Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in modern societies, and most forms of CVD are linked to dysfunction of arteries. Vascular oxidative stress is a likely common underlying mechanism of multiple forms of CVD. The term oxidative stress describes the disturbance of the redox homeostasis in favor of increased levels of reactive oxygen species (ROS). The major consequence of oxidative stress is increased inactivation of nitric oxide (NO) by ROS which results in endothelial dysfunction, and the latter was established as important predictor (risk factor) of increased mortality in CVD. Paradoxically however, using antioxidants to prevent CVD has been demonstrated to be ineffective in clinical trials, which most likely reflects our incomplete understanding of the oxidative stress.

Vascular oxidative stress; In cells of aerobic organisms, an often byproduct of various enzymatic oxido-reductive reactions is a transfer of free electron to molecular oxygen resulting in the generation of superoxide anion (O_2 + e- → O_2^-), i.e., the oxygen molecule with an unpaired extra electron (oxygen free radical). Exceptional is in this context the NADPH oxidase (Nox) family enzymes which produce O_2^- (or H_2O_2) as their primary and sole function. Superoxide is a highly unstable molecule and, as such, initiates a cascade of the free radical reactions ending with the production of substances like: peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH*), and hypochloric acid (HClO) (Fig. 1). All these substances are highly chemically reactive (*Reactive Oxygen Species*, ROS) as they are potent acceptors or donors of the electron and thereby act as oxidants or reductants, respectively. In normal concentrations, ROS serve as messengers in physiological cellular signalization. However, at increased concentrations, ROS become toxic. Cellular effects of ROS are related to the fact that they preferentially attack: (i) nitric oxide (NO) that limits NO bioavaiability and results in endothelial dysfunction. Simultaneously, peroxynitrite is formed (O_2^- + NO → $ONOO^-$), which is a source of highly toxic hydroxyl radical, OH*, and which results in S-nitrosylation of proteins and their consecutive modifications; (ii) protein thiol-moieties (mostly contained in cysteine) that affords posttranslational modification of proteins that would be important for cell signaling; (iii) lipid unsaturated bounds that results in a destruction of membrane phospholipids, and (iv) nucleic acids, that may result in genomic changes. Cellular protection against ROS is provided by cellular low-molecular anti-oxidants and enzymes, like superoxide dismutase (SOD), catalase, and glutathione peroxidase. The term oxidative stress describes the disturbance of the redox homeostasis in favor of increased levels of ROS resulting in the development of undesired effects of ROS.

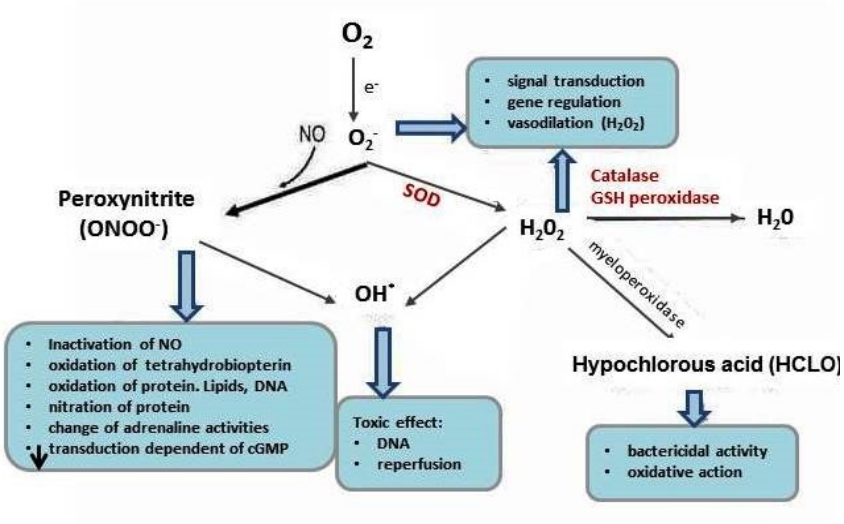

Fig. 1. *Reactions mediated by O2-. A major mechanism by which O2- contributes to vascular disease is via O2- -mediated inactivation of NO. This reaction is essentially diffusion-limited (Km 1.9 x 1010 mol/L/sec). Much slower are two O2- dismutation reactions to H_2O_2: a faster one is that with an aid of superoxide dismutase (SOD) (1,8 x 109 mol/L/sec), and ~10 thousand times slower reaction is that occurring spontaneously (i.e., without SOD) (8,0 x 104 mol/L/sec). Peroxynitrite and H_2O_2 may be a source of highly toxic hydroxyl radical (OH*). Usually, H_2O_2 is inactivated directly to H_2O (without generation of OH*) by catalase and glutathione peroxidase. However, in the presence of reduced forms of iron (Fe^{2+}) and copper (Cu^+), H_2O_2 undergoes Fenton reaction, which becomes a source of OH*, and this is a reason why iron and copper may become a source of the redox toxicity. In the presence of the inflammatory cells-derived myeloperoxidase, H_2O_2 may be metabolized to a strong oxidant and bactericidal substance, hypochloric acid.*

It is believed that cardiovascular risk factors (hypercholesterolemia, hypertension, ageing, smoking, obesity, diabetes and others) mediate the production of excess vascular O_2^-. Superoxide, acting: (i) per se or via H_2O_2; (ii) via NO inactivation or (iii) via peroxinitrite and S-nitrosilation, would activate various signaling molecules (e.g., MAP kinases, thyrosine kinases, phosphatases, transcription factors), which ultimately mediate pro-atherosclerotic vascular activation, inflammation and remodeling (e.g., induction of endothelial adhesion molecules).

NADPH oxidase and its isoforms; Primary vascular source of ROS is the NADPH oxidase (Nox) family enzymes which produce ROS as their primary and sole function. Moreover, Nox-derived ROS may promote ROS formation from other, normally dormant enzyme sources (mitochondria, oksydaza ksantynowa, uncoupled eNOS) considered as secondary sources of ROS. Seven isoforms of Nox have been described in mammals differing in their activation mechanism, cellular localization, and type of the ROS produced. In cardio-vascular system in humans (and in guinea-pig, my own unpublished data) homologes Nox1, Nox2, Nox4, and Nox5 have been identified. However in mouse and rat, only Nox1, Nox2 and Nox4 are present. Best studied are Nox1 and Nox2 isoforms, which are partially plasmalemma-bound enzymes releasing O2- intra- and extracellularly. Nox4 and Nox5 are bound to the intracellular membranes and their biological function is less understood. Numerous studies have reported increased activity and expression levels of Nox1/2 isoforms in the vascular wall in rodent models of oxidative stress. Likewise, in humans with coronary artery disease and diabetes the overexperssion of Nox1/2 and also of Nox5 was noted. Consequently, it is believed that it is the upregulation of mostly Nox1/2 that underlies the mechanism of the vascular oxidative stress.

However, it has recently been reported that the pharmacological induction of Nox4 confers vascular protection, and that Nox4 gene deletion is deleterious for the endothelium. Possible explanation for this paradox would be that while Nox1/2 produce O_2^-, Nox4 is a producer of H_2O_2. Of these, only O_2^- inactivates NO. In addition, cellular signaling induced by O_2^- and H_2O_2 may differ substantially.

The present project is aimed at studying the individual role of various Nox homologes in the mechanism of vascular oxidative stress induced by diabetes. In particular, the project is aimed at verifying **the hypotheses that**: (1) the mechanism of vascular oxidative stress involves both the upregulation of the „toxic" Nox1/Nox2 and the downregulation of the "protective" Nox4; (2) Nox1/2 i Nox4 mutually regulate their expression, and (3) the endothelial NO is a likely intermediary in this mutual regulation.
In order to verify these hypotheses, three research tasks will be performed, and they are aimed at:
1. Studying cardiac expression of Nox1/2 and Nox4 protein in two models of the oxidative stress (i.e., seasonal and that associated with the type I diabetes) and in two species differing in their Nox5 expression. In this context, I have already performed a number of p**reliminary experiments,** which revealed, **in agreement with the hypothesis**, that the all four studied models of the oxidative stress were associated with increased: cardiac O_2^- production, enzymatic Nox activity, and Nox1/Nox2 expression, and simultaneously with decreased Nox4 and endothelial NO synthase (eNOS) expression (Western blot). In addition, cardiac O_2^- production showed a positive, linear correlation with the Nox2, but not Nox4 expression, and the expression of Nox4 and Nox2 showed an inverse linear correlation.
2. Studying the effect of exercise training and nitrite supplementation on cardiac Nox1/2 and Nox4 expression and on numerous indices of: (i) oxidative stress; (ii) NO homeostasis, and (iii) pro-inflammatory activation of the endothelium in rats with streptozotocin-induced diabetes. Training and nitrite are known to prevent oxidative stress and Nox2 overexpression in various animal models of the oxidative stress. Recently, it became evident that dietary nitrites can be converted (with an aid of body's reductases) to directly bioavailable NO.
3. Studying the effect of the prolonged eNOS inhibition (supplementation of eNOS inhibitor, L-NAME) on cardiac expression of Nox1/2 and Nox 4 and other indices (see point 2) in otherwise untreated rats.

If my hypothesis is correct: (i) diabetes should result in increased expression of Nox1/2, decreased expression of Nox4 and eNOS, and increased endothelial expression of adhesion molecules (ICAM-1, VCAM-1); (ii) chronic eNOS inhibition should produce the effects similar to those induced by the diabetes, and (iii) diabetes-induced effects should be prevented by the exercise training and nitrites.
Positive verification of the hypothesis may provide a starting point to the search for new strategies of the anti-oxidative therapy in CVD. These strategies should be probably aimed either at a selective elimination of one particular ROS, instead of all kinds of ROS or on an individual inhibition (or activation) of one particular Nox isoform, instead of acting on all Noxs together.