discrepancies between the measurements of $[Ca^{2+}]_M$ with dyes or targeted proteins are also qualitative in some cases and significant changes in the behaviour or kinetics of $[Ca^{2+}]_M$ appear when comparing measurements obtained with both kinds of methods [3, 4]. We have made here a systematic comparison of the response of two fluorescent dyes, rhod-2 and rhod-FF, and two Ca^{2+}-sensitive proteins, aequorin and pericam. Our results show that measurements obtained with aequorin and pericam are consistent in terms of dynamic Ca^{2+} changes. Instead, fluorescent dyes failed to follow Ca^{2+} changes adequately, especially during repetitive stimulation. In particular, measurements obtained with rhod-2 or rhod-FF evidenced the previously reported Ca^{2+}-dependent inhibition of mitochondrial Ca^{2+} uptake [5], but data obtained with aequorin or pericam under the same conditions did not. The reason for the loss of response of fluorescent dyes is unclear. Loading with these dyes produced changes in mitochondrial morphology and membrane potential, which were small and reversible at low concentrations (1-2 mM), but produced large and prolonged damage at higher concentrations. Our results suggest that $[Ca^{2+}]_M$ data obtained with these dyes should be taken with care and confirmed with other methods.

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doi:10.1016/j.bbabio.2010.04.380

15P.16 Inhibition of nitric oxide synthase protects hypercholesterolemic mice mitochondria against permeability transition

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Atherosclerosis is associated with elevated levels of oxidized products derived from nitric oxide (NO) and superoxide radicals indicating nitroxidative stress. We have recently shown that hypercholesterolemic LDL receptor knockout mice (*LDLR*$^{-/-}$) mitochondria release high levels of reactive oxygen species (ROS). The aim of this work was to verify the effect of a nitric oxide synthase (NOS) inhibitor (L-NAME) on the membrane permeabilization and redox state of *LDLR*$^{-/-}$ liver mitochondria. Mitochondrial permeability transition (MPT) (cyclosporine sensitive swelling and calcium release), ROS (H2DCF-DA and Amplex-red) and NO production rates (DAF-FM diacetate), and protein S-nitrosothiol content were determined in *LDLR*$^{-/-}$ and control liver mitochondria before and after administration of L-NAME, *in vitro* (50 μM) and *in vivo* (1 mg/Kg/day, during 14 days). The *LDLR*$^{-/-}$ mitochondria presented higher levels of nitrotyrosine (Western Blot), which was undetectable in control mitochondria. *In vitro* L-NAME protected *LDLR*$^{-/-}$ mitochondria against MPT. However, in control mitochondria, L-NAME favored MPT. These results were also observed after *in vivo* chronic L-NAME treatment. Under all conditions, L-NAME reduced mitochondria ROS and NO production rates. Mitochondrial protein S-nitrosothiol content decreased only in L-NAME treated control but not in *LDLR*$^{-/-}$ mitochondria. These results suggest that *LDLR*$^{-/-}$ mitochondria are under nitroxidative stress which is normalized by L-NAME treatment, thus correcting their higher susceptibility to MPT. On the other hand, inhibiting physiological NO production in control mitochondria promotes MPT which is associated with decreased protein S-nitrosothiol content. Therefore, mitochondrial nitric oxide synthase activity seems to be directly involved in the nitroxidative stress in the atherosclerosis prone *LDLR*$^{-/-}$ mice. These findings might be relevant for the vascular wall cell death that occurs in atherogenesis.

doi:10.1016/j.bbabio.2010.04.381

15P.17 Mitochondria energy metabolism and store-operated calcium entry in *mdx* mouse myoblasts

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Duchene muscular dystrophy (DMD) is a neuromuscular genetic disease leading to progressive damage of muscle and premature death. DMD is caused by mutation in the dystrophin encoding gene leading to lack of dystrophin. Patients with DMD exhibit aberrant calcium homeostasis and altered energy metabolism. As dystrophin seems to appear not before muscle cells differentiation any phenotypic changes in *mdx* myoblasts have been unexpected. In contrary to such assumption, a significant increase in nucleotide-dependent receptors activity in *mdx* myoblasts was described. Here we found that myoblasts derived from *mdx* mouse exhibit significantly decreased oxygen consumption, enhanced mitochondrial membrane potential and ROS production, stimulated lactate synthesis but unchanged ATP content. Interestingly, in *mdx* myoblasts stably transfected with minidystrophin-encoding gene some features of wild phenotype were restored. This latter observation strongly indicates that all changes observed in *mdx* myoblasts in comparison to the wild cells were related to the point mutation in dystrophin gene. Moreover, changes in mitochondrial metabolism correlated with enhanced rate of thapsigargin-induced store-operated Ca^{2+} entry. Although a direct link between these events can not been excluded, changes in SOC activity due to enhanced expression of proteins involved in store-operated Ca^{2+} channel formation and/or activation have to also be considered. In sum, these results confirm our earlier findings indicating that the point mutation in dystrophin-encoding gene may give variety of phenotypic changes at the early stage of muscle cell differentiation.

doi:10.1016/j.bbabio.2010.04.382

15P.18 Mitochondria of activated macrophages utilize glycolytic ATP to maintain membrane potential and prevent apoptosis

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We have previously investigated the bioenergetic consequences of activating J774.A1 macrophages (MΦ) with interferon (IFN)γ and lipopolysaccharide (LPS) and found that there is a nitric oxide (NO)-