490. Physiological basis of respiratory disease

P4728

Late-breaking abstract: Comparative analysis of cardiopulmonary and clinical responses to six minute walking test and maximal exercising test in obese women

Luciana Di Thommazo-Luporini, Soraia Pilon Jürgensen, Viviane Castello, Camila Negrão Dias, Rafael Luís Luporini, José Carlos Bonjorno-Júnior, Claudio Ricardo de Oliveira, Aparecida Maria Catai, Audrey Borghi-Silva. *Physiotherapy, Federal University of Sao Carlos, São Carlos, SP, Brazil Medicine Department, Federal University of Sao Carlos, Sao Carlos, SP, Brazil Interunits Post Graduate Program in Bioengineering, University of Sao Paulo, Sao Carlos, SP, Brazil*

Background: The six minute walking test on the treadmill (tread6MWT) can be an efficient method to evaluate the functional capacity in obese population comparable to cardiopulmonary exercising test (CPET).
Aims: To compare the cardiopulmonary and subjective responses to tread6MWT and CPET in obese and eutrophic women.
Methods: Fourteen obese women were recruited to obese group (OG) and 15 women to eutrophic group (EG). Both groups performed a CPET and a tread6MWT. Cardiopulmonary variables and dyspnea level were registered. Absolute limits of agreement between cardiopulmonary and subjective responses to CPET and tread6MWT were assessed by Bland–Altman analysis.
Results: OG presented higher oxygen uptake (V'O2), minute ventilation (V'E), and systolic blood pressure (SBP) than EG ($p<0.05$) in both tests. There is a

strong correlation (r=-0.76) between V'O2 and body mass index in the CPET, as well as heart rate (HR) in the peak of both tests (r=0.77) in OG. The dyspnea was higher during CPET than tread6MWT ($p<0.05$) in both groups. It was observed the agreement of both tests to identify relative V'O2, V'E, SBP and HR at the peak of exercise, presenting a mean difference between the tests of: 6.0±5.6 (mL/kg/min), 29.0±16.9 (L/min), 17.5±19.4 (mmHg) and 32.9±19.4 (bpm), respectively [BIAS±SD].
Conclusions: The tread6MWT was able to promote metabolic and cardiopulmonary responses in agreement to the CPET. The tread6MWT seems to be an appropriate method to evaluate the functional limitation in obese women without submitting them to such a significative dyspnea as the CPET does.
Financial support: FAPESP (09/01842-0) and CAPES.

P4729
Late-breaking abstract: Time-dependent effect of acute hypoxia on brain excitability in healthy humans
Samuel Verges[1], Thomas Rupp[1], Marc Jubeau[2], Bernard Wuyam[1], Stéphane Perrey[3], Patrick Levy[1], Guillaume Millet[2]. *[1]HP2 Laboratory, U1042, Joseph Fourier University, INSERM, Grenoble, France; [2]Exercise Physiology Laboratory, Jean Monnet University, Saint-Etienne, France; [3]Movement To Health Laboratory, Montpellier-1 University, Montpellier, France*

Some studies have shown altered cortex excitability in hypoxemic patients suffering from COPD or OSAS. Recently, contradictory results regarding the effect of hypoxia (H) on cortex excitability have been reported in healthy subjects, possibly depending on H exposure duration. We evaluated the effects of 1 and 3 hours H on motor cortex excitability, intracortical inhibition and supraspinal voluntary activation (VA) using transcranial magnetic stimulation (TMS). TMS to the quadriceps cortex area and femoral nerve electrical stimulations were performed in normoxia and H (FiO_2 = 12%) in 10 healthy subjects. Motor-evoked potentials (MEPs at 50-100% maximal voluntary contraction - MVC), recruitment curves (MEPs at 30-100% maximal stimulator power output at 50% MVC), cortical silent periods (CSP) and VA were measured. One hour H did not modify any parameters of brain excitability but reduced VA probably due to the repetition of contractions 1-h apart (98±2% vs. 95±4%; p=0.01). Conversely, 3 h H significantly increased i) MEPs of the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) at all force levels (*e.g.* at 50% MVC, RF: +26±35%, VL: +15±24%, VM: +17±15%) and stimulator power outputs (*e.g.* at 70% maximal power, RF: +17±23%, VL: +18±37%, VM: +8±15%), and ii) CSP at all force levels (*e.g.* at 50% MVC, RF: +23±39%, VL: +27±31%, VM: +24±37%) (all $p<0.05$), but did not modify VA (98±1% vs. 97±2%; p=0.21). These data demonstrate a time-dependent H-induced increase in cortex excitability and intra-cortical inhibition, without changes in VA. The impact of these cortical changes on physical or cognitive performances needs to be elucidated to better understand the effects of hypoxemia in patients.

P4730
Late-breaking abstract: Association between serum surfactant protein D (SP-D) and lung function measurements in self-reported healthy twins
Sofie Lock Johansson[1], Quiha Tan[2], Anders Schlosser[1], Ingrid Titlestad[3], Uffe Holmskov[1], Kirsten Kyvik[4], Grith Sørensen[1]. *[1]Department of Cardiovaskular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark; [2]Department of Epidemiology, University of Southern Denmark, Odense, Denmark; [3]Department of Pulmonary Medicine, Odense University Hospital, Odense, Denmark; [4]Institute of Regional Health Services Research, University of Southern Denmark, Odense, Denmark*

Introduction: Serum SP-D is suggested to serve as a biomarker in various pulmonary diseases, and has been showed negatively correlated to FEV1 in COPD.
Aim: The objective of the present study was to investigate the association between serum SP-D and lung function in normal Danes.
Material and methods: Data of serum SP-D originates from 1,476 self-reported healthy adult twins.
Association between variables were analyzed by using a multiple linear regression model using SP-D as response variable and pre-bronchodilatator FEV1 and FVC as explanatory variables. Intra-pair dependency was taken into account, and data was adjusted for sex, age and BMI.
Results: There was a significant difference in mean serum SP-D levels in smokers with and without obstruction. ($p<0.05$) See table 1.
The association for SP-D with FEV1 and FVC was found to be negative in smokers ($p< 0.001/0.001$), but positive in non-smokers (p:0.003/0.002).

SP-D and FEV1%, divided by smoking and airway obstruction

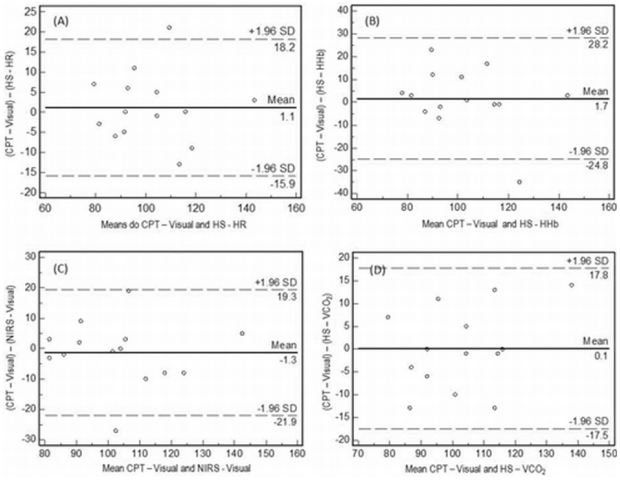
	Non-smokers		Smokers	
	NO (N=911)	WO (N=38)	NO (N=417)	WO (N=48)
SP-D	966±490	1061±529	1347±753	1850±1068
FEV, % pred.	100±12	83±13	96±11	76±11

Means ± standardeviations. NO: FEV1/FVC >0.7, WO: FEV1/FVC <0.7

Conclusion and perspectives: Findings indicate opposite phenotypic correlation between SP-D and FEV1 in smoking and non-smoking individuals. Further analysis of available data will include multivariate twin modelling to investigate whether there is a genetic correlation between the traits and genetic association analysis to find out whether such a genetic correlation could be explained by single nucleotide polymorphisms within candidate genes such as the SFTPD gene.

P4731
Late-breaking abstract: Determination of anaerobic threshold through different methodologies during ramp protocol in elderly healthy men
Michel Reis[1], Ross Arena[2], Danilo Berton[3], Adriano Barroco[3], Aparecida Catai[1], J. Alberto Neder[3], Audrey Borghi-Silva[1]. *[1]Department of Physioterapy, Federal University of Sao Carlos, Sao Carlos, SP, Brazil; [2]Department of Orthopaedics, University of New Mexico, Albuquerque, NM, United States; [3]Department of Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil*

For assessing the cardiopulmonary integration during aerobic exercise, anaerobic threshold (AT) has been an important index of performance. Additionally, the respiratory compensation threshold (RCT) has been useful for determining performance of quasi-maximum intensities (Wasserman et al., 1983). The aim of this study to identify the anaerobic threshold (AT) obtained from the V-slope method, visual inspection of oxihemoglobin (O2Hb) and deoxihemoglobin (HHb) curves and compare findings with the heteroscedastic (HS) method applied to VCO2, heart rate (HR) and HHb data. Fourteen healthy-men were subjected to cardiopulmonary testing (CPX) on a cycle-ergometer until physical exhaustion. Biological signals collected during CPX included: ventilatory variables; spectroscopy by NIRS; and HR by a cardiofrequencymeter. We observed temporal equivalence and similar values of power, VO2 (mL/min), VO2 (mL kg^{-1} min^{-1}) and HR at AT by the detection methods performed. In addition, by the Bland-Altman plot (Fig. 1), HR confirmed the good agreement between the methods with biases between -1.3 and 3.5 bpm.



In conclusion: (i) all detection methods were sensitive in identifying AT, including the HS applied to HR and (ii) the methods showed a good correlation in the identification of AT. Thus the results support the HR seems to be a valid parameter in determining the AT of the individuals in our study (Grants: FAPESP)

P4732
Cytokine expression in the diaphragm of rats breathing against subacute hypoxic conditions
M. Dominguez, S. Pascual, M. Sabaté, F. Sánchez, C. Casadevall, E. Barreiro, J. Gea. *Respiratory Medicine-CEXS, Hospital Mar-IMIM. UPF. CIBERES-ISCIII, Barcelona, Spain*

COPD patients show muscle damage and an increase in the expression of local cytokines in their diaphragms. The paracrine role of these cytokines still remains controversial.
Objective: To analyze the effects of subacute hypoxia on the diaphragm muscle.
Methods: Wistar rats (n=8/group) were exposed to: (1) hypoxia (FIO2 0.10) +placebo, (2) normoxia +placebo, (3) hypoxia +Infliximab [monoclonal antibody that results in the blockade of TNF-α receptors], and (4) normoxia +Infliximab for 2 weeks in all cases. At the end of the study period diaphragm and gastrocnemius muscles as well as blood samples were obtained. Molecular and cellular indices of muscle damage, oxidative stress, cytokine expression and activation of regeneration pathways were obtained using morphometry, Western-blot, spectrophotometry, ELISA, luminometry and RT-PCR.
Results: Although rats exposed to hypoxia showed higher levels of expression of different cytokines (TNF-α, IL-6, INF-g) in their diaphragms than the control animals (normoxia), no differences were observed in muscle damage, oxidative stress and biomarkers of muscle regeneration. Inhibition of TNF-α action in hypoxic animals resulted in an even higher expression of local cytokines with no relevant changes in the other variables when compared with hypoxic animals receiving placebo. No changes were observed in either limb muscle or blood in any of the groups.

Conclusions: Hypoxia induces local inflammation in respiratory muscles of hypoxic rats. This effect appears to be selective for respiratory muscles and can be related to changes in their mechanical loading and its mismatching with the oxygen delivery to the muscle.
Funded: SAF07-62719, CIBERES, SEPAR & SOCAP.

P4733
The impact of aerobic exercise on lung inflammation and remodeling in experimental emphysema
Isabela Guimarães[1], Gisele Padilha[1], Miquéias Lopes-Pacheco[1], Patricia Marques[1], Mariana Antunes[1], Nazareth Rocha[2], Edson Assis[3], Hugo Faria-Neto[3], Raquel Magalhaes[1], Debora Xisto[1], Patricia Rocco[1]. *[1]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Department of Physiology, Fluminense Federal University, Niterói, Brazil; [3]Laboratory of Immunopharmacology, FIOCRUZ, Rio de Janeiro, Brazil*

This study investigated the impact of aerobic exercise on lung inflammation and remodeling in experimental emphysema. 32 BALB/c mice were assigned into 2 groups. In control (C) animals, saline was intratracheally (*it*) injected, whereas emphysema mice received porcine pancreatic elastase (ELA, 0.1 UI, *it*). Saline and ELA were *it* injected once a wk during 4 wks. After the last wk, C and emphysema groups were further randomized into subgroups: sedentary and exercise. Exercise mice ran on a motorized treadmill, at moderate intensity (8-12 $m.min^{-1}$), 5% grade, 30 min/day, 3 times a wk for 4 wks. 24-h after the last session, lung mechanics and morphometry, as well as cytokines and total cell count in bronchoalveolar lavage fluid (BALF) and blood were measured. Echocardiographic analysis was done before and after emphysema induction and at the end of the experiment. The sedentary emphysema group presented, compared to C: 1) reduced lung static elastance; 2) increased lung hyperinflation and elastic fiber content; 3) augmented levels of KC [murine interleukin (IL)-8 homolog], tumor necrosis factor-α, interferon-γ, and IL-10; and 4) pulmonary arterial hypertension, evidenced by increased pulmonary flow acceleration. Aerobic exercise: 1) improved lung mechanics, 2) reduced lung hyperinflation, and the number of cells and levels of these cytokines in BALF and blood, 3) diminished elastic fiber content, and 4) restored pulmonary flow acceleration to C values. In conclusion, in the present elastase-induced emphysema model, 4 weeks of aerobic exercise modulated the inflammatory process and acted on lung remodeling, improving pulmonary function.
Supported by: INCT-INOFAR, FAPERJ, PRONEX, CNPq

P4734
Regular and moderate exercise prevents airway remodeling in a murine model of chronic allergic asthma
Cynthia Samary[1], Carla Araujo[1], Isabela Guimaraes[1], Johnatas Silva[1], Adriana Silva[1], Mariana Antunes[1], Patricia Marques[1], Bruno Dias[2], Ilka Abreu[2], Nazareth Rocha[3], Vera Capelozzi[4], Patricia Rocco[1]. *[1]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Laboratory of Inflammation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Department of Physiology, Fluminense Federal University, Niterói, Brazil; [4]Department of Pathology, University of Sao Paulo, Sao Paulo, Brazil*

The present study investigated whether regular and moderate aerobic exercise might prevent airway remodeling in experimental chronic allergic asthma. For this purpose, 48 BALB/c mice were assigned into 2 groups: sedentary (S) and trained (Tr). Tr group ran on a motorized treadmill, at moderate intensity (8-12 $m.min^{-1}$), 5% grade, 30 min/day, 3 times a week for 8 wks. At 8 wks, animals were further randomized into 2 subgroups to be immunized and challenged with ovalbumin (OVA) or to receive saline using the same protocol (C). Aerobic exercise continued until the end of the protocol. Echocardiographic analysis was done before, at 4 and 8 weeks of training, and after asthma induction. Twenty-four hours after the last challenge, trained, compared to sedentary mice, presented: 1) an increase in systolic output, left ventricular mass, and end-diastolic volume; 2) a reduction in airway resistance, viscoelastic pressure, static elastance, eosinophil infiltration, smooth-muscle actin expression, and collagen fiber content in airways and lung parenchyma; 3) a decrease of transforming growth factor-β levels in bronchoalveolar lavage fluid (BALF) and blood; 4) an increase in interferon-γ in BALF and blood; 5) an augment of interleukin (IL)-10 in blood but a reduction in BALF; and 6) a decrease in IL-5 and IL-13 only in BALF. In conclusion, regular and moderate aerobic exercise was effective in preventing airway and lung parenchyma remodeling in the present murine model of chronic allergic asthma, improving lung function.
Supported by: INCT-INOFAR, CNPq, PRONEX, FAPERJ, CAPES

P4735
Repeated mannitol or NaCl hyperosmolar exposure of bronchial epithelial cells to mimic exercise-induced airways damage
Laura Chimenti[1,2], Roberta Santagata[2], Vincenzo Bellia[1], Ramon Farrè[1], Maria R. Bonsignore[2]. *[1]Unity of Biophysics and Bioengineering, Faculty of Medicine, University of Barcelona, Barcelona, Spain; [2]DIBIMIS, Section of Pneumology, University of Palermo, Palermo, Italy*

Hyperosmolarity of the airway surface lining fluid might be involved in exercise-induced airway epithelial damage. Hyperosmolarity causes release of interleukin 8 (IL-8) by bronchial epithelial cells (BEC) in vitro, but the effects of repeated hyperosmolar exposure on BEC are unknown. 16HBE cells were exposed to NaCl or Mannitol (Mann) (320, 640, 960, 1280 mOsm/kg H2O) in culture medium for 10 or 40 min for 3 consecutive days; at 24 h after each exposure, supernatants were collected and stored at -80 C for subsequent IL-8 measurements (R&D System, UK). Cell viability was examined by MTT assay. JNK phosphorylation (pJNK) was assessed by Western Blot. Repeated exposure to NaCl or Mann for 10 min at any concentration did not affect IL-8 release. Exposure of 16-HBE cells to NaCl or Mann at 640-1280 mOsm/kg H2O for 40 min increased IL-8 concentration at days 1 and 2 compared to untreated cells ($p<0.0001$); however, IL-8 release decreased at days 2-3 compared to day 1 ($p<0.05$). Repeated NaCl or Mann treatment for 10 min decreased cell viability by 10-20% ($p<0.0001$), while hyperosmolar exposure for 40 min decreased cell viability in an dose-dependent manner at day 1 and dramatically at days 2-3 (-60% for NaCl, -40% for Mann at highest concentrations, $p<0.0001$). NaCl was cytotoxic compared to Mann ($p<0.05$). PJNK expresssion increased dose-dependently with hyperosmolarity at days 1-2 but decreased at day 3; IL-8 release was blocked by a specific JNK inhibitor (SP600125). Therefore, hyperosmolar exposure acutely activates BEC through JNK activation, whereas prolonged hyperosmolar exposures repeated for 3 days decrease IL-8 release likely due to major epithelial damage.

P4736
Acute exposure to mechanical forces deteriorates lung structure and function in a mouse model of emphysema
Margit Szabari[1,2], Susumu Sato[3], Harikrishnan Parameswaran[1], Elizabeth Bartolák-Suki[1], Zoltán Hantos[2], Béla Suki[1]. *[1]Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary; [2]Biomedical Engineering Department, Boston University, Boston, United States; [3]Department of Pulmonary Medicine, Kyoto University Hospital, Kyoto, Japan*

Mechanical forces have been suggested to accelerate the deterioration of lung structure and function in emphysema. To test this, we used C57BL/6 mice treated with porcine pancreatic elastase (N=48) or left intact as controls (N=16). At 2, 7 or 21 days after treatment, mice were ventilated (Vt=8 ml/kg, 240/min) for 1 h with or without deep inspirations (DI, inflation to 35 hPa airway pressure twice/min). FRC was measured in a plethysmograph and tissue elastance (H) was calculated from respiratory impedance. After the experiment, lungs were fixed at 20 hPa pressure and sections were stained with hematoxylin-eosin or a modified Verhoeff method to visualize elastin. Independent of time, DI increased FRC ($p<0.001$) and independent of DI, FRC increased with time ($p<0.001$). Compared to control, H decreased in the no-DI 7- and 21-day groups ($p<0.001$, $p=0.009$). Compared to no-DI, H decreased in the DI groups ($p<0.001$). DI had a significant effect on FRC and H in controls. Alveoli were grouped as small, medium and large airspaces according to their equivalent diameters (D). DI reduced D of the small airspaces in the 21-day group ($p<0.001$) suggesting that as the DIs rupture a septal wall, many small airspaces surrounding the newly formed larger airspace reduce their size. The coefficient of variation of elastin increased with time in treated groups ($p<0001$) reflecting heterogeneous remodeling, but this was not affected by DIs. Our results suggest that acute mechanical forces rupture septal walls with a subsequent increase in FRC and a decrease in small airspace diameters in a time dependent manner during disease progression.
Supported by grants NIH HL090757 and OTKA 66700.

P4737
Aerobic exercise training attenuates the decrease in heart rate variability induced by exposure to cigarette smoke in mice
Alessandra C. Toledo[1], Josemara Brito[2], Renato M. Magalhães[1], Agostinho C. Caleman-Neto[1], Bernardo Camilo[1], Francine Almeida[1], Petra Mello[1], Clarice R. Olivo[1], Fernanda D.T.Q.S. Lopes[1], Chin J. Lin[2], Paulo H.N. Saldiva[2], Mílton A. Martins[1]. *[1]Internal Medicine, School of Medicine of University of Sao Paulo, Sao Paulo, Brazil; [2]Pathology, School of Medicine of University of Sao Paulo, Sao Paulo, Brazil*

Smoking has been shown to influence the tone of the autonomic nervous system as reflected by heart rate variability (HRV) a predictor of increased cardiac risk and aerobic exercise (AE) training has been described as capable to modulate the HRV.
Objective: This study evaluated the temporal effects of aerobic exercise in the HRV in mice exposed to cigarette smoke (CS).
Methods: C57Bl6 mice were divided in 4 groups: Control, Smoke, Exercise and Smoke/Exercise. Smoke groups were exposed to CS for 30min/day (twice), 5days/week for 12 weeks. Exercise groups were trained at moderate intensity for 60min/day, 5days/week for 12 weeks. HRV was measured at baseline and 2, 4, 6, 8, 10 and 12 weeks after the last CS exposure and/or AE session. HRV was measured by a cuff (noninvasive tool) applied to the base of the mouse tail coupled to PowerLab system. The following parameters were used: heart rate (HR), HRV for time domain (standart deviation of normal beats [SDNN] and root mean square of successive differences in the heart beat interval [RMSSD]) and frequency domain (low frequency [LF], high frequency [HF] and LF/HF ratio).
Results: Exposure to CS decreased SDNN and RMSSD values after 6 weeks ($p<0.001$, compared to control group) where it remained until 10 weeks and AE training reverted this effect. Exposure to CS also decreased HF only after 6 weeks ($p<0.01$) compared to control group and this effect was reverted to AE training.

Conclusion: Our results suggest that AE training at moderate intensity have beneficial effects on cardiac autonomic nervous function, a clinically relevant predictor of cardiovascular morbidity and mortality, in mice exposed to cigarette smoke.

P4738

Site of ROS production by mitochondria of skeletal muscle of patients with COPD

Luis Puente-Maestu[1], Javier de Miguel[1], Luis Álvarez-Sala[2], Alberto Tejedor[3], Alberto Lázaro[3], Elena Ojeda[1], Teresa Gómez[1], Alvar Agustí[4]. *[1]Neumología, Hospital Gregorio Marañón/Universidad Complutense de Madrid, Madrid, Spain; [2]Medicina Interna, Hospital Gregorio Marañón/Universidad Complutense de Madrid, Madrid, Spain; [3]Nefrología Experimental, Hospital Gregorio Marañón/Universidad Complutense de Madrid, Madrid, Spain; [4]Neumología, Servicio Neumologia (Hospital Clínic de Barcelona), Fundación Caubet-Cimera and CIBER Enfermedades Respiratorias (CIBERES), Barcelona, Cataluña, Spain*

Rationale: Exercise-induced oxidative stress is well documented in patients with chronic obstructive pulmonary disease (COPD).
Objective: To study the specific site(s) of reactive oxygen species (ROS) production in the *Vastus Lateralis* (VL) of COPD patients.
Methods: VL biopsies were obtained during lung cancer surgery in 11 COPD (67±7 yrs; FEV_1 = 54.1±12pp) and 10 controls (66±10 yrs; FEV_1 = 92±13pp). Mitochondrial respiration ($V'O_2m$) and ROS output before and after inhibition with rotenone (complex-I), malonate (complex-II) and antimycin-A (complex-III) were determined in mitochondria and submitochondrial fragments.
Results: $V'O_2m$ were 2.8±0.7 vs 2.7±0.5 mmol min^{-1} kg^{-1} (n.s) and respiratory control rate 7.2±1.5 vs 6.1±1.5; p=0.097 (controls/COPD). H_2O_2 outputs by mitochondria oxidizing complex-I substrates were 51±6 and 150±35 pmol h^{-1} mg^{-1} (p<0.001) respectively or 0.8% and 2.1% of the $V'O_2m$. While antimycin-A greatly increased (×7) H_2O_2 output both in mitochondria and submitochondrial particles in both groups, rotenone only did it in submitochondrial particles. In mitochondria it instead decreased H_2O_2 output as it did malonate, both upstream blockers of the electron flux to complex-III.
Conclusions: Only the ROS generated at complex-III are secreted by the mitochondria. We interpret that this is due to the spatial orientation of complex-I (towards the matrix) and III towards de intermembrane space), being in the first case largely neutralized by the antioxidant system of the mitochondrial matrix.

P4739

Role of systemic and muscle oxidative stress and cytokines in patients with lung cancer cachexia

Esther Barreiro, Pilar Ausin, Francisco Sanchez, Ester Puig-Vilanova, Juana Martinez-Llorens, Victor Curull, Joan Broquetas, Joaquim Gea. *Pulmonology Dpt-URMAR, IMIM-H.Mar,CIBERES, UPF, UAB, PRBB, Barcelona, Spain*

Oxidative stress and proinflammatory cytokines are involved in muscle mass loss in several conditions such as COPD and cancer cachexia.
Objectives: To explore redox balance, inflammation, proteolysis, signaling pathways and muscle structure in the vastus lateralis of patients with lung cancer cachexia.
Methods: Molecular markers of oxidative stress and proteolysis, proinflammatory cytokines, signaling pathways, and muscle fiber types, size, and damage were assessed in the vastus lateralis and blood of patients with lung cancer with and without cachexia, patients with COPD and cachexia, and healthy volunteers (controls). All patients and control subjects were clinically evaluated (body composition and exercise capacity).
Results: Compared to controls, COPD patients and patients with lung cancer exhibited exercise limitation and muscle dysfunction, increased muscle oxidative stress and activation of proteolytic pathways. Cachexia in both COPD and lung cancer patients, compared to healthy controls, also induced greater levels of muscle oxidative stress, proteolysis, damage and atrophy, along with increased systemic oxidative stress, and worsened peripheral muscle dysfunction and exercise tolerance.
Conclusions: Within peripheral muscles, lung cancer induced functional, structural, and molecular alterations, which were significantly worsened when cachexia developed. These results may offer potential therapeutic targets for the management of COPD and cancer associated cachexia.
Funded by: SEPAR 2007, MTV3-07-1010, 2009-SGR-393, FIS 05/2458 & CIBERES.

P4740

Iron deficiency as a novel biomarker of functional impairment in patients with chronic obstructive pulmonary disease (COPD)

Diego A. Rodriguez[1], Josep Roca[2], Alba Ramirez-Sarmiento[3], Isabel Blanco[2], Joaquim Gea[3], Anael Barberan[2], Yolanda Torralba[2], Ane Arbillaga[2], Joan Albert Barbera[2], Jordi Vilaro[4], Mauricio Orozco-Levi[3]. *[1]Respiratory, Hospital Clínic i Provincial de Barcelona (ICT), CIBERES, IDIBAPS; Universidad de Barcelona and Grupo de Investigación en Lesión, Respuesta Inmune y Función Pulmonar, IMIM-Hospital del Mar; CEXS-Univers; [2]Respiratory, Hospital Clínic i Provincial de Barcelona (ICT), CIBERES, IDIBAPS; Universidad de Barcelona, Barcelona, Spain;[3]Respiratory, Grupo de Investigación en Lesión, Respuesta Inmune y Función Pulmonar, IMIM-Hospital del Mar; CEXS-Universidad Pompeu Fabra, Barcelona, Spain; [4]Respiratory, Hospital Clínic i Provincial de Barcelona (ICT), CIBERES, IDIBAPS; Universidad de Barcelona and Facultad de Ciencias de la Salud Blanquerna, Universidad Ramol Llull, Barcelona, Spain*

Introduction: The role of iron deficiency (IrDe) on exercise intolerance has not been explored in patients with COPD. We hypothesized that IrDe could represent a potentially treatable factor in the functional impairment of COPD patients.
Methods: We evaluated 80 COPD patients (FEV1 43±15% pred; PaO2 71±11 mmHg; BMI 27±4 kg/m^2; Charlson Index 2.5±1.2; 18% current smokers) without anemia. IrDe was defined as a ferritin level <100 μg/L, or a ferritin level between 100-299 μg/L, and a transferrin saturation <20%. We measured both cycloergometer endurance time (ET) and six minute walking distance (6mWD) to evaluate associations between training responses and IrDe.
Results: Forty percent (n=32) of the patients showed IrDe. Iron deficiency did not show association with demographic or pulmonary function variables. But, patients with IrDe showed lower ET (226±25 vs. 362±21 sec, p<0,001) and lower 6MWD (81±5 vs. 100±6%pred., p<0.01) than those without iron deficiency. Accordingly, IrDe was associated with increased the risk of decreased ET (OR: 4.16; IC: 1.48-11.7; p = 0.007), and decreased 6MWD (OR de 3.82; IC: 1.22-8.81; p=0.018). Moreover, endurance training response assessed by ET was lower in IrDe patients (Δ265±64 vs. Δ459±82 sec) than in the COPD without such deficiency.
Conclusions: IrDr shows a high prevalence in COPD patients and discloses a significant association with aerobic capacity. Evaluation of the potential benefit of a specific treatment of IrDe in COPD appears to be warranted.

P4741

Predictors for developing hypoxic respiratory failure in COPD – A 3-year follow-up

Eirunn Waatevik Saure[1], Tomas M.L. Eagan[2], Robert L. Jensen[3], Per S. Bakke[1], Marianne Voll-Aanerud[2], Einar Thorsen[1], Jon A. Hardie[1]. *[1]Institute of Medicine, University of Bergen, Bergen, Norway; [2]Department of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway; [3]Internal Medicine, Pulmonary Division, University of Utah, Salt Lake City, UT, United States*

Background: The risk of developing respiratory failure rises with increasing severity of COPD. Still, it is unknown which clinical and pulmonary function measurements are associated with subsequent development of hypoxic respiratory failure.
Methods: 401 subjects from the Bergen COPD Cohort Study, aged 40-75 years, GOLD stage II-IV, underwent repeated clinical and pulmonary function measurements including arterial blood gases over 3 years. Sex, age, smoking, and baseline measures of FEV1, RV/TLC-ratio, PaO2, PaCO2, and Fat Free Mass Index (FFMI) were analyzed as possible predictors for developing hypoxemia. We used both bivariate and multivariate Cox proportional hazards analyses, with the time from baseline normoxemia until the first event of hypoxemia (PaO2<8kPa) as a measure of event free time. 73 (18%) of the 401 patients were hypoxemic at baseline and excluded from the analyses.
Results: Within the three years of follow-up, a total of 46 patients (14%) developed hypoxemia. In bivariate Cox proportional hazards analyses, baseline FEV1, RV/TLC, FFMI, PaO2, and PaCO2 were significantly associated with developing hypoxemia. After multivariate Cox proportional hazards analyses, the following measures remained significantly associated with developing hypoxemia:

Variable	HR (95% CI)	p
RV/TLC ratio (%)	1.014 (1.003–1.025)	0.012
PaO2 (kPa)	0.220 (0.129–0.375)	0.000
FFMI (kg/m^2)	0.907 (0.830–0.992)	0.032

Conclusion: High RV/TLC-ratio, low PaO2, and low fat free mass index were predictors for developing hypoxemic respiratory failure in a 3 years follow-up of COPD patients.

P4742

Plasma vitamin D concentration and clinical characteristics in clinically stable patients with moderate COPD

Elisabeth Romme[1], Lidwien Graat-Verboom[1], Erica Rutten[2], Frank Smeenk[1], Martijn Spruit[2], Emiel Wouters[3]. *[1]Respiratory Medicine, Catharina Hospital, Eindhoven, Netherlands; [2]Program Development Centre, Centre of Expertise for Chronic Organ Failure, Horn, Netherlands; [3]Respiratory Medicine, Maastricht University Medical Center, Maastricht, Netherlands*

Introduction: Vitamin D deficiency is associated with poor functional performance in patients with COPD. However, most research is limited to patients with severe COPD, whilst vitamin D deficiency also occurs in GOLD stage II patients (Janssens et al. Thorax 2010). Therefore, we aimed to investigate differences in clinical characteristics in clinically stable GOLD stage II patients with and without vitamin D deficiency.
Methods: 159 patients with COPD GOLD stage II (age 69±9 yrs; FEV_1 65±9% pred; 58% men) recruited at the outpatient consultation office of the Catharina Hospital were included. Vitamin D deficiency was defined as plasma 25(OH)D concentration <50 nmol/L. BMI, FEV_1, RV/TLC, arterial pO_2, MRC dyspnea,

six-minute walk distance (6MWD) and score on the BODE index were assessed. The number of sunshine hours 2 months before blood sampling was determined using data from the Royal Netherlands Meteorological Institute.
Results: Vitamin D deficient patients had higher RV/TLC, lower pO_2, worse 6MWD, higher BODE score and less sunshine hours.

	Vit D deficient, N=77	Normal vit D, N=82
Men, %	56	61
Age, y	69±9	69±9
BMI, kg/m^2	27±6	27±4
FEV1, % pred	64±9	66±9
RV/TLC, % pred	126±16	120±18*
pO2, mmHg	77±10	82±11*
Modified MRC	0.6±0.9	0.5±0.8
6MWD, m	316±117	364±105*
BODE, points	2.2±1.8	1.5±1.3*
Sunshine hours, hr	127±65	161±55*

*p≤0.05.

After correction for confounders, vitamin D deficiency was associated with lower pO_2, higher BODE score and less sunshine hours (all p≤0.03).
Conclusions: Vitamin D deficiency is common in COPD GOLD stage II patients. Moreover, patients with vitamin D deficiency were characterized by lower arterial pO_2, worse exercise performance and higher BODE score.

P4743

Effects of sildenafil and the association of sildenafil-sixtasentan on the NO/CO transfer factor in low landers exposed to high altitude

Jean-Benoît Martinot[1], Claire de Bisschop[2], Cristina Scoditti[3], Chantal Dedobbeleer[4], Maria Overbeek[5], Robert. Naeije[4], Hervé Guenard[6].
[1]Pneumology, Ste. Elisabeth, Namur, Belgium; [2]Physiologic Adaptations to Physical Activities, Poitiers University, Poitiers, France; [3]Pneumology, University of Bari, Bari, Italy; [4]Cardiology, Erasme University Hospital, Brussels, Belgium; [5]Pneumology, VU University Medical Center, Amsterdam, Netherlands; [6]Department of Physiopathology and Cardiology, Erasme University Hospital, Brussels, Belgium; [7]Laboratory of Physiology, University of Bordeaux, Bordeaux, France

Little is known about the CO/NO lung transfer in low landers (LL) exposed to hypoxic environment without previous acclimatisation. The acute expected rise of the arterial pulmonary pressure (sPAP) can modify the capillary lung volume (Vc) and the conductance for CO (DmCO). We studied the effects of sildenafil and the association sildenafil-sixtasentan on the lung diffusion in 26 LL subjects transported to Cerro de Pasco (4300 m) in 2 or 3 days. sPAP was acquired using a Cx50 echocardiographic system. The tests were done at arrival and repeated 5 days later under a placebo, double blind randomised trial. The data are corrected for haemoglobin concentration.
Results:

Variables (units)	Sea level	Altitude Day 2 & 3	Placebo	Sildenafil	Sildenafil + Sixtasentan
DmCO/VA	102.3 (9.8)	107.9 (9.7)*	106,8 (20,9)	104,6 (20,5)	109,8 (21,5)
Vc corr/VA	100.1 (12.3)	131.8 (20.3)*	115,7 (22,7)	111,8 (21,9)	120 (23,5)

Data are presented as mean (sd). VA: alveolar volume. No statistically significant difference at 5% between groups was observed UNDER TRIAL. *Statistically difference at 5% vs SEA.

sPAP increased between sea level and altitude from a mean (± sem) of 23.1 (3.9) to 32 (7.6) mmHg without significant differences in cardiac index and mitral E/Ea ratio.
Conclusion: Acute exposure to high altitude leads to a recruitment of lung capillaries associated to the expansion of the exchange surface (DmCO/VA) and to a 48% increase in sPAP. Acclimatisation is accompanied by a spontaneous decrease of Vc after one week. The arterial pulmonary vasodilators had no effect on Vc, suggesting that the mean capillary lung pressure remained constant.

P4744

Exercise pathophysiology in patients with chronic mountain sickness

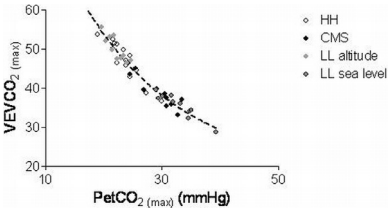
Herman Groepenhoff[1], Mart Van der Plas[2], Sophia Beloka[3], Vitalie Faoro[3], Marieke Overbeek[1], Fransico Villafuerte[4], Robert Naeije[5]. *[1]Pulmonolgy, VU University Medical Center, Amsterdam, Netherlands; [2]Pulmonolgy, OLVG, Amsterdam, Netherlands; [3]Kinesiology, Free University, Erasme, Brussels, Belgium; [4]Physiology, University, Lima, Peru; [5]Physiology, Free University, Erasme, Brussels, Belgium*

Introduction: Patients with chronic mountain sickness (CMS) are characterized by excessive erythrocytosis, severe hypoxemia and occasionally pulmonary hypertension, that may lead to exercise limitation. However, cardio-pulmonary exercise test (CPET) parameters in this patient group have not been established yet.
Methods: 12 CMS, 14 healthy high- (HH) and 10 lowlanders (LL) were included to perform a maximal CPET. CMS and HH performed their test at 4350m above sea level (Cerro de Pasco, Peru). LL also underwent CPET at sea level.

Results: CMS compared to HH and LL at sea level reached the same VO2max but decreased SpO2. However CMS had increased haemoglobin (HB). VE/VCO2 slope was decreased in CMS compared to HH and LL at altitude but not different from sea level LL (table 1). Figure 1 shows that the ventilatory response to exercise, was decreased in CMS compared to HH and LL at altitude ($p<0.001$) but not different from sea level LL.

	LL sea level	LL	HH	CMS
VO2max, ml/kg	39±8$^{\#}$	31±7	32±7	32±5
SpO2max, %	98±2$^{\#*\$}$	78±7*	88±3$^{\$}$	81±5
HB, gr/dl	14±2$^{\#*\$}$	16±2$^{\$}$	18±2$^{\$}$	24±2
VE/VCO2slope	34±4$^{\#*}$	50±4$^{\$}$	47±5$^{\$}$	39±5

$p<0.05$: $^{\#}$vs LL; *vs HH; $^{\$}$ vs CMS.



Conclusion: Patients with CMS have a preserved aerobic capacity with a ventilatory response identical to LL at sea level but blunted ventilatory adaptation compared to HH and LL at altitude likely explained by preserved oxygen delivery because of increased hemoglobine. This study was supported by a grant from Pfizer