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## 44. Respiratory muscle pathophysiology

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**Evidence for early onset of skeletal muscle abnormalities in smoking mice**  
 Harry Gosker<sup>1</sup>, Ramon Langen<sup>1</sup>, Ken Bracke<sup>2</sup>, Guy Joos<sup>2</sup>, Emiel Wouters<sup>1</sup>, Annemie Schols<sup>1</sup>, Brusselle Guy<sup>2</sup>. <sup>1</sup>*Department of Respiratory Medicine, NUTRIM School for Nutrition, Toxicology and Metabolism; Maastricht University, Maastricht, Netherlands;* <sup>2</sup>*Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium*

Skeletal muscle atrophy and loss of muscle oxidative phenotype are typical in COPD progression. A potential role of cigarette smoking in muscular pathogenesis is yet unknown. For this purpose, wild-type male C57Bl/6 mice (n=26) were subjected to cigarette smoke exposure (CSE) or air exposure (AE) for 24 weeks. Animals were sacrificed and soleus (Sol), tibialis (Tib), gastrocnemius (Gas) and plantaris (Pla) muscles were dissected, weighed and homogenized. Oxidative enzyme activities of citrate synthase (CtS),  $\beta$ -hydroxyl-CoA dehydrogenase (HAD) and cytochrome *c* oxidase (COX) were analyzed spectrophotometrically and myosin heavy chain (MyHC) isoforms by gelectrophoresis. In subgroups (N=8 each) airspace enlargement and serum inflammatory profile (Biorad Bio-Plex Cytokine Panel) were measured. Airspace enlargement was found after CSE as compared to AE (41 vs 37  $\mu$ m; p<0.001). Sol tended to be lighter after CSE than AE (9.4 vs 10.0 mg; p=0.052), whereas weights of Tib, Gas, and Pla were similar. Oxidative enzyme activities tended to be consistently lower after CSE than AE including CtS in Sol (88%; p=0.05) and Tib (76%; p=0.036), HAD in Sol (85%; p=0.076) and COX in Gas (75%; p=0.086). MyHCs in Tib, Pla, and Gas were not different. In Sol MyHC IIA was significantly lower and IIB tended to be higher in CSE than in AE. Serum levels of Eotaxin and TNF $\alpha$  were twice as high (p<0.05) after CSE as compared to AE. Our data show that CSE for 24 weeks already induces muscle abnormalities that may contribute to muscular pathogenesis in COPD. *Financial support: Concerted Research Action, Ghent University, Belgium; Netherlands Asthma Foundation award #3.2.05.038 (HG).*

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**Decreased expression of genes involved in skeletal muscle oxidative metabolism in COPD patients with muscle wasting**

Alexander Remels<sup>1</sup>, Harry Gosker<sup>1</sup>, Joaquim Gea<sup>2</sup>, Pawel Sliwinski<sup>3</sup>, Michael Polkey<sup>4</sup>, Juan Galdiz<sup>5</sup>, Annemie Schols<sup>1</sup>. <sup>1</sup>*Department of Respiratory Medicine, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University, Maastricht, Netherlands;* <sup>2</sup>*Muscle and Respiratory System Research Unit, IMIM-Hospital del Mar, Barcelona, Spain;* <sup>3</sup>*Department of Respiratory Medicine, Institute of Tuberculosis and Lung Diseases, Warsaw, Poland;* <sup>4</sup>*Respiratory Muscle Lab, Royal Brompton Hospital, London, United Kingdom;* <sup>5</sup>*Pneumology Department and Research Unit, Cruces Hospital, Vizcaya, Basque Country, Spain*

Skeletal muscle pathology significantly impairs clinical outcome in COPD. Within our European Network ENIGMA we aim to increase insight in the molecular mechanisms underlying skeletal muscle abnormalities in COPD. In the present clinical study we assessed if COPD phenotyping by body composition may also reflect abnormal expression of genes involved in muscle metabolism. Vastus lateralis muscle biopsies were taken from 74 COPD patients across 4 countries (the Netherlands, Spain, UK and Poland). Patients with muscle wasting were identified based on a below normal fat-free mass index (FFMi<15 or FFMi<17; female/male). Real Time QPCR was used to investigate the expression of genes involved in skeletal muscle oxidative capacity. Expression levels of citrate synthase (citric acid cycle),  $\beta$ -hydroxyacyl CoA dehydrogenase (fatty acid oxidation), cytochrome *c* oxidase subunits III (mitochondrial encoded) and IV (nuclear encoded) (electron transport chain) and the regulatory gene mitochondrial transcription factor A were significantly lower, while transcript levels of hexokinase II (glycolysis) were higher

in patients with muscle wasting (n=41) compared to non-wasted patients (n=33) (p<0.05). Lung function (FEV<sub>1</sub>: 37.4±13.0 vs 35.0±13.3% pred) was comparable among the groups. These data show that muscle wasted COPD patients in particular are at risk for a decreased muscle oxidative metabolism. This may point towards involvement of an integrated mechanism in regulation of muscle mass as well as in determination of skeletal muscle oxidative capacity.

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**Neural respiratory drive in patients with COPD**

H.D. Wu<sup>1</sup>, Caroline Jolley<sup>2</sup>, Y.R. Lu<sup>1</sup>, J. Steier<sup>2</sup>, J. Moxham<sup>2</sup>, M.I. Polkey<sup>3</sup>, Y.M. Luo<sup>1</sup>, N.S. Zhong<sup>1</sup>. <sup>1</sup>State Key Laboratory of Respi Dis, Guangzhou Medical College, Guangzhou, China; <sup>2</sup>Respiratory Muscle Lab, King's College Hospital, London, United Kingdom; <sup>3</sup>Respirator Muscle Lab, Royal Brompton Hospital, London, United Kingdom

It was hypothesized that there was neural respiratory inhibition in patients with COPD (Polkey et al. AJRCCM, 959-964 1995). However, few data are available to support or refute the hypothesis because of the lack of reliable methods to assess neural respiratory drive in patients with COPD. To test the hypothesis, 15 patients with moderate-severe COPD were studied. Transdiaphragmatic pressure (Pdi) and the diaphragm EMG were recorded by a combined multipair electrode balloon catheter during treadmill exercise at constant load (80% of maximal oxygen consumption derived from an incremental exercise test). Oxygen consumption was also measured. Root mean square (RMS) of the diaphragm EMG increased initially and reached a plateau. RMS of the diaphragm EMG at the end of exercise was significantly larger than that at the beginning of exercise (171±16 vs 38±16 μV, p <0.001). There was a good relationship between RMS of the diaphragm EMG and oxygen consumption (r=0.92±0.06). Although Pdi increased over the exercise, Pdi at the end of exercise (24.9±15.2) was significantly smaller rather than larger than oesophageal pressure (36.1±16.9) (p<0.01). The ratio of Pdi to RMS of EMG at the beginning of exercise was larger than at the end of exercise (0.27 vs 0.17). We conclude that diaphragm EMG dissociated from Pdi during exercise, probably because dynamic hyperinflation and abdominal muscle activity attenuates pressure generation. Diaphragm EMG provides different data to Pdi in terms of assessment of neural drive. There is a failure of translation of neural drive to pressure generation by the diaphragm in patients with COPD during constant exercise at high workload.

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**Signaling of angiotensin-1 in human skeletal cells: role of NADPH oxidase**

Mahroo Mofarrahi<sup>1</sup>, Frederik Vilhardt<sup>2</sup>, Sabah Hussain<sup>1</sup>. <sup>1</sup>Critical Care and Respiratory Divisions/Department of Medicine, McGill University, Montreal/QC, Canada; <sup>2</sup>Institute of Cellular and Molecular Medicine, Copenhagen University, Copenhagen, Denmark

Angiotensin-1 (Ang-1) is a ligand for endothelial-specific Tie-2 receptors. Ang-1 stimulates skeletal myoblast migration and proliferation and induces specific gene expression profile. In this study, we investigated whether NADPH oxidase-derived oxygen radicals play an important role in Ang-1-induced migration and proliferation of skeletal myoblasts. Immortalized human myoblasts were exposed to PBS or Ang-1 (300 ng/ml) or infected with adenoviruses expressing Ang-1 or GFP. Cells were studied 48 h after viral transduction. Ang-1 (300 ng/ml) elicited a significant rise in O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> production compared with cells exposed to PBS. This production was significantly attenuated by pharmacological inhibitors of NADPH oxidase (DPI and apocynin). Cell migration was evaluated in a monolayer of skeletal myoblasts by measuring the rate of healing of mechanically generated wounds over a 24h-period. The rate of wound healing was significantly greater (75% healing) in cells expressing Ang-1 compared with cells expressing GFP (53% healing). In the presence of N-acetylcysteine and apocynin, the rate of healing induced by Ang-1 was reduced to 12% and 43%, respectively thereby confirming the importance of oxygen radicals in this process. In addition, expression of a dominant negative p47<sup>phox</sup> subunit of NADPH oxidase attenuated the induction of skeletal myoblast migration by Ang-1. Finally, overexpression of p47<sup>phox</sup> protein using lentiviruses elicited a significant increase in cell migration, a response which was inhibited by DPI and apocynin. We conclude that Ang-1 stimulates NADPH oxidase complex to release oxygen radicals, which in turn activate cell migration and that this response is independent of Tie-2 receptors.

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**Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists**

Ioannis Vogiatzis<sup>1</sup>, Dimitris Athanopoulos<sup>1</sup>, Robert Boushel<sup>3</sup>, Jordan Guenette<sup>2</sup>, Maria Koskolou<sup>1</sup>, Harrieth Wagner<sup>4</sup>, Charris Roussos<sup>1</sup>, Peter Wagner<sup>4</sup>, Spyros Zakynthinos<sup>1</sup>. <sup>1</sup>Department of Critical Care Medicine and Pulmonary Services, University of Athens, Athens, Greece; <sup>2</sup>School of Human Kinetics, University of British Columbia, Vancouver, Canada; <sup>3</sup>Department of Exercise Science, Concordia University, Montreal, Canada; <sup>4</sup>Department of Medicine, University of California, San Diego, United States of America

We investigated whether the greater degree of exercise-induced diaphragmatic

fatigue previously reported in highly-trained athletes in hypoxia (compared to normoxia) could have a contribution from limited respiratory muscle blood flow. Seven trained cyclists completed three constant load 5-min exercise tests at inspired O<sub>2</sub> fractions (F<sub>I</sub>O<sub>2</sub>) of 0.13, 0.21 and 1.00 in balanced order. Work rates were selected to produce the same tidal volume, breathing frequency and respiratory muscle load at each F<sub>I</sub>O<sub>2</sub> (63±1, 78±1 and 87± % of normoxic maximal work rate, respectively). Respiratory muscle blood flow (QR) was measured by near-infrared spectroscopy over the left 7<sup>th</sup> intercostal space using indocyanine green. After hypoxic exercise, twitch transdiaphragmatic pressure fell by 33.3±4.8%, significantly (p<0.05) more than after normoxic and hyperoxic exercise (25.6±3.5 and 26.6±3.3% reduction, respectively), confirming greater fatigue in hypoxia. Despite lower power output in hypoxia, neither cardiac output (QT) nor leg blood flow (QL) (27.6±1.2 and 15.9±0.9 l/min, respectively) was significantly different compared to normoxia (28.4±1.9 and 15.7±0.9 l/min) and hyperoxia (27.8±1.6 and 14.7±0.4 l/min). Although a substantial QT-QL difference, >10 l/min, was presumably available for other tissues, QR was surprisingly not different between hypoxia and normoxia (49.4±11.6 and 44.8±7.9 ml/100ml/min, respectively). We conclude that when respiratory muscle load is similar during heavy but submaximal normoxic and hypoxic exercise, respiratory muscle blood flow does not sufficiently increase to possibly lessen the greater diaphragmatic fatigue observed in hypoxia.

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**Damage and expression of TNF receptors in respiratory muscles of COPD patients: temporal sequence**

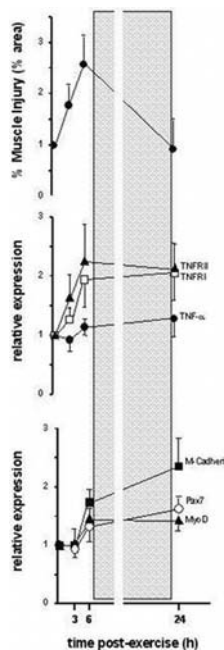
Carme Casadevall, Carlos Coronell, Alba Ramirez-Sarmiento, Mauricio Orozco-Levi, Esther Barreiro, Joaquim Gea. *Respiratory Medicine-URMAR-CEXS, Hospital del Mar-IMM-UPF, CibeRes, Barcelona, Spain*

The role of TNF-α and its receptors (TNFR-I and -II) in muscle repair it's a hot topic in respiratory physiology. We have recently reported that both receptors are overexpressed in respiratory muscles following a ventilatory exercise.

**Aim:** The aim of this study was to clarify the temporal sequence of the expression of these pro-inflammatory markers with regard to the presence of muscle damage.

**Methods:** We included 17 severe COPD patients who underwent an exhaustive ventilatory effort (load equivalent to 40-50% MIP). External intercostal samples were obtained both at baseline and post-exercise (cohorts at 3, 6 and 120 h). Muscle injury was quantified according to Reid's method (MacGowan, AJRCCM 01). Transcriptional expression of relevant pro-inflammatory and repair markers were assessed by real-time RT-PCR.

**Results:** see figure.



TNFRs and repair markers were overexpressed concomitantly with the appearance of muscle damage. However, these high expressions persisted even when abnormal muscle morphology returned to normal values. No changes were observed in transcripts from IL-1 beta, IL-6 and TNF-α.

**Conclusions:** our findings suggest that TNFR signaling could play a relevant role in the repair process subsequent to acute injury in respiratory muscles of COPD patients.

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**Aminophylline increases respiratory muscle activity during hypercapnia in humans**

Masanori Yokoba<sup>1</sup>, Masato Katagiri<sup>1</sup>, Yasuto Nishii<sup>1</sup>, Tomoko Yanaihara<sup>1</sup>, Yasumasa Okada<sup>2</sup>, Noriyuki Masuda<sup>1</sup>, Paul Easton<sup>3</sup>, Tadashi Abe<sup>1</sup>. <sup>1</sup>*Respiratory Medicine, Kitasato University, Sagamihara/Kanagawa, Japan;* <sup>2</sup>*Tsukigase Rehabilitation Center, Medicine, Keio University, Izu/Shizuoka, Japan;* <sup>3</sup>*Critical Care Medicine, University of Calgary, Calgary, AB, Canada*

It has been reported that aminophylline increases respiratory muscle contractility in COPD patients with hypoxic hypercapnia (Chest. 92. 27S-31S). We reported previously that aminophylline increases the hypoxic ventilatory response by increasing neural drive in humans (Respir Physiol Neurobiol. [Epub]). However, the effect of aminophylline in standard clinical dosage on respiratory muscles during hypercapnia is still uncertain. We examined phasic EMG activities of transversus abdominis (TA) and parasternal intercostal (PARA) muscles using fine wire electrodes, in 5 healthy subjects. Before and after aminophylline, we recorded airflow, end-tidal CO<sub>2</sub> (ETCO<sub>2</sub>), and moving averaged electromyogram (EMG) to computer, during resting and CO<sub>2</sub> stimulated breathing elicited by rebreathing 5.8% CO<sub>2</sub> in O<sub>2</sub>. EMG activity was expressed as a percentage of maximum EMG activity recorded for each subject (%EMGmax). The mean serum aminophylline level was 15.4 mg/l. With aminophylline, minute ventilation, tidal volume and respiratory rate all increased significantly, beginning from the lowest levels of hypercapnia. Mean EMG activity of both TA and PARA increased significantly with aminophylline compared to baseline without aminophylline, at matching levels of hypercapnia. Specifically, with aminophylline, TA EMG activity increased significantly during hypercapnia (22% greater than without aminophylline at CO<sub>2</sub>Low (55 Torr), 24% greater at CO<sub>2</sub>Med (65 Torr) and 36% greater at CO<sub>2</sub>Hi (75Torr)). PARA EMG activity showed equivalent significant increases with aminophylline at CO<sub>2</sub>Low and CO<sub>2</sub>Med. We conclude that aminophylline increases both expiratory and inspiratory muscle activities during hypercapnia.

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**Effects of swallowing and inspiratory efforts on pharyngeal cortical control and pharyngeal muscle strength**

Eric Verin<sup>1</sup>, Yacine Ouahchi<sup>2</sup>, Jean-Paul Marie<sup>3</sup>. <sup>1</sup>*Physiology, Rouen University Hospital, Rouen, France;* <sup>2</sup>*EA 3830 GRHV, Rouen University, Rouen, France;* <sup>3</sup>*ENT Department, Rouen University Hospital, Rouen, France*

The pharynx is involved in two antagonist and vital functions, swallowing and ventilation. The aim of this study was to determine how pharyngeal cortical motor organization and pharyngeal muscle strength could be modified by inspiratory or swallowing tasks. Mylohyoid (MH) motor evoked potentials (MEP) and twitch pharyngeal pressure (tPphar) induced by non-focal magnetic stimulation were recorded in nine right-handed healthy volunteers during four experiments spaced by one-week. Focal cortical stimulation were used to perform pharyngeal cortical topographic mapping. Baseline was first evaluated and its reproducibility was assessed one week later. Thereafter, the subjects had to performed swallowing or inspiratory tasks fifteen minutes everyday during one week, in a random order. Non focal cortical stimulations evoked MHMEPs (482±53 µV) and evoked a positive tPphar (10.1±1.4 cmH<sub>2</sub>O). During swallowing and sniff maneuvers there was an increase in MHMEP amplitudes (p<0.05) and an excellent reproducibility between M1 and M2. After one week of the different tasks there was an increase in MHMEP amplitudes (p<0.05) and tPphar (p<0.05) after inspiratory tasks. The changes in topographic mapping was marked by a displacement of the site of maximum response and an increase of MH representation after swallowing practice (p<0.05).

In conclusion, our study demonstrated that in healthy subjects, swallowing or ventilatory specific-tasks modify cortical control of pharyngeal muscles and increased pharyngeal muscle strength. These strategies should now be evaluated in dysphagic patients.