

## Session 2: Integrated Cardiovascular and Mitochondrial Physiology.

### II. Oxygen Delivery, Hypoxia, and Altitude



#### **2-01. Measuring and modelling the effects of altered oxygen delivery on muscle metabolism.**

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I consider four main issues, based as far as possible on quantitative analysis of published data in terms of models of mitochondrial function and regulation *in vivo*.

(A) *Methodological issues in measurement of O<sub>2</sub> delivery and O<sub>2</sub> usage in vivo:* There are several ways to study muscle 'metabolism' (by which I mean ATP turnover in the service of force generation) *in vivo*. First, for oxidative metabolism, there are whole body V<sub>O<sub>2</sub></sub> measurements, and (less convenient) invasive arteriovenous difference (AVD) measurements of muscle O<sub>2</sub> consumption [5]. Second, <sup>31</sup>P magnetic resonance spectroscopy (MRS) can measure ATP production by oxidative and nonoxidative means (the latter including glycolysis to lactate and adjustments of transient ATP demand/supply imbalance by changes in phosphocreatine (PCr) concentration) [1,10,11]. There is a tradition of assessing 'mitochondrial capacity' (inferred maximal rate of oxidative ATP synthesis *in vivo*) by analysing relationships between oxidative ATP synthesis rate and the concentrations of metabolites of presumed regulatory relevance (e.g. ADP) or their correlates (e.g. PCr) [11,13,16]. Third, near-infrared spectroscopy (NIRS) and <sup>1</sup>H MRS of deoxymyoglobin can be used to report tissue PO<sub>2</sub>. NIRS estimates of muscle O<sub>2</sub> content can be used to measure rates of O<sub>2</sub> usage [22], and to make semi-quantitative inferences about vascular O<sub>2</sub> supply abnormalities [11,12]. Recently NIRS has been suggested as a measure of O<sub>2</sub> AVD, and in combination with V<sub>O<sub>2</sub></sub> has been used to estimate the kinetics of blood flow [4]. (I argue that this is not a useful calculation, for algebraic reasons, and because in the data analysed [4] blood flow is always tightly coupled to O<sub>2</sub> use). Interpretation of NIRS data [12] is still hampered by disagreement about whether the signal is mainly from capillary deoxyhaemoglobin or myocyte deoxymyoglobin; if the latter [21], then like <sup>1</sup>H MRS of deoxymyoglobin [19], NIRS usefully reports *p*<sub>O<sub>2</sub></sub> in the vicinity of the mitochondrion (see (D) below). Fourth, <sup>13</sup>C MRS labelling methods can be used to measure TCA cycle flux *in vivo* [9], although current estimates of basal oxidative ATP synthesis by this method are substantially too high, for technical reasons. The same is true of a fifth technique, <sup>31</sup>P MR saturation transfer, which can be used with <sup>13</sup>C MRS to assess mitochondrial coupling *in vivo* [17].

(B) *Understanding oxidative ATP synthesis rates in relation to metabolite concentrations:* None of these methods tell us all we want to know, but some key measurements are available. I want to ask to what extent we understand these, a question with several aspects. First, can we fit our measurements of rates and metabolite concentrations into a model consistent with known biochemistry and physiology, both classical, and the newer insights of Metabolic Control Analysis (MCA)? Probably yes, to some extent. Secondly, how much explanatory power do such models have? Does e.g. measured [ADP] predict the rate of mitochondrial O<sub>2</sub> consumption? In some circumstances, yes e.g. within a single experiment. However, there is substantial

variation between published human studies in inferred maximal rates and relationships between ATP synthesis rates and e.g. [ADP]. Some of this is due to disagreement about resting [PCr] measured by  $^{31}\text{P}$  MRS (necessary for calculation of [ADP]), and some is no doubt methodological in other ways, but what remains might be interesting physiology. In addition to longstanding disagreements about candidate force-flow relationships, it is argued (in conformity with MCA [3]) that 'parallel-activation' or 'feed-forward' influences are important in control of mitochondrial ATP synthesis *in vivo* [14], detectable in  $^{31}\text{P}$  MRS experiments as a lack of correlation between fluxes and concentrations, and in particular as flux changes 'too large' for the changes in e.g. [ADP] [14]. This conclusion can be avoided if one posits a higher-order force-flow relation [7], although whether this corresponds to mitochondrial behaviour *in vitro* remains controversial. Recently attention has been focussed [20] on the direct effects of PCr and free Cr on  $\text{O}_2$  consumption *in vitro*, independent of [ADP] [23]. Implications for mitochondrial regulation *in vivo* are linked to mitochondrial creatine kinase [20], although no detailed predictions have been made. While this is in theory a way in which a hyperbolic flux-ADP relationship *in vitro* could appear *in vivo* as the sigmoid relationship [7] needed to explain the dynamic-range problem without parallel activation, the required effect is much larger than has been shown *in vitro*. However, our understanding of these mechanisms remains incomplete.

(C) *Understanding oxidative ATP synthesis rates in relation to glycolysis:* A third question is, given some task-defined ATP demand, can we predict, nontrivially, how ATP generation will be split between oxidative and glycolytic means? One approach is to use the measurements we have (e.g. pH, PCr, ADP) to predict the oxidative ATP synthesis rate, and then infer glycolytic ATP synthesis essentially by difference. As well as the problems already mentioned, this is hampered by disagreement about how much the pH change which accompanies lactate accumulation itself reduces mitochondrial capacity [6,8]. Assessing this definitively requires agreement on the relevant flux-force relationships, which, as we have seen, is lacking. A related approach, concentrating on possible activators of glycolysis, is hampered by similar disagreement about whether open- or closed-loop influences dominate [2,15], and if the latter, which metabolites measurable *in vivo* are potential predictors of flux *in vivo*.

(D) *Modelling the effect of impaired  $\text{O}_2$  delivery on oxidative metabolism:* This is physiologically and pathologically important. There have been semi-quantitative approaches. In vascular disease [11] and experimental hypoxia [18] the mitochondrial capacity inferred from  $^{31}\text{P}$  MRS measurements is reduced at low cell  $\text{PO}_2$  inferred from NIRS or  $^1\text{H}$  MRS (see (A) above), apparently consistent with the known dependence of mitochondrial metabolism on the concentration of its substrate  $\text{O}_2$  [10]. At present, though, a rate of  $\text{O}_2$  usage cannot be 'read off' from cell  $\text{PO}_2$  and (say) [ADP], any more than it can be from [ADP] and pH in exercise where [see (B) and (C) above], although pH falls,  $p_{\text{O}_2}$  is not 'limiting'.

1. Blei ML, Conley KE, Kushmerick MJ (1993) Separate measures of ATP utilization and recovery in human skeletal muscle [correction in *J. Physiol.* (1994) 475: 548]. *J. Physiol.* 465: 203-222.
2. Conley KE, Kushmerick MJ, Jubrias SA (1998) Glycolysis is independent of oxygenation state in stimulated human skeletal muscle *in vivo*. *J. Physiol.* 511: 935-945.
3. Fell DA, Thomas S (1995) Physiological control of metabolic flux: the requirement for multisite modulation. *Biochem. J.* 311: 35-39.
4. Ferreira L, Townsend D, Lutjemeier B, Barstow T (2005) Muscle capillary blood flow kinetics estimated from pulmonary  $\text{O}_2$  uptake and near-infrared spectroscopy. *J. Appl. Physiol.* 98: 1820-1828.
5. Grassi B, Poole D, Richardson RS, Knight D, Erickson B, Wagner PD (1996) Muscle  $\text{O}_2$  uptake kinetics in humans: implications for metabolic control. *J. Appl. Physiol.* 80: 988-998.
6. Harkema S, Meyer RA (1997) Effect of acidosis on control of respiration in skeletal muscle. *Am. J. Physiol.* 272: C491-500.
7. Jeneson JAL, Wiseman RW, Westerhoff HV, Kushmerick MJ (1996) The signal transduction function for oxidative phosphorylation is at least second order in ADP. *J. Biol. Chem.* 271: 27995-28.
8. Jubrias SA, Crowther G, Shankland E, Gronka R, Conley KE (2003) Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle *in vivo*. *J. Physiol.* 533: 589-599.

9. Jucker B, Dufour S, Ren J, Cao X, Previs S, Underhill B, Cadman K, Shulman GI (2000) Assessment of mitochondrial energy coupling in vivo by  $^{13}\text{C}/^{31}\text{P}$  NMR [correction in *PNAS USA* (2001) 98: 3624]. *Proc. Natl. Acad. Sci. USA* 97: 6880-6884.
10. Kemp GJ (2004) Mitochondrial dysfunction in chronic ischemia and peripheral vascular disease. *Mitochondrion* 4: 629-640.
11. Kemp GJ, Roberts N, Bimson WE, Bakran A, Frostick SP (2002) Muscle oxygenation and ATP turnover when blood flow is impaired by vascular disease. *Spectroscopy Int. J.* 16: 317-334.
12. Kemp GJ, Roberts N, Bimson WE, Bakran A, Harris P, Gilling-Smith G, Brennan J, Rankin A, Frostick SP (2001) Mitochondrial function and oxygen supply in normal and in chronically ischaemic muscle: a combined  $^{31}\text{P}$  magnetic resonance spectroscopy and near infra-red spectroscopy study *in vivo*. *J. Vasc. Surg.* 34: 1103-1110.
13. Kemp GJ, Thompson CH, Taylor DJ, Hands L, Rajagopalan B, Radda GK (1993) Quantitative analysis by  $^{31}\text{P}$  MRS of abnormal mitochondrial oxidation in skeletal muscle during recovery from exercise. *NMR Biomed.* 6: 302-310.
14. Korzeniewski B (1998) Regulation of ATP supply during muscle contraction: theoretical studies. *Biochem. J.* 330: 1189-1195.
15. Lambeth MJ, Kushmerick MJ (2002) A computational model for glycogenolysis in skeletal muscle. *Ann. Biomed. Eng.* 30: 808-827.
16. McCully K, Fielding R, Evans W, Leigh JJ, Posner J (1993) Relationships between in vivo and in vitro measurements of metabolism in young and old human calf muscles. *J. Appl. Physiol.* 75: 813-819.
17. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GL, Shulman GI (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140-1142.
18. Richardson RS, Leigh JS, Wagner PD, Noyszewski E (1999) Cellular  $\text{PO}_2$  as a determinant of maximal mitochondrial  $\text{O}_2$  consumption in trained human skeletal muscle. *J. Appl. Physiol.* 87: 325-331.
19. Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD (1995) Myoglobin  $\text{O}_2$  desaturation during exercise. Evidence of limited  $\text{O}_2$  transport. *J. Clin. Invest.* 96: 1916-1926.
20. Smith SA, Montain SJ, Zientara GP, Fielding RA (2004) Use of phosphocreatine kinetics to determine the influence of creatine on muscle mitochondrial respiration: an in vivo  $^{31}\text{P}$ -MRS study of oral creatine ingestion. *J. Appl. Physiol.* 96: 2288-2292.
21. Tran T, Sailasuta N, Kreutzer U, Hurd R, Chung Y, Mole P, Kuno S, Jue T (1999) Comparative analysis of NMR and NIRS measurements of intracellular  $\text{PO}_2$  in human skeletal muscle. *Am. J. Physiol.* 276: R1682-R1690.
22. Van Beekvelt M, Colier W, Wevers R, Van Engelen B (2001) Performance of near-infrared spectroscopy in measuring local  $\text{O}_2$  consumption and blood flow in skeletal muscle. *J. Appl. Physiol.* 90: 511-519.
23. Walsh B, Tonkonogi M, Soderlund K, Hultman E, Saks V, Sahlin K (2001) The role of phosphorylcreatine and creatine in the regulation of mitochondrial respiration in human skeletal muscle. *J. Physiol.* 537: 971-978.



## **2-02. Spatial profiles of mitochondrial oxygen consumption in myocardium *in situ* during ischemia.**

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In the normal heart myocardial blood flow and oxygen consumption are both heterogeneous, but highly matched to each other. Myocardial ischemia and infarction as a result of coronary stenosis are major causes of death in humans. Myocardial infarction may show a patchy pattern. We investigated how oxygen delivery, as reflected by coronary blood flow, matches to oxygen consumption at the local level during increasing partial coronary stenosis of the left anterior descending (LAD) artery in porcine left ventricle.

In *in vitro* and *in vivo* myocardium we have demonstrated that TCA cycle and other metabolic fluxes can be quantitated from a single high-resolution carbon-13 NMR spectrum in a small tissue sample, making it possible to assess spatial patterns of oxygen consumption [1,2]. Heart muscle samples are taken exactly 5.5 min after the start of infusion of  $^{13}\text{C}$ -enriched acetate. A high-resolution NMR spectrum is obtained from tissue sample extracts, showing single carbon NMR peaks, often split into multiplets due to J-coupling between adjacent isotopes. The NMR free induction decays are analyzed in the time domain and yield absolute line intensities. The multiplet intensities are analyzed by a model for isotope traffic in the TCA cycle and communicating amino acid pools. The 160 differential equation model is integrated for each point on a trajectory in parameter

space to find an optimal fit. Flux quantitation therefore depends on a pre-steady-state of carbon-13 isotope enrichment in glutamate.

In small (<1 ml) tissue samples, blood flow was measured with radioactive microspheres, and oxygen consumption in the same sample with the carbon-13 method. In one group of animals LAD pressure was 65-70 mmHg downstream of the stenosis (group I,  $n = 7$ ), and in another group LAD pressure was 30-35 mmHg (group II,  $n = 7$ ). During normal perfusion, blood flow ( $\sim 5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  dry mass) and oxygen consumption ( $\sim 19 \text{ }\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  dry mass) are well correlated ( $r=0.85$ ). In group I, blood flow decreased by 31.9 % on average during stenosis, in group II by 40.9 %. The correlation between blood flow and oxygen consumption on a local level decreased markedly with decreasing blood flow and with decreasing LAD pressure downstream of the stenosis.

We conclude that during progressing partial coronary occlusion, in addition to the global decrease of the oxygen supply-to-consumption ratio, oxygen delivery to oxygen consumption matching is increasingly heterogeneous. This implies that local vasodilation reserve is not uniformly matched to regional demand and that during stenosis some areas are more affected by ischemia than others.

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1. Beek JHGM van, Mil HGJ van, Kanter FJJ de, King RB, Alders DJC, Bussemaker JA (1999)  $^{13}\text{C}$ -NMR double labeling method to quantitate local myocardial oxygen consumption using frozen tissue samples. *Am. J. Physiol.* 277: H1630-H1640.
2. Alders DJC, Groeneveld ABJ, De Kanter FJJ, Beek JHGM van (2004) Myocardial oxygen consumption in porcine left ventricle is heterogeneously distributed in parallel to heterogeneous oxygen delivery. *Am. J. Physiol.* 287: H1353-H1361.



### **2-03. Oxygen- and flux-dependence of ROS-formation of lung alveolar epithelial cells.**

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ROS are produced in the cytoplasm and by mitochondria [3]. However, results are divergent on changes in ROS production upon varying cellular oxygen supply [1,2]. In the present study we measured ROS-formation during 15 min (37 °C) exposure of lung alveolar A549 cells to hypoxia (2% O<sub>2</sub>), normoxia (21 % O<sub>2</sub>) and hyperoxia (35 % O<sub>2</sub>) by chemiluminescence (CL; 30  $\mu\text{M}$  lucigenin) and ESR-spectroscopy (CMH, CPH, ACP, PPH; 500  $\mu\text{M}$ ). Both, CL and ESR indicate the lowest accumulation of ROS during exposure to hypoxia and an increase in ROS-formation with increasing oxygenation. It has to be noted, however, that decreasing the concentration of CMH also decreases the rate of ROS-formation in normoxic cells. In contrast to results obtained by CL and ESR, DCF-fluorescence was increased in hypoxia. In all cases, return to 21 % O<sub>2</sub> increased ROS-formation in hypoxic but decreased ROS in previously hyperoxic cells. N-acetyl-cysteine decreased CL in all states of oxygenation but oxygen-dependency of CL was still apparent. Decreased ROS-formation in hypoxia was associated with slightly increased NADH, measured by fluorescence microscopy, and a decrease in cellular oxygen consumption, measured by high-resolution respirometry (OROBOROS Oxygraph). In intact cells, addition of rotenone and antimycin A increased ROS-formation, whereas stimulation of complex I and II respiration by increasing ADP decreased ROS measured by CL in digitonin-permeabilized cells. Both, in the non-stimulated and ADP-stimulated state, ROS-formation was lower in hypoxia than normoxia. Our results confirm apparent discrepancies on changes in ROS formation upon changing oxygenation that can only be explained on the basis of different species of ROS that are seen by different detection methods. It might also be, however, that different ROS-indicators affect the rate of cellular ROS-formation in different ways, which needs further exploration.

1. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc. Natl. Acad. Sci. USA* 95: 11715-11720.
2. Michelakis ED, Hampl V, Nsair A, Wu XC, Harry G, Haromy A, Gurtu R, Archer SL (2002) Diversity in mitochondrial function explains differences in vascular oxygen sensing. *Circ. Res.* 90: 1307-1315.
3. Nohl H (1994) Generation of superoxide radicals as byproduct of cellular respiration. *Ann. Biol. Clin. (Paris)* 52: 199-204.



## **2-04. The effect of hypoxia upon the expression pattern of isoforms and the kinetics of cytochrome c oxidase in astrocytes and neurons.**

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The brain is the organ with the highest energy demand in mammalian organisms. Neurons and astrocytes, two different brain cell types, are structurally, functionally, and metabolically tightly coupled with astrocytes playing a central role in regulation of cerebral energy metabolism in dependence on neuronal activity. Oxygen is, besides glucose, the most important substrate to fulfil neuronal energetic requirements and also the substrate of cytochrome c oxidase (COX), the enzyme that is engaged in mitochondrial oxidative energy metabolism. Mammalian COX is composed of three catalytic, mitochondrially encoded and ten regulatory, nuclear encoded subunits. The regulatory COX subunit IV plays an important role in adjusting energy production to energetic requirements by binding of ATP to the N-terminus of subunit IV and thereby causing an allosteric inhibition of COX activity at high energy level, i.e. high ATP/ADP ratio [1]. It was found that this COX subunit is expressed in isoforms (IV-1 and IV-2). While isoform IV-1 is ubiquitously transcribed in all adult mammalian tissues including brain, isoform IV-2 showed so far high transcription levels only in the lung [2].

Besides the expression of COX IV-1 isoform in astrocytes and neurons from mouse and rat brains, we detected also mRNA transcripts for the COX IV-2 isoform in neurons pointing at a cell type specific expression of COX subunit IV isoforms in the brain. Under conditions of oxygen deprivation mRNA transcription of COX IV-2 is induced in astrocytes and upregulated in neurons. So far, yeast has been the only organism known to express two isoforms (Va and Vb), homologous to the mammalian subunit IV, in dependence on oxygen concentration [3]. The functional consequences of an increased expression of COX IV-2 isoform, that structurally differs from COX IV-1 isoform in its N-terminus, are reflected in the abolition of allosteric inhibition of COX by ATP at high ATP/ADP levels. We conclude that the expression of COX IV-2 isoform under hypoxia suppresses the sensitivity of COX to its allosteric regulator ATP and overrules the regulation of COX by the cellular energy level.

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1. Arnold S, Kadenbach B (1999) The intramitochondrial ATP/ADP-ratio controls cytochrome c oxidase activity allosterically. *FEBS Lett.* 443: 105-108.
2. Hüttemann M, Kadenbach B, Grossman LI (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. *Gene* 267: 111-123.
3. Allen LA, Zhao XJ, Caughey W, Poyton RO (1995) Isoforms of yeast cytochrome c oxidase subunit V affect the binuclear reaction center and alter the kinetics of interaction with the isoforms of yeast cytochrome c. *J. Biol. Chem.* 270: 110-118.

## **2-05. How Epo-overexpressing mice adapt to chronic excessive erythrocytosis.**

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Excessive erythrocytosis is usually associated with hypertension and thromboembolism resulting in severe cardiovascular complications. The key player of regulating red blood cell number is erythropoietin (Epo) that controls red blood cell production in a HIF-1-dependent manner. To test the impact of hypoxia-independent expression of Epo, we generated a transgenic (tg) mouse line termed tg6 that, due to constitutive expression of human Epo, reached hematocrit values of 0.8 to 0.9 without alteration of blood pressure, heart rate or cardiac output. In tg mice plasma volume was not elevated whereas blood volume was 25 % of the body weight compared to 8 % in wildtype (wt) siblings. While plasma viscosity did not differ between tg and wt, tg whole-blood viscosity increased to a lower degree (4-fold) than expected. Apart from the nitric oxide-mediated vasodilatation, adaptation to high hematocrit in tg mice involves regulated elevation of blood viscosity by increasing erythrocyte flexibility.

Knowing that Epo exerts also non-erythropoietic but tissue-protective effects, we tested the impact of several insults on our tg6 mice. We observed that elevated Epo levels protected from light-induced (but not from inherited) retinal degeneration as well as from experimentally induced myocardial infarction. Finally, we provide evidence that upon exposure of tg6 mice to 6 % oxygen, cerebral Epo regulated the hypoxic ventilatory response.



## **2-06. Mitochondrial respiratory function in human skeletal muscle fibers studied at high altitude.**

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This study examined the effects of acclimatization to hypoxia (4559 m) on mitochondrial substrate utilization and respiratory function. The Bergstrom technique was used to obtain muscle biopsies of the vastus lateralis from 10 healthy Danish male subjects (25±2 yrs) at sea level and again after 6 to 9 days at high altitude. High-resolution respirometry (OROBOROS Oxygraph-2k [1]) allowed quantification of mitochondrial respiratory capacities from saponin-permeabilized skeletal muscle fibers (2-6 mg).

At sea level, state 3 respiration (in the presence of ADP) with parallel electron input into respiratory complexes I+II (glutamate, malate and succinate) was 58±4 pmol·s<sup>-1</sup>·mg<sup>-1</sup>, 1.6-fold higher than with glutamate+malate or succinate+rotenone. Flux with complex I+II substrates indicates the capacity of the phosphorylation system as shown by the 1.5-fold higher respiration after uncoupling by FCCP. These findings obtained with permeabilized muscle fibers agree with results on isolated mitochondria [2]. Respiratory capacity with octanoylcarnitine+malate was 48 % of that with glutamate+malate. Respiratory coupling was quantified through the stimulation by ADP or inhibition of ATP synthase by oligomycin and subsequent uncoupling by FCCP. Respiratory control ratios with succinate or octanoylcarnitine were 2.8. Compared to these results at sea level, 6 to 9 days of high altitude exposure did not induce a detectable change in any of the respiratory capacities nor in coupling. These findings indicate that mitochondrial function is not a limiting factor for  $V_{O_{2max}}$  during early acclimatization to high altitude,

strengthening the concept of a dominant role for systemic oxygen delivery during intense dynamic exercise [3].

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1. Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir. Physiol.* 128: 277-291.
2. Rasmussen UF, Rasmussen HN (2000) Human quadriceps muscle mitochondria: a functional characterization. *Mol. Cell. Biochem.* 208: 37-44.
3. Calbet JAL, Boushel R, Radegran G, Sondergaard H, Wagner PD, Saltin B (2003) Why is  $\text{VO}_2\text{max}$  after altitude acclimatization still reduced despite normalization of arterial  $\text{O}_2$  content? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284: R304-R316.

## **2-07. Different regimen of intermittent hypoxia training (IHT) as modulators of heart mitochondrial membrane permeability transition.**

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IHT is believed to induce myocardial protection. We hypothesized that inhibiting of mitochondrial permeability transition pore (mPTP) opening by means of antioxidant system promotion is one of the key elements of such protection, and mitochondrial swelling rate depends on degree of hypoxic exposure. Four groups of male adult Wistar rats participated in the study. Animals of Gr. 1 underwent daily sham IHT (control group). Gr. 2-4 were exposed for 2 weeks to IHT in two regimen:  $R_1$ - breathing in normobaric chamber with 11 %  $\text{O}_2$  gas mixture, 15-min sessions with 15 min rest intervals, 5 times daily (Gr. 2);  $R_2$  - breathing with 8 %  $\text{O}_2$ , 5-min sessions with 15 min rest intervals, 5 times daily (Gr. 3-4). Gr. 1-3 were examined next day after IHT, Gr. 4 - in 45 days after IHT stopping. Phenylarsineoxide (PAO)-induced mitochondrial swelling rate was investigated spectrophotometrically ( $\lambda = 520 \text{ nm}$ ) in isolated heart mitochondria by a decrease in their optical density after 20 min of incubation with PAO. The intensity of lipid peroxidation and antioxidant defense mechanisms in rat organism were estimated before and after IHT by measuring of malon dialdehyde (MDA) content and the activity of superoxide dismutase (SOD) and catalase (CAT) in blood and liver. It was shown that the training with moderate hypoxia ( $R_1$ ) did not essentially influence mPTP opening nor free radical production: The magnitude of mitochondrial swelling in Gr. 2 under incubation with PAO in concentrations  $10^{-5}$  and  $10^{-4} \text{ mol/l}$  was almost the same as in Gr. 1; MDA content decreased by 10 and 20 % in blood and liver, respectively; a slight increase in superoxide dismutase activity by 29 и 23%, a decrease in catalase activity by 9 % (ns) and 21 %, respectively, was observed. The training with more severe hypoxia ( $R_2$ ) provoked a two-fold decrease in PAO-induced mitochondrial swelling (Gr. 3 compared with Gr. 1). And even in 45 days (Gr. 4) the protective effects of  $R_2$  on mitochondria were well-preserved. These effects were completely abolished in the presence of cyclosporin A ( $10^{-5} \text{ mol/l}$ ), indicating that mitochondrial swelling was due to mitochondrial permeability transition pore opening. Simultaneously,  $R_2$  training caused pronounced increases in MDA content both in blood and liver by 67 and 32 %, respectively, and considerable augmentation in activities of SOD (49 and 32 %) and CAT (18 and 43 %). Moreover, in 45 days the activity of SOD exceeded initial levels three-fold both in blood and liver. Taken together, moderate intermittent hypoxia which provokes some inhibition of lipid peroxidation with slight increase in antioxidant activity, does not influence PAO-induced mitochondrial swelling. A more severe regime of IHT, stimulating both the increase of lipid peroxidation and strongly pronounced augmentation of antioxidant system, causes a stable increase in resistance of mitochondrial membrane to PAO. Probably, intensified free radical production during more severe hypoxia could serve as a trigger in signal transduction cascades and lead to increase in antioxidant defence and

thus to mitochondria protection. IHT can be used as protective procedure preventing mitochondria from damage.

## **2-08. Structure and function of muscle mitochondria at endurance training connected with intermittent hypoxia.**

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Endurance training (ET) induces muscle mitochondrial biogenesis and adaptations of mitochondrial function [1]. ET in hypoxia is thought to modulate these effects [2]. Intermittent hypoxic training (IHT) has been recently shown to improve oxygen transport to and within muscle cells [3]. But, the effects of this modality of hypoxia exposure on muscle mitochondria structure and respiration at ET are poorly understood. The aim of this study was to compare muscle mitochondrial adaptations induced by severe ET combined with IHT to those occurring with ET only at the same relative workload.

Male adult Wistar rats were subjected to swimming training for 4 weeks (30 min daily, the workload corresponded to 70-75 %  $V_{O_{2max}}$ ). The IHT course was added in the last 2 weeks of ET; the rats underwent the IHT sessions: breathing with hypoxic mixture containing 12 %  $O_2$  for 15 min with 15-min rest intervals, 5 times daily. We found that ET+IHT induced a greater increase in the numerical density, the volume density, and size of mitochondria in the red gastrocnemius compared to the similar effects of ET. Whereas ET without IHT stimulated preferential adaptation of the subsarcolemmal mitochondria, ET+IHT affect both the subsarcolemmal and intermyofibrillar mitochondria. In case of combined action of ET+IHT, the internal structure of mitochondria in terms of compartmental spaces and membranes was well-preserved. ET+IHT led to a highly expressed increase in the values of mitochondrial respiratory control ( $J_3/J_4$ ) and ADP/O ratio under  $\alpha$ -ketoglutarate oxidation compared to values under succinate oxidation. The combination of ET with IHT, therefore, was found to be the most productive model for stimulating mitochondrial biogenesis and increasing of the NADH-dependent oxidation pathway role in muscle energy production.

1. Hood DA (2001) Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J. Appl. Physiol.* 90: 1137-1157.
2. Hoppeler H, Fluck M (2003) Plasticity of skeletal muscle mitochondria: structure and function. *Med. Sci. Sports Exerc.* 35: 95-104.
3. Rozova KV, Gavenauskas BL, Mankovskaya IN (2004) Influence of intermittent hypoxia on ultrastructure of skeletal muscle under intensive exercise. *Clin. Exp. Pathology (Ukraine)* 3: 63-68.

## **2-09. Effects on gene regulation by reactive oxygen species during intermittent hypoxic training.**

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Genetic mechanism relevant to reactive oxygen species (ROS) during intermittent hypoxic training were studied, so that the theory on exercise and hypoxic acclimatization is provided.

One hundred and twenty male Sprague-Dawley rats were randomly divided into three groups: normoxia group, acute hypoxia group and intermittent hypoxic group. Normoxia group included normoxia control and normoxia training sub-groups; acute hypoxia group included acute hypoxia control, acute hypoxia exercise and acute hypoxia training sub-groups; intermittent hypoxic group included intermittent hypoxic control and intermittent hypoxic training sub-groups. Therefore there are totally 12 sub-groups, each one with 10 rats. During the 4 weeks experimental period, we employed the 14.5 % and 12.6 % concentrations of oxygen (equal to altitude 3000 m and 4000 m respectively) in the

hypoxic chamber. The rats of acute hypoxia training were introduced to treadmill running on an incline of 0 at 25 m/min for 1 h every day for 4 weeks. The rats of intermittent hypoxic were exposed to hypoxia for 12 h every day for 4 weeks. In addition, intermittent hypoxic training rats were kept running out of chamber with 1 h training bouts at the speed of 25 m/min every day. Mitochondria ROS were assessed by methods of DCFH-DA. The mRNA level of HIF-1 $\alpha$ , VEGF, NF- $\kappa$ B (p65), c-fos, c-jun, MnSOD, CuZnSOD, GSH-Px in heart tissue and EPO in kidney tissue were investigated by RT-PCR.

The results show that ROS generation is required for the induction of HIF-1 mRNA. During hypoxia, these findings reveal that mitochondria-derived ROS are both required and sufficient to initiate HIF-1 $\alpha$  stabilization during hypoxia. And hypoxia activated transcription of EPO, VEGF-MnSOD, NF- $\kappa$ B, c-fos, c-Jun. We therefore conclude that ROS participate in the signalling pathways involved in the activation of multiple transcription factors. It is also indicated that there is a close relation between hypoxic acclimatization and gene expression of HIF-1, EPO, VEGF, MnSOD, NF- $\kappa$ B, c-fos, c-Jun and hypoxic acclimatization.

1. Sethi S, Singh MP, Dikshit M (2000) Mechanisms involved in the augmentation of arachidonic acid-induced free-radical generation from rat neutrophils following hypoxia-reoxygenation. *Thromb. Res.* 98: 445-450.
2. Sasaki H, Ray PS, Zhu et al (2001) Hypoxia/reoxygenation promotes myocardial angiogenesis via an NF kappa B-dependent mechanism in a rat model of chronic myocardial infarction *J. Mol. Cell. Cardiol.* 33: 283-294.
3. Laura L. Loftis LL, Cheryl A et al. (2000) Brief hypoxic stress suppresses postbacteremic NF- $\kappa$ B activation and TNF- bioactivity in perfused liver. *J. Cell Biol.* 279: R99-R108.
4. Suzuki M, Takeuchi H, Kakita T (2000) The involvement of the intracellular superoxide production system in hepatic ischemia-reperfusion injury. In vivo and in vitro experiments using transgenic mice manifesting excessive CuZn-SOD activity. *Free Radical Biol. Med.* 29: 756-763.

## **2-10. Effect of muscle mitochondria heterogeneity on oxygen transport in muscle fibers at rest and work.**

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Muscle O<sub>2</sub> supply is very heterogeneously distributed with high values near capillary and low ones at the lethal corner. Using the ideas concerning O<sub>2</sub> supply in some biological objects we tried to examine the possible ways of  $p_{O_2}$  equalization in muscle fibers with mitochondrial heterogeneity.

Contemporary literature is lacking a unified opinion as to physiological role of the two mitochondrial populations - subsarcolemmal (SS) and intermyofibrillar (IMF) - in skeletal and cardiac muscle which differ in their morphological and biochemical properties [1]. The aim of the study is to investigate the impact of heterogeneity of the mitochondria on muscle O<sub>2</sub> supply by means of mathematical modeling [2]. We have calculated the  $p_{O_2}$  distribution in myocytes under different values of the apparent Michaelis constant for SS and IMF clusters, both varying within the range of 0.5 to 10 mmHg and mitochondria oxygen capacities  $q_{O_2,SS}$  and  $q_{O_2,IMF}$  varying within the range of 1 to 30 ml·min<sup>-1</sup>·100 g<sup>-1</sup> at rest and work.

Our investigation at rest revealed that a 2-fold increase in oxygen consumption rate in the SS mitochondria only with unchanged oxygen utilization rate in IMF mitochondria may markedly displace the estimated  $p_{O_2}$  histogram to the left. So we suggest that an increased O<sub>2</sub> consumption in the SS mitochondrial cluster (for example, by partial uncoupling of respiratory chain in mitochondria of SS cluster) allows to burn 'extra' oxygen, converting the energy to heat, which is readily eliminated by capillary blood flow and by heat dissipation from the fiber surface near blood capillaries.

Investigation at work reveals that one of the approaches for equalizing  $p_{O_2}$  distribution and increasing  $p_{O_2}$  at the lethal corner is to change the values of  $q_{O_2,SS}$  and  $q_{O_2,IMF}$ . According to computations, a significant increase in  $q_{O_2,SS}$  gives only a slight increase in  $p_{O_2}$  at the lethal corner. In this case, a worthy strategy of fight for oxygen might be

linked with the hyperplasia of the mitochondria that are located near capillaries and densely packed under the sarcolemma. Another approach is to change the values of  $K_m$  for  $O_2$  in mitochondrial SS and IMF. The calculations have demonstrated that a rise of  $K_{m,SS}$  by 6 to 8 times changes the  $O_2$  gradients in the fiber, increases the  $p_{O_2}$  value at the lethal corner, reduces the percentage of tissue zones with low values  $p_{O_2}$  and shifts the  $p_{O_2}$  histogram to the right. Since SS-mitochondria take only small volume of muscle fiber, they can, without noticeable damage to general energy production, increase oxygen flux inside the fiber to IMF mitochondria thereby performing an important function of equalizing the cell  $p_{O_2}$ . It contributes to the reduction of oxygen debt production and weakens the local hypoxia in a working skeletal muscle.

1. Bizeau M, Willis W, Hazel JR (1998) Differential responses to endurance training in subsarcolemmal and intermyofibrillar mitochondria. *J. Appl. Physiol.* 85: 1279-1284.
2. Lyabakh KG, Mankovskaya IN (2002) Oxygen transport to skeletal muscle working at  $V_{O_{2max}}$  in acute hypoxia: theoretical prediction. *Comp. Biochem. Physiol. A.* 132: 53-60.

