exercise training improves heart function and cardiovascular disease risk profile are not understood. The purpose of the present investigation was to study the effect of long-term exercise training on the sensitivity of calcium-induced MPTP opening and the mitochondrial uncoupling protein 3 (UCP3) expression. The animals were divided into 2 groups: 1st group - intact adult animals (control rats), 2nd group - adult animals, which are subject to dosed physical load carried by the forced swimming five days a week for six weeks (trained rats). It was shown that the sensitivity of MPTP-opening to Ca<sup>2+</sup> in the trained rats heart decreased compared with control animals. Thus, in the trained rats heart mitochondria had a significant increase cNOS activity almost twice, slight increase in the NOS activity compared with control, and slight increasing the hydrogen peroxide. The expression level of UCP3 was reduced by 65% in heart mitochondria of long-term exercise training rat compared with the control. These results suggest that decreased the expression level of UCP3 in heart mitochondria of the trained rats may play the certain role in the complex mechanism of adaptive response of the heart. that may be aimed mainly on the efficiency of oxidative phosphorvlation in mitochondria and, consequently, to increase the synthesis ATP. Thus, long-term exercise training contribute to reducing the sensitivity of the MPTP opening to the action of calcium ions by increasing the activity of mitochondrial constitutive NOS and synthesis of nitric oxide – an endogenous inhibitor of MPTP opening.

## References

[1] Naito H et al. (2001) Med. Sci. Sports Exerc. 33: 729-734.

[2] Crompton M (1999) Biochem. J. 341: 233-249.

[3] Halestrap AP et al. (1998) Biochim. Biophys. Acta 1366: 79-94.

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## 15P.5 Mitochondrial bioenergetic profile of human hepatocarcinoma cell lines

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Understanding how cancer cells derive their vital energy from microenvironmental nutrients is fundamental for developing anticancer therapies and diagnostic approaches. Recent advances, expanding the Warburg hypothesis, indicate that bioenergetic features of cancer cells are highly variable. Nowadays OXPHOS is re-evaluated and the common idea is that mitochondria are not only bystanders but they are involved in cell transformed state. This study focuses on OXPHOS complexes in two human hepatocellular carcinoma cell lines, HepG2 and JHH-6, with different growth/ differentiation characteristics. First, by high resolution respirometry and western blot analyses we evaluated the functionality of OXPHOS complexes in digitonin-permeabilized cells and their expression in isolated mitochondria. Markedly lower expression levels were observed in undifferentiated JHH-6 with respect to HepG2. Also the activities of ETS carriers and the phosphorylating respiration were lower in JHH-6, where in addition a low phosphorylating system (PhS) capacity limited the ETS capacity much more that in HepG2. We then explored F<sub>1</sub>F<sub>0</sub> ATP synthase oligomerization and IF<sub>1</sub> inhibitor content by 2-DE analysis and immunoblotting of mitochondrial digitonin extracts. In both cell lines BN-PAGE extracted ATP synthase mostly in monomeric form, with the amount of bound IF<sub>1</sub> being very low despite its overexpression in JHH-6. The effects of pharmacological inhibition of glycolysis or ATP synthase were evaluated on mitochondrial membrane potential, ATP production and oxygen consumption. The results documented that the two cell lines had different mitochondrial plasticity. In particular, subsequent to glycolysis blocking HepG2 were able to activate silent mitochondria, while in JHH-6 there was an optimization of the ATP synthesis by the PhS, without improvement of the ETS. Together our data are consistent with the hypothesis that in JHH-6, but not in HepG2, ATP synthase functions in reverse and consumes glycolytic ATP. Whereas, when glycolysis is inhibited ATP synthase becomes able to supply ATP, though in limited extent, by switching its activity to ATP synthase. Thus, OXPHOS may play a different role, depending on the growth/differentiation characteristics of tumor cells, in energy production and cell adaptation and survival in situations of glucose limitations. Analysis of mitochondrial bioenergetic profile may emerge as a predictive factor for tumor resistance of metabolic restriction-based therapy.

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## 15P.6 Evolutionary approach to problems of medicine

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The liver of the adult lamprey (Lampetra fluviatilis L.) in the last winter of its life-cycle (lampreys are monocyclic animals) is a unique natural model imitating the pathological states of the human liver (biliary atresia, cholestasis, and steatosis). In this period the lamprey liver enables one to study the mechanisms of necrosis and apoptosis in their dynamics. The purpose of our study is to clarify the role of mitochondria in metabolic depression of lamprey hepatocytes, and reveal apoptosis as it is known mitochondrial depolarization promotes apoptotic and necrotic cell death. The following methods have been used: (1) measurement of adenine nucleotides concentration in liver tissue and hepatocytes by an HPLC; (2) fluorometric determination of mitochondrial membrane potential distribution in hepatocytes exploiting a laser confocal microscopy and flow cytometry, using parameter-specific fluorescent probes (TMRM, Mitotracker Green FM, DiOC<sub>6</sub>, JC-1); (3) detection of hepatocytes death by necrosis and apoptosis with sequential acridine orange and propidium iodide staining. In winter ATP concentration in the lamprey hepatocytes decreases to extraordinarily low values  $(0.1-0.2 \mu mol/g wet mass)$ , by a factor of about ten as compared with the values in the autumn. The Atkinson energy charge potential lays within 0.2-0.3 for 25-30% of the experimental individuals and does not exceed 0.5 for the others. Mitochondria of lamprey hepatocytes develop high  $\psi_{mit}$  in autumn and spring during the last period of their life circle. In autumn it is seems such energization relates with delivery of nutrients to hepatocytes by a bloodstream. In spring there is intensive lipolysis in hepatocytes supporting vitellogenesis and spermatogenesis. Confocal FRET has revealed heterogeneity of hepatocytes. The experiments have distinguished energized and de-energized mitochondria in hepatocytes. We have observed a sharply increase of necrotic and apoptotic hepatocytes in spring before spawning. It may look surprising that in spring the lamprey liver mitochondria "overcome" the energetic depression and "get alive" under the influence of hormonal activated lipolysis connected with vitellogenesis. Hepatocytes return to physiological normal energy situation but for the very short period. In May-June after spawning lampreys die.

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