IOC68. *Mitochondrial Physiology Network* 17.08: 1-12 (2012)
Version 4: 2013-10-08

68th International Workshop on HRR

2012 April 11-16
Schröcken, Vorarlberg, Austria

The **68th Workshop on High-Resolution Respirometry (HRR)** is the **27th** International Oxygraph Course held in Schröcken since 1988. The workshop includes experiments with biological samples, providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab**, applications of the **TIP2k**, the new **O2k-Fluorescence LED2-Module**, and perspectives of HRR in mitochondrial physiology. Parallel to four O2k-Basic groups, a TPP-Basic group will focus on **O2k-MultiSensor** applications for measurement of mt-membrane potential, Ca^{2+}, and acidification rate (pH).

An international team of experienced tutors guides small working groups step-by-step through the approach of HRR. Five O2k (10 chambers) are available for do-it-yourself applications of both hardware and software. Combined with an introduction and demo experiments, it is best to put the O2k into action yourself.

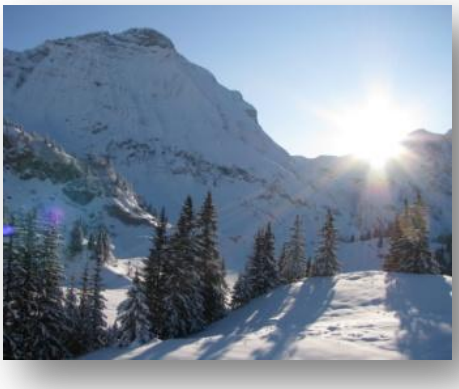
Lunch breaks provide an opportunity for skiing, relaxing walks and talks, to enjoy the refreshing scenery of the secluded alpine environment, or use the spare time for specific tutorials. With DatLab 5 (new) we accomplish data analysis on-line, providing final results and their graphical presentation by the end of an experimental run. Thus we gain sufficient time to see the Titration-Injection microPump TIP2k with feedback-control in action and practice its simple and automatic operation.

Lecturers and Tutors

Erich Gnaiger
Mario Fasching
Andrea Eigentler
Mona Fontana-Ayoub
Meissner Barbara

Guest tutor

Carolina Doerrier Velasco, Univ Granada, ES

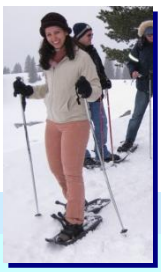


Programme IOC68

1 Wednesday, April 11

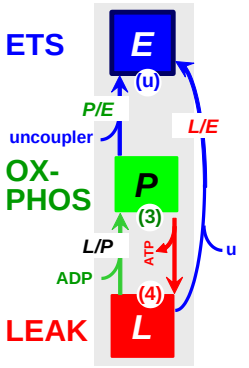
	Arrival	
15:00	**Arrival in Bregenz: Meeting point Bregenz train station at 3:00 pm; approx. 1 hour bus drive to Schröcken and Hochtannberg (Salober). Transfer/walk to Hotel Körbersee**	
18:30	*Welcome reception at Hotel Körbersee.*	
19:00	*Dinner*	
21:00	**Get-together: Introduction of participants and their research interests**	

2 Thursday, April 12

	Workshop 1	*Weblink*
07:30-08:30	*Breakfast*	
	Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result	Gnaiger 2008 POS
08:00	*Organize loan of skiing equipment*	
08:30-09:00	**Erich Gnaiger: Get O2k-Connected with your OROBOROS-USB: a guided tour to the O2k**	get O2k-Connected
09:00-10:00	**Instrumental quality control 1: The oxygen sensor OROBoPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.**	O2k-Calibration
10:00	Organize special lunch or *bad weather* task groups (fibre and homogenate prep, fluorometry and spectrophotometry, NO, pH and Ca, Bioblast wiki login) and *loan of skiing equipment*	
11:00	*Practice - skiing, walk & talk, or* individual O2k-tasks / *Lunch*	
14:00	*Coffee / Tea*	
14:30-16:00	**O2k-Demo experiment 1: Respiration of intact cells and on-line DatLab Analysis: Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (add-on O2k-Fluorescence LED2-Module)**	O2k-Fluorometry Workshop
16:00	*Coffee / Tea*	
16:30-17:15	**DatLab Guide through the menues: DL-Demo files and DL-Excel templates**	DatLab 4 Guide
17:15-18:00	**Mario Fasching: O2k-MultiSensor overview**	
18:30	*Dinner*	
O2k-Basic 20:00-21:00	**DatLab O_2 flux analysis: Flux per volume, flux per mass, flow per cell, flux control ratio**	DatLab4 Flux Analysis
TPP-Basic 20:00-21:00	**Join Mario Fasching for the parallel introductory potentiometric O2k-MultiSensor group with ion selective electrodes (ISE) Hands-on: Assembly and maintenance of TPP and reference electrode**	

3 Friday, April 13

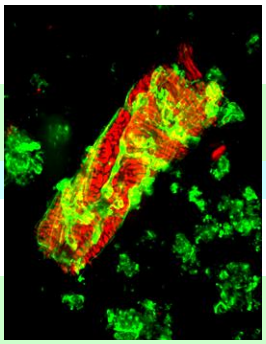
	Workshop 2		Weblink
07:30	*Breakfast*		
O2k-Basic	**O2k instrumental setup**	**OROBoPOS service**	O2k-Start
08:30-09:15	Groups A and B	Groups C and D	POS Service
09:15-10:00	Groups C and D	Groups A and B	
TPP-Basic 08:30-10:00	**TPP-Basic: Hands on: Set up of the instrument with TPP and reference electrodes**		O2k-MultiSensor-Manual
10:00	*Practice - skiing, walk & talk, or* individual O2k-tasks / *Lunch*		
14:00	*Coffee / Tea*		
O2k-Basic 14:30-16:00	**Instrumental quality control 2: O2k-Background test and on-line analysis of oxygen flux.**		O2k-Background
TPP-Basic 14:30-16:00	**TPP-Basic: Introduction in Planning and Performing an TPP experiment; Hands on: Instrumental background oxygen flux in the presence of the TPP electrode**		O2k-TPP and Ca ISE-Module
16:00	*Coffee / Tea*		
O2k-Basic 16:30-18:00	**Hands-on (4 groups): O2k-Background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 - 200 µM. A and B: O2k-Background with automatic TIP2k titration. C and D: O2k-Background with manual titrations.**		O2k-Background
TPP-Basic 16:30-18:00	**TPP-Basic: Hands on: TPP calibration and TPP chemical background**		
18:30	*Dinner*		
20:00-21:00	**Hot MiP-Topics: 10+5 min presentations of abstracts by participants**		



4 Saturday, April 14

	Workshop 3		Weblink
O2k-Basic 08:30-09:30	**Experimental design 1: Coupling control of mitochondrial respiration: LEAK, OXPHOS, ETS, ROX**		Glossary: Respiratory states - Bioblast
O2k-Basic 09:30-10:00	**Experimental design 2: Coupling Control Protocol with intact cells: ROUTINE, LEAK, ETS, ROX**		Cells: PCP
TPP-Basic 08:30-10:00	**From the TPP^+ signal to mitochondrial membrane potential - Guide through the Excel templates.**		
10:00	*Practice - skiing, walk & talk, or* individual O2k-tasks / *Lunch*		
14:00	*Coffee / Tea*		
O2k-Basic 14:30-16.00	**O2k-Demo experiment 2: SUIT protocol with myocardial homogenate and on-line DatLab analysis**		Pesta 2012 Methods Mol Biol
TPP-Basic 14:30-16:00	**Hands-on: TPP calibration and extending the TPP method for the determination of mitochondrial membrane potential beyond isolated mitochondria: TPP calibration and experiment with biological sample**		
16:00	*Coffee / Tea*		

O2k-Basic 16:30-17:30	Hands-on: SUIT experiment continued with DatLab Analysis and guide through Excel templates	DatLab4 Flux Analysis
O2k-Basic 17:30-18:00	Carolina Doerrier and Andrea Eigentler: Mitochondrial respiration in permeabilized fibres versus homogenate from mouse myocardium. Application study with the PBI-Shredder	PBI-Shredder
TPP-Basic 16:30-18:00	Hands-on: continued, data evaluation	
18:30	*Dinner*	
O2k-Basic 20:00-21:00	Experimental design 3: Substrate and coupling control of mitochondrial respiration.	
TPP-Basic 20:00-21:00	Hands-on: continued	

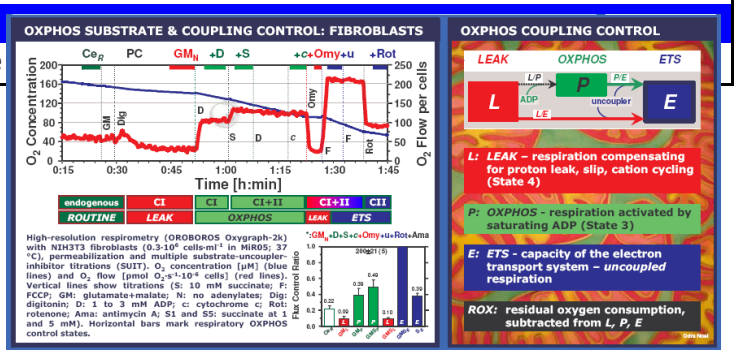


5 Sunday, April 15

	Workshop 4	Weblink
07:30	*Breakfast*	
O2k-Basic 8:45-09:30	Working groups: Elaborate answers to the "Questions for the O2k-Course"	IOC-Questions
9:30-10:15	IOC-Questions - discussion of "Answers"	IOC-Questions
TPP-Basic 08:45-10:15	Final analysis of TPP experiment - discussion	
10:15	*Coffee / Tea*	
10:45-11:45	O2k-Demo experiment 3: Respiration and steady-state feedback control of oxygen levels with the TIP2k.	TIP2k Manual
11:45-12:00	Barbara Meissner: The O2k-Workshop continues with the Bioblast wiki - MiPNet Reference Laboratories, O2k-Publications	www.bioblast.at
12:00	*Lunch*	
13:00-13:45	Mario Fasching: Introduction into trouble shooting	
14:00	*Snowshoe Walk (rental of snowshoes) to the Alpmuseum: Guided tour and reception: 15 €*	www.alpmuseum.at
17:30	*Coffee / Tea*	
18:00-19:00	Erich Gnaiger: MitoPathways through Complexes I+II - perspectives of comparative mitochondrial physiology	Gnaiger 2009 Int J Biochem Cell Biol
19:00	*Dinner*	
20:30-21:00	Panel Discussion - Feedback IOC68	O2k-Feedback
	Farewell party	

6 Monday, April 16

	Departure
	Early morning: Departure



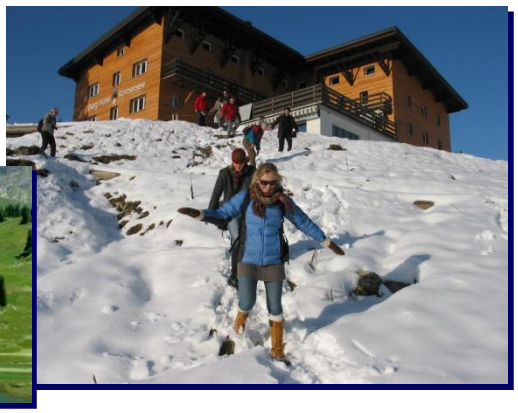
IOC68-Participants

Name	Lab - Fields of interest
Albertini Eva	**AT_Innsbruck_Jansen Duerr P -** Institute for Biomedical Aging Research, Austrian Academy Sciences. - FAHD1, senescence, aging, Mus musculus, C. elegans
Arbo Ingrid	**NO_Trondheim_Rognmo O -** Norwegian Univ Science and Technology. - Mitochondrial function, exercise training, disease
Bakkerud Fredrik	**NO_Trondheim_Rognmo O -** NTNU, Medical Faculty ISB. - Mitochondrial function, exercise training, disease
Barth Sonja	**AT_Graz_Graier W -** Dept Med Biochem Med Molec Biol, Univ Graz. - Calcium, endocannabinods
Bouitbir Jamal (Hot topics IOC68-03)	**CH_Basel_Kraehenbuehl S -** Universitätsspital Basel, Dept Biomedizin. - ROS, statins, mitochondrial biogenesis, antioxidant capacity. ***TPP-Group*** (cancelled)
Cannon Daniel T. (Hot topics IOC68-02)	**US_CA Torrance_Rossiter HB -** Inst Membrane Systems Biol, Fac Biol Sci, Univ Leeds, UK. - Exercise training, skeletal muscle, primary pulmonary arterial hypertension
Distefano Giovanna (Hot topics IOC68-01)	**US_PA Pittsburgh_Goodpaster B -** Univ Pittsburgh, Div Endocrinol Metabolism, Dept Medicine, Cellular Rehabilitation Lab. - Aging, exercise, skeletal muscle, mitochondrial respiration
Doerrier Velasco Carolina A. (tutor)	**ES_Granada_Acuna Castroviejo D -** Ctr Investig Bioméd, Parque Tecnol Ciencias de la Salud, Univ Granada.
Eigentler Andrea (tutor)	**AT_Innsbruck_Gnaiger E -** DSL, Dept Visc, Transplant Thorac Surgery, Med Univ Innsbruck
Esfandiary Azadeh (Hot topics IOC68-05)	**DE_Giessen_Weissmann N -** Justus-Liebig-Univ Giessen, Med Klinik II, Innere Medizin. - Mitochondria, cardiovascular diseases. ***TPP-Group***
Fasching Mario (organizer, tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS** - ***TPP-Group***
Felser Andrea	**CH_Basel_Kraehenbuehl S -** Universitätsspital Basel, Dept Biomed. - Mitochondria, ROS, apoptosis. ***TPP-Group***
Fontana-Ayoub Mona (tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**
Gnaiger Erich (organizer, tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**; DSL, Dept Visceral, Transpl Thoracic Surgery, Medical Univ Innsbruck
Jespersen Nichlas Riise	**DK_Aarhus_Jespersen NR -** Univ Hospital Skejby. - Ischemia-reperfusion injury, respiratory capacity, ROS production
Johannsen Darcy	**US_LA Baton Rouge_Noland RC -** Pennington Biomed Res Ctr. - Respiration, human skeletal muscle, metabolism, chronic disease, obesity, type 2 diabetes
Kapitsinou Pinelopi	**US_TN Nashville_Kapitsinou P -** Vanderbilt Univ Med Ctr. - Hypoxia, signaling, endothelial cell bioenergetics
Kluckova Katarina	**CZ_Prague_Neuzil J -** Lab Molec Therapy, Inst Biotechnol, Acad Sci Czech Republic. - Cancer, inhibition, CI, CII
Lund Jim (Hot topics IOC68-04)	**NO_Tromso_Larsen TS -** Cardiovasc Research Group, UiT, Univ Tromso. - Exercise, obesity, heart
Meers Grace	**US_MO Columbia_Rector RS -** VA Hospital. - Mitochondrial function, NAFLD
Meissner Barbara (tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**

Noland Robert C.	**US_LA Baton Rouge_Noland RC -** Pennington Biomed Res Ctr. - Insulin resistance, diabetes, lipid and glucose metabolism, mitochondria, peroxisome, oxidation, oxygen consumption, membrane potential, ATP, oxidative stress
Ojuka Edward	**ZA_Cape Town_Ojuka E -** ESSM UCT Dept Human Biol Sports Sci, Inst South Africa Newlands, Univ Cape Town. - Nuclear respiratory factors
Olek Robert	**PL_Gdansk_Kaczor JJ -** Jedrzej Sniadecki Univ, School Phys Education Sport, Biochem Dpt. - Hypercaloric diets, mitochondrial function
Palladino Michael J.	**US_PA Pittsburgh_Palladino MJ -** Dpt Med-Pharmacol Chem Biol, Pittsburgh Inst Neurodegenerative Diseases (PIND), Univ Pittsburgh. - PC3, Drosophila
Peyta Laure	**FR_Tours_Dumas JF -** Inserm U1069 - N2C. - Hepatocytes, oxidative phosphorylation, ROS, membrane potential
Podramägi Taavi	**EE_Tartu_Seppet EK -** Univ Tartu. - ***TPP-Group***
Rognmo Oivind	**NO_Trondheim_Rognmo O -** Fac Med, ISB, Norwegian Univ Sci Technol. - ***TPP-Group***
Schmitz Lysann	**DE_Cologne_Larsson NG -** Max Planck Inst Biol Ageing. - OXPHOS dysfunction
Stottrup Nicolaj B.	**DK_Aarhus_Jespersen NR -** Univ Hospital Skejby. - Ischemia-reperfusion injury, respiratory capacity, ROS production
Uusijärvi Johan	**SE_Stockholm_Weitzberg E -** Karolinska Hospital, Dept Pharmacol Physiol. - Cyanide, hydroxicobalamine, sodiumthiosulfate
Woodlief Tracey	**US_PA Pittsburgh_Goodpaster B -** Univ Pittsburgh, Div Endocrinol Metabolism, Montefiore Hospital. - Lipid induced ROS production
Ying Li	**SG_Singapore_Summer S -** Dept Cardiovasc Metabolism Diseases, Duke-NUS Medical School. - Metabolic diseases, cancer, neuroscience
Ziolkowski Wieslaw	**PL_Gdansk_Kaczor JJ -** Jedrzej Sniadecki Univ, Sch Physical Education Sport, Biochem Dpt. - Exercise, skeletal and cardiac mitochondria, dystrophy. ***TPP-Group***

Accommodation and Location

Hotel Körbersee www.koerbersee.at
T +43 5519 265; hotel@koerbersee.at



MiPNet Abstracts –
Hot topics in Mitochondrial Physiology: pp 9-12

Online: http://www.bioblast.at/index.php/IOC68 Abstracts MiPNet17.08
Continue the discussion: http://www.bioblast.at/index.php/Talk:IOC68

New in the O2k-Manual [MiPNet 17.05]

As innovation within our *open innovation* approach, the 'Manual for the O2k-Fluorescence LED2-Module' evolves as a guided tour through the Bioblast wiki

- **O2k-Catalogue: O2k-Fluorescence LED2-Module**

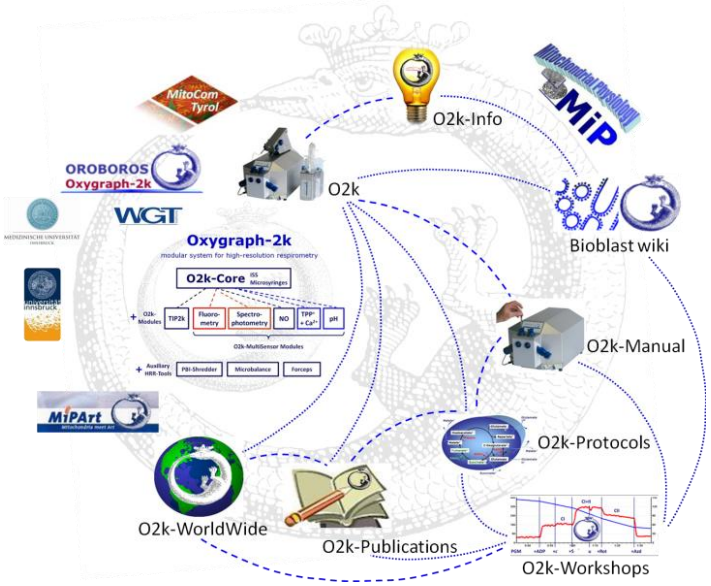
Respiratory states and flux control ratios [MiPNet12.15]

Coupling control states

- *E* Electron transfer system (ETS)
- *L* LEAK
- *P* OXPHOS (mt-preparations)
- *R* ROUTINE (intact cells)

Coupling control ratios (*CCR*)

- *L/E* LEAK *CCR*
- *P/E* Phosphorylation system *CCR*
- *R/E* ROUTINE *CCR*



Further information

O2k-Manual – www.oroboros.at/?O2k-Manual
O2k-Protocols – www.oroboros.at/?O2k-Protocols
O2k-Publications – www.oroboros.at/?O2k-Publications

Reading

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopisies of human muscle. Methods Mol Biol 810: 25-58.

Lemieux H, Semsroth S, Antretter H, Hoefer D, Gnaiger E (2011) Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart. Int J Biochem Cell Biol 43: 1729–1738.

Pesta D, Hoppel F, Macek C, Messner H, Faulhaber M, Kobel C, Parson W, Burtscher M, Schocke M, Gnaiger E (2011) Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. Am J Physiol Regul Integr Comp Physiol 301: R1078–R1087.

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int J Biochem Cell Biol* 41: 1837–1845.

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial Dysfunction in

Drug-Induced Toxicity (Dykens JA, Will Y, eds) John Wiley: 327-352. – *A methodological introduction into high-resolution respirometry.*

Hütter E, Renner K, Pfister G, Stöckl P, Jansen-Dürr P, Gnaiger E (2004) Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. Biochem J 380: 919-928.

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir Physiol* 128: 277-297. – *A detailed introduction into high-resolution respirometry with particular emphasis on kinetics and measurements at low oxygen.*

Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold* (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: 431-442. – *Isolated mitochondria and permeabilized muscle fibres, MiR05.*

Acknowledgements

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www.oroboros.at/?MitoCom-Tyrol

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Gentle Science Shapes the World – http://wiki.oroboros.at/index.php/Gentle_Science

MiPNet Abstracts IOC68: 10+5 min

Hot topics in Mitochondrial Physiology

Online: http://www.bioblast.at/index.php/IOC68 Abstracts MiPNet17.08
Continue the discussion: http://www.bioblast.at/index.php/Talk:IOC68

IOC68-01 Distefano G, Dube JJ, Helbling NL, Ritov VB, Stefanovic-Racic M, Toledo FGS, Ng J, Goodpaster B, Coen P (2012) Human skeletal muscle mitochondria respiration: The influence of aging, adiposity and aerobic fitness. MiPNet17.08.

Background: Aging is associated with reductions in skeletal muscle mitochondria function as evidenced by a decreased capacity for ATP production and mitochondrial protein content [1,2,3]. Aging is also associated with changes in body composition, including increased adiposity, and a loss of aerobic fitness. Both are factors that confound an examination of the relationship between mitochondrial function and aging per se. The objective of this study was to determine whether the respiratory properties of permeabilized skeletal muscle fibers are altered with chronological age, or more related to age associated changes in adiposity and aerobic fitness.

Methods: A total of 63 participants were assigned to one of the following groups: Young (Y, 26.9 ± 0.9 yrs, *n*=30), Middle-aged (M, 41.2 ± 2.4 yrs, *n*=13), or Elderly (77.7 ± 1.1 yrs, *n*=20). Following an overnight fast, a percutaneous muscle biopsy of vastus lateralis was obtained. Maximal coupled (St.*P*), maximal non-coupled (St.*E*), and LEAK state (St.*L*) respiration was determined in saponin permeabilized muscle fiber bundles using high-resolution respirometry. V_{O2peak} was determined by a graded exercise test. Total body fat and fat free mass were assessed by whole body DEXA.

Results: The Y group had significantly greater levels of St.*P* respiration (220 ± 15 pmol O_2 $s^{-1}mg^{-1}$) compared to M (166 ± 13 pmol O_2 $s^{-1}mg^{-1}$, $P = 0.02$) and O groups (170 ± 13 pmol O_2 $s^{-1}mg^{-1}$, $P = 0.014$). There was no difference in St.*P* respiration between M and O groups. Similar group differences were also observed for St.*E* and St.*L* respiration. The Y group exhibited a higher V_{O2peak} (46 ± 2.9 ml $min^{-1}kg^{-1}$) compared to M (28 ± 1.8 ml $min^{-1}kg^{-1}$, $P<0.01$) and O (21 ± 2.2 ml $min^{-1}kg^{-1}$, $P<0.01$) groups. When the three groups were combined, St.*P* respiration was positively correlated with V_{O2peak} ($R = 0.631$, $P<0.01$), and negatively correlated with age ($R = -0.324$, $P = 0.01$), BMI ($R = -0.371$, $P<0.01$), fasting glucose ($R = -0.252$, $P = 0.047$), and fat mass ($R = -0.516$, $P = <0.01$).

Conclusions: Our data suggest that age related changes in body composition and aerobic fitness may be more important to mitochondrial dysfunction than chronological age per se.

1. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI (2003) Mitochondrial dysfunction in the elderly: Possible role in insulin resistance. Science 300: 1140-1142.
2. Conley KE, Jubrias SA, Esselman PC (2000) Oxidative capacity and ageing in human muscle. J Physiol 526: 203-210. 3. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102: 5618-5623.

Keywords: Mitochondrial respiration, Permeabilized fibers, Skeletal muscle, Aging
MiPNetLab: US PA Pittsburgh Goodpaster B

IOC68-02 Cannon DT, Oudiz R, Casaburi R, Rossiter HB (2012) The effects of exercise training on skeletal muscle mitochondrial function in patients with primary pulmonary arterial hypertension. MiPNet17.08.

Primary pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary circulation resulting in increased pulmonary vascular resistance, right ventricular hypertrophy and right ventricular failure. PAH results in poor quality of life due to dyspnoea and exercise intolerance, and unsurprisingly, an increased mortality risk. Therefore, therapies to improve muscle metabolic function and exercise tolerance are crucial to preserve quality of life and patient prognosis. Numerous studies support the safety and efficacy of exercise training in patients with COPD, and early concerns about accelerating the rate of decline in patient health have mostly not been substantiated. Evidence for benefits with rehabilitative exercise training in PAH have recently been reported (Mereles et al 2006). While limited data are available for exercise training in PAH, none have examined mitochondrial function after exercise training. Recently, our group have demonstrated reduced maximum respiratory rate and Complex I dysfunction using high-resolution respirometry in an animal model of PAH (Wuest et al 2012). However, whether this mitochondrial dysfunction is reversible through exercise training or pharmacologic intervention (Piao et al 2010) remains to be determined.

Therefore, our study aims to exploit a multi-disciplinary exercise and education intervention study of PAH. This investigation will explore the efficacy of exercise training in mild and severe PAH. Control groups of mild and severe patients will receive health education and exercise training in a cross-over design. In conjunction with traditional measures of cardiorespiratory fitness, pulmonary function, muscle oxygenation during exercise, cardiac MR imaging, and quality-of-life assessment, knee-extensor muscle biopsies will be taken for measurement of mitochondrial function. This is to be done using a substrate-uncoupler-inhibitor-titration protocol of high-resolution respirometry. Additionally, biochemical analyses for respiratory enzymes and mitochondrial protein expression will be made.

1. Mereles D, Ehlken N, Kreuscher S, Ghofrani S, Hoeper MM, Halank M, Meyer FJ, Karger G, Buss J, Juenger J, Holzapfel N, Opitz C, Winkler J, Herth FF, Wilkens H, Katus HA, Olschewski H, Grunig E (2006) Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. Circulation 114: 1482-1489.
2. Piao L, Marsboom G, Archer SL (2010) Mitochondrial metabolic adaptation in right ventricular hypertrophy and failure. J Mol Med 88: 1011-1020.
3. Wuest RC, Myers DS, Stones R, Benoist D, Robinson PA, Boyle JP, Peers C, White E, Rossiter HB (2012) Regional skeletal muscle remodeling and mitochondrial dysfunction in right ventricular heart failure. Am J Physiol Heart Circ Physiol 302: H402-H411.

Keywords: Exercise training, Respiration, Permeabilised fibres, Skeletal muscle
MiPNetLab: UK Leeds Peers C

IOC68-03 Bouitbir J, Charles A-L, Echaniz-Laguna A, Kindo M, Daussin F, Auwerx J, Piquard F, Geny B, Zoll J (2012) Effects of statins on mitochondrial function in cardiac and skeletal muscles. MiPNet17.08.

Statins protect against cardiovascular-related mortality but induce skeletal muscle toxicity [1]. To investigate mechanisms of statins, we tested the hypothesis that statins optimized cardiac mitochondrial function but impaired vulnerable skeletal muscle by inducing different level of reactive oxygen species (ROS). In atrium of patients treated with statins, ROS production was decreased and oxidative capacities were enhanced together with an extensive augmentation of mRNAs expression of PGC-1 family. However, in deltoid biopsies from patients with statin-induced muscular myopathy, oxidative capacities were decreased together with ROS increase and a collapse of PGC-1 mRNA

expression. Several animal and cell culture experiments were conducted and showed by using ROS scavengers that ROS production was the triggering factor responsible of atorvastatin-induced activation of mitochondrial biogenesis pathway and improvement of antioxidant capacities. Conversely, in skeletal muscle, the large augmentation of ROS production following treatment induced mitochondrial impairments, and reduced mitochondrial biogenesis mechanisms. Quercetin, an antioxidant molecule, was able to counteract skeletal muscle deleterious effects of atorvastatin in rat [2]. Our findings identify statins as a new activating factor of cardiac mitochondrial biogenesis and antioxidant capacities, and suggest the importance of ROS/PGC-1 signalling pathway as a key element in regulation of mitochondrial function in cardiac as well as skeletal muscles.

1. Bouitbir J, Charles AL, Rasseneur L, Dufour S, Piquard F, Geny B, Zoll J (2011) Atorvastatin treatment reduces exercise capacities in rats: involvement of mitochondrial impairments and oxidative stress. J Appl Physiol 111: 1477-1483.
2. Bouitbir J, Charles AL, Echaniz-Laguna A, Kindo M, Daussin F, Auwerx J, Piquard F, Geny B, Zoll J (2011) Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a "mitohormesis" mechanism involving reactive oxygen species and PGC-1. Eur Heart J (in press).

Keywords: Statins, ROS, Permeabilized fibres, Skeletal muscle, Cardiac muscle
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IOC68-04 Lund J, Hafstad AD, Hagve M, Larsen TS, Aasum E (2012) High intensity exercise prevents impairment of respiratory capacity in cardiac mitochondria from obese mice. MiPNet17.08.

Cardiac inefficiency and increased myocardial oxygen consumption are hallmarks of diabetes-induced cardiomyopathy, and is most likely related to ROS generation and mitochondrial dysfunction. We have recently shown that exercise prevent development of cardiac dysfunction and inefficiency. In the present study we investigated the effect of high intensity training (HIT) on mitochondrial respiration in cardiac mitochondria from obese mice. Because long chain fatty acids are supposed to increase LEAK oxygen consumption, experiments were performed both in the absence and presence of albumin-bound palmitate.

Methods: Diet-induced obese (DIO) mice were obtained by feeding C57BL/6J mice a high fat diet for 8 weeks. DIO mice were thereafter subjected to either a sedentary lifestyle (SED) or HIT (interval running, 10x4 min at 85-95% of V_{O2max}) for 8-10 weeks. Sedentary mice fed normal chow were included as lean controls (CON). Mitochondrial oxygen consumption was measured in isolated myocardial mitochondria (Oxygraph-2k, Oroboros Instruments) with glutamate and malate as substrates (5 and 2.5 mM, respectively). ADP (300 µM) was added to achieve maximal mitochondrial OXPHOS capacity (*P*). Mitochondrial LEAK oxygen consumption (*L*) was recorded after depletion of ADP and addition of oligomycin (4 µg/mL). In order to estimate the mitochondrial proton leak through the adenine nucleotide translocator (ANT), atractyloside (25 µM) was also added. The protocol was performed in the presence and absence of 75 µM palmitate.

Results: *P* was reduced and *L* was increased in cardiac mitochondria from DIO mice. HIT was found to normalize *P* and to cause an increase in *L*. Palmitate was found to increase *L* in all groups, although the latter response was less pronounced in mitochondria from HIT mice. Both basal and palmitate-dependent mitochondrial uncoupling was reduced by ANT inhibition.

Conclusion: Diet-induced obesity is associated with a reduction in cardiac mitochondrial OXPHOS capacity, while mitochondrial uncoupling was unaltered. Exercise training restored respiratory capacity and increased uncoupling in isolated cardiac mitochondria from obese mice. Exercise was also found to reduce the long chain fatty acid-induced uncoupling.

Keywords: Exercise training, Respiration, Isolated mitochondria, Heart muscle, Obesity
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IOC68-05 Esfandiary A, Pak O, Weissmann N, Sommer N (2012) Mitochondrial respiration in experimental right heart hypertrophy. MiPNet17.08.

Right heart failure is the leading cause of death in diseases which induce increased right heart afterload (e.g. pulmonary hypertension). Right heart hypertrophy can compensate for increased afterload, before right heart failure develops. Increased right heart afterload and right heart hypertrophy may induce mitochondrial alterations that determine right heart performance. This study aims to investigate the role of mitochondria in right heart hypertrophy. Right heart hypertrophy was induced in C57Bl6/J wild type mice by pulmonary artery banding (PAB). Control mice received sham surgery. Three weeks later, the development of right heart hypertrophy was evaluated by measurement of right ventricular systolic pressure (RVP) and weight of the right ventricle. Mitochondrial respiration of freshly prepared heart biopsies from the right and left ventricles was quantified by high-resolution respirometry. We used a substrate-inhibitor protocol with pyruvate, ADP, malate, octanoylcarnitine, rotenone, succinate and antimycin A. PAB caused a significant increase in RVP and right ventricular weight in comparison to sham operated animals. Systolic arterial pressure and the ratio of the weight of the left ventricle to body weight were not significantly changed. We could not detect significant differences in respiration of the right ventricular biopsies after PAB compared to sham operation. Thus, respiration was not altered in the right ventricle during right ventricular hypertrophy induced by PAB. Further studies need to be performed to evaluate the role of mitochondrial membrane potential, ROS and other metabolic pathways.

Keywords: Right heart hypertrophy, Right ventricular failure, Cardiac afterload, Heart ratio, Mitochondria, Respiration, PAB (pulmonary artery banding), RVP (right ventricular pressure)

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