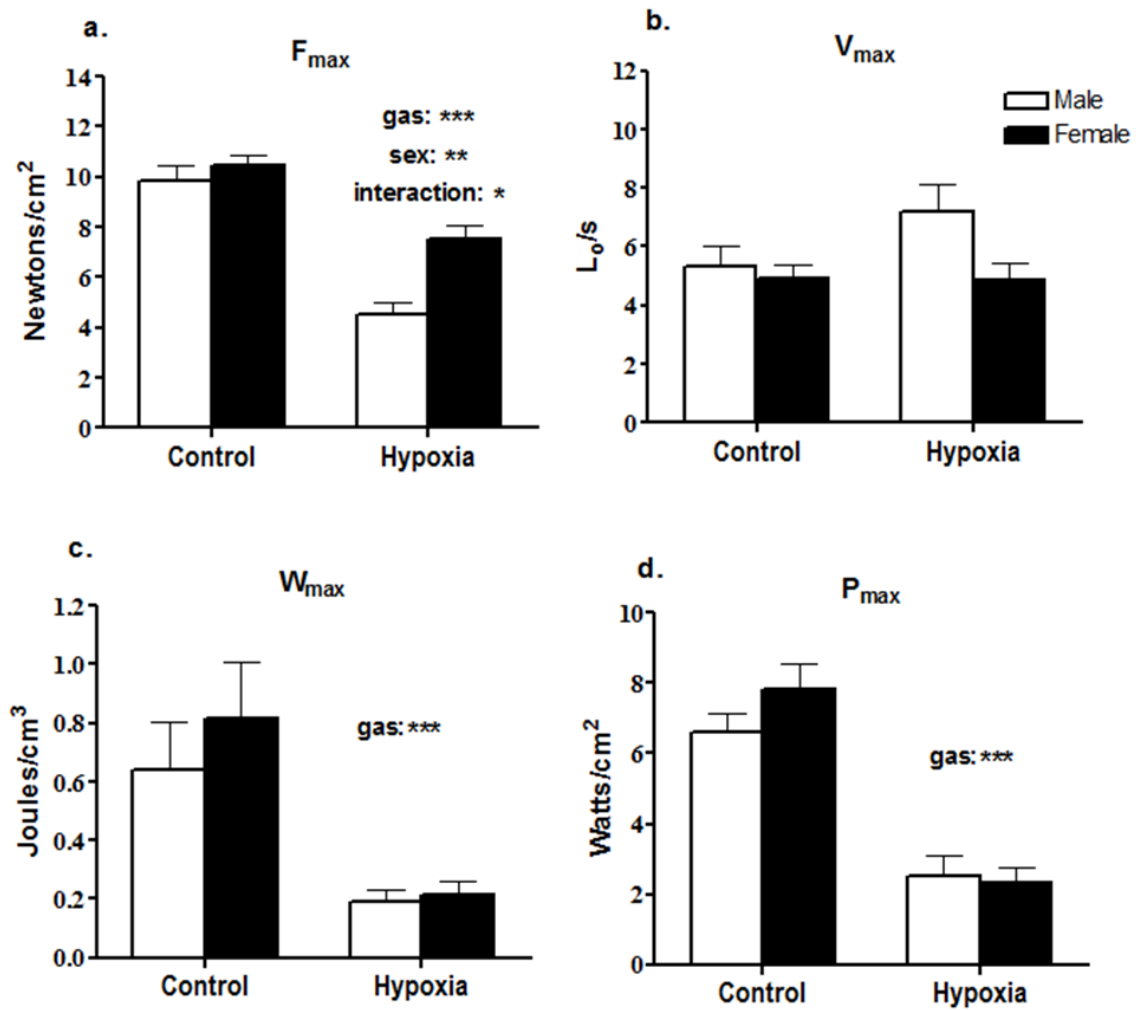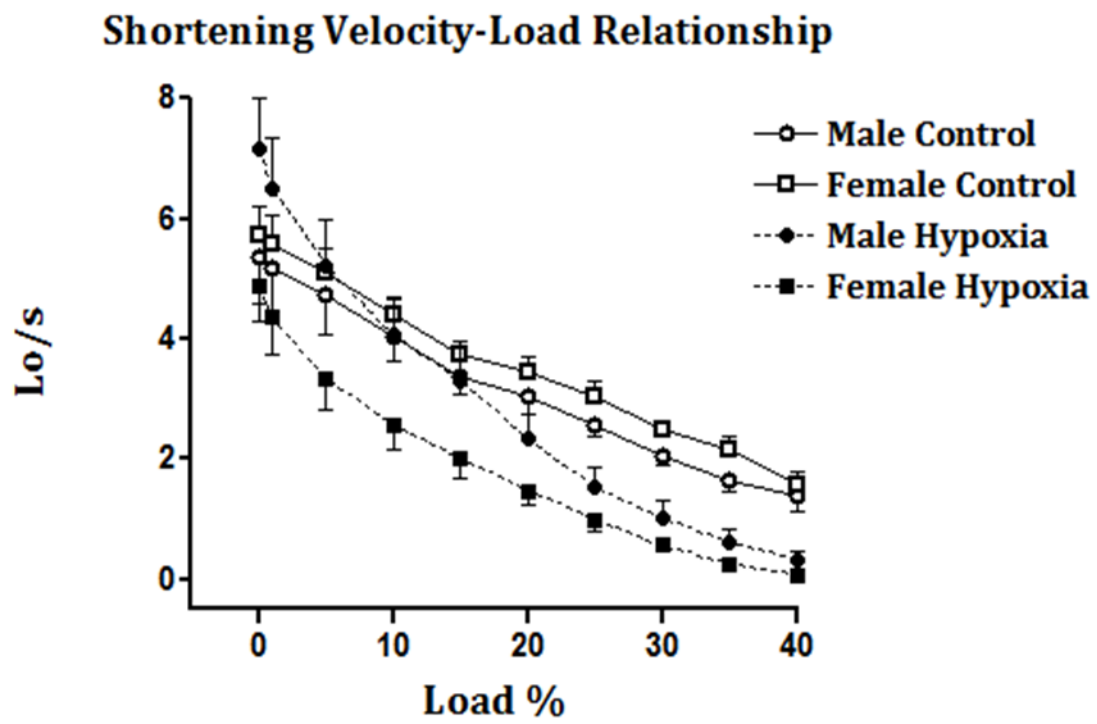**Figure 1: Male and female sternohyoid F*max*, V*max*, W*max* and P*max***

**Figure 2: Male and female sternohyoid muscle shortening velocity-load relationship in control and hypoxia**

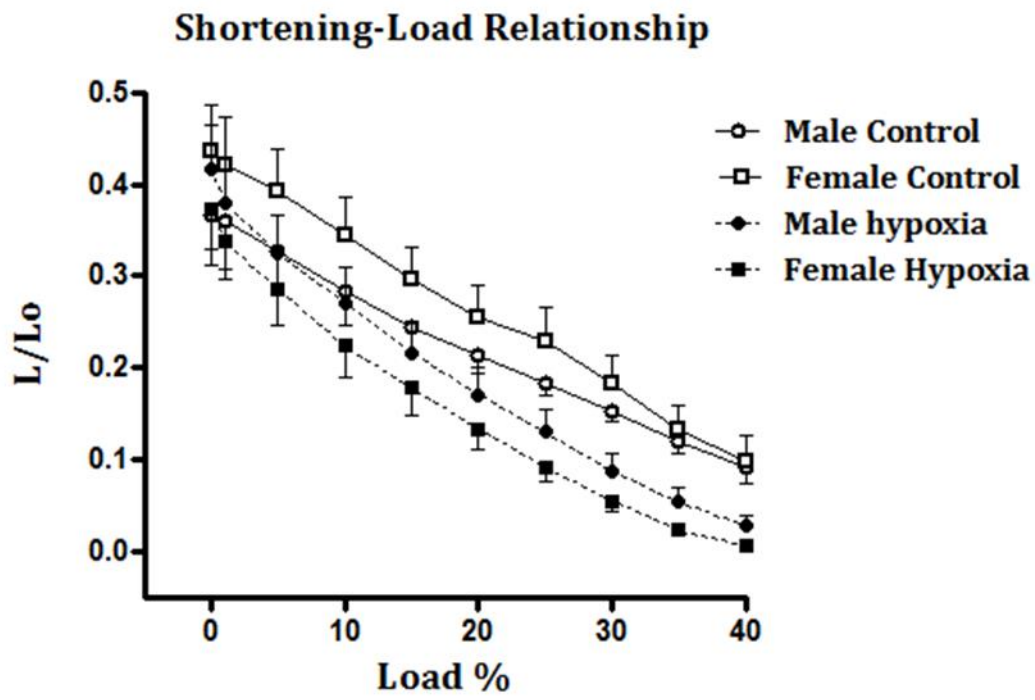**Figure 3: Male and female sternohyoid muscle shortening -load relationship in control and hypoxia**



Shortening-Load Relationship

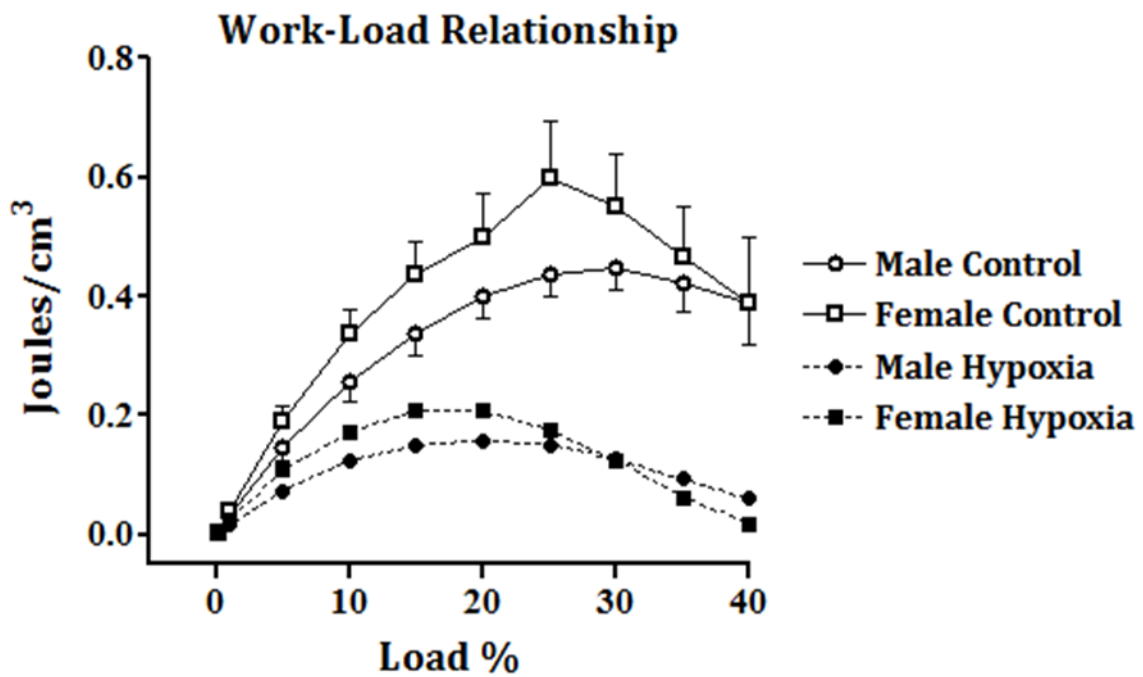**Figure 4: Male and female sternohyoid muscle work-load relationship in control and hypoxia**



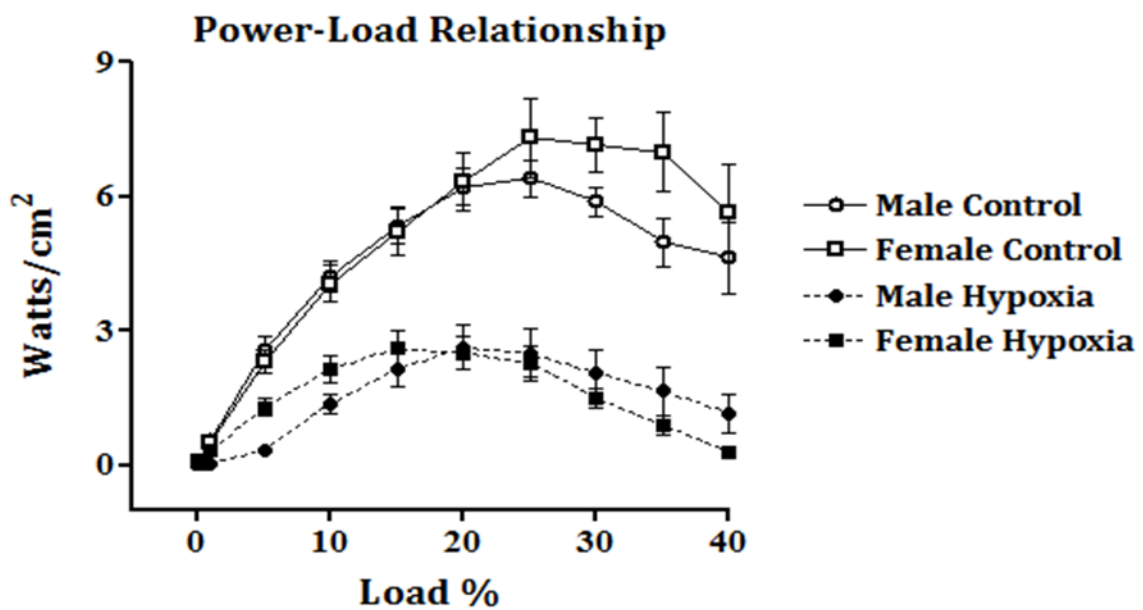**Figure 5: Male and female sternohyoid muscle power-load relationship in control and hypoxia**

**Figure 6: Sternohyoid isotonic fatigue index: male and female sternohyoid muscle power in control and hypoxia after 2mins of repeated stimulation**