The endothelium, the monolayer of endothelial cells, is the inner lining of the entire vascular system. Endothelial dysfunction is closely related to excessive production of reactive oxygen species (ROS) and leads to the development of civilization cardiovascular diseases. Because anoxia (a total depletion in the level of oxygen) followed by reoxygenation (restoration of oxygenation) (i.e., A/R) significantly augments the mitochondrial ROS formation leading to oxidative stress, we hypothesize that A/R affect significantly endothelial mitochondrial bioenergetic function. Moreover, adaptation of endothelial mitochondria function caused by chronic hypoxia (reduced oxygen content) may lead to better tolerance to A/R damage by: (i) lowering the level of mitochondrial Q content and (ii) rearranging the bioenergetic properties of endothelial mitochondria.

It has been shown in our laboratory that chronic hypoxia induces numerous changes endothelial mitochondrial function, particularly a general decrease in mitochondrial respiration and increase in mitochondrial ROS formation. Recently, we have observed that chronic hypoxia leads to a 50% reduction in Q amount in endothelial mitochondria (unpublished data). Therefore, questions arise (i) how will earlier endothelial cell adaptation to chronic hypoxia affect A/R tolerance of endothelial mitochondria and (ii) what is the role of a strongly reduced level of mitochondrial Q in this phenomenon?

The main purpose of this research project is to understand the effect of A/R stress on the bioenergetic properties of mitochondria isolated from endothelial cells cultured under chronic hypoxia compared to cells grown under normal oxygenation conditions.

Hypoxic oxidative stress is an important aspect of many cardiovascular diseases associated with endothelial dysfunction. Therefore, studying the effect of reduced Q level on ROS production under conditions of hypoxia and A/R stress may be important in understanding the bioenergetic and physiological functions of endothelial mitochondria. These studies will answer the questions of how hypoxic cells cope with A/R, and how important mitochondrial Q and the mitochondrial energy-dissipating systems (uncoupling protein 2, UCP2 and mitochondrial large-conductance Ca^{2+}-activated potassium channel, mitoBK$_{Ca}$) are in this phenomenon. In vitro models of ischemia/reperfusion, including A/R model on isolated mitochondria, are important, because they allow detailed study of the mechanism of mitochondrial adaptations to oxidative stress.

The study of adaptation of endothelial mitochondrial function due to chronic hypoxia in association with A/R tolerance is pioneering. The role of UCP2 and the mitoBK$_{Ca}$ in bearing endothelial A/R stress is unknown. Since mitochondria are a potential site of pharmacological intervention aimed at widely understood endothelial cell protection under conditions of oxidative stress, these studies may be helpful in further perspective in developing new therapies or verifying the existing therapies (including therapies related to Q supplementation) in cardiovascular diseases treatment.

General research plan:

Studies will be performed using endothelial cells (EA.hy926 and HUVEC) cultured under normoxic (21% O_2) and hypoxic conditions (1% O_2). Mitochondria isolated from hypoxic and normoxic endothelial cells will undergo 30-min anoxia followed by a 5-min reoxygenation. Then, measurements of the bioenergetic properties of mitochondria will be carried out.

Task 1. Analysis of the effects of A/R on bioenergetic properties of phosphorylating mitochondria isolated from hypoxic and normoxic endothelial cells

Task 2. Analysis of the effects of A/R on the bioenergetic properties of nonphosphorylating mitochondria isolated from hypoxic and normoxic endothelial cells

The following bioenergetics parameters will be measured in isolated endothelial mitochondria: respiratory rate, $\Delta\Psi$, the Q reduction level and ROS formation under different respiratory conditions. These measurements will allow to estimate the bioenergetic condition of endothelial mitochondria after A/R stress. Comparison of mitochondria isolated from hypoxic and normoxic endothelial cells will estimate the effect of previous chronic hypoxia on A/R mitochondrial stress tolerance. Research will explain the role of hypoxia-induced decrease in mitochondrial Q content in tolerating A/R stress. Our study will indicate whether UCP2 and mitoBK$_{Ca}$ contribute to suppressing A/R stress in endothelial mitochondria and thus have a protective role.