We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Mechanisms of HBO-Induced Vascular Functional Changes in Diabetic Animal Models

Ivana Jukic, Mihael Mišir, Martina Mihalj, Zrinka Mihaljevic, Sanela Unfirer, Dijana Kibel and Aleksandar Kibel

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.76569>

Abstract

The mechanisms by which HBO exerts its potentially beneficial effects are not completely clear. Interactions of mechanisms affecting endothelial dysfunction, NO synthesis, EETs and HETE formation, CYP expression changes, oxidative stress and antioxidant defense system changes, and multiple effects on inflammation take place that might be considered as mediating factors for the observed positive (or negative) clinical effects in diabetes mellitus (for instance in chronic diabetic wounds). Studies on vasculature in diabetic animal models can provide us with more information that can help us understand its effects on blood vessel function. This chapter discusses the most relevant studies that have assessed the potential mechanisms of HBO-induced vascular functional changes in diabetic animal models.

Keywords: hyperbaric oxygen, diabetes mellitus, endothelial dysfunction, cytochrome P450, nitric oxide, arachidonic acid metabolites

1. Introduction

Hyperbaric oxygen (HBO) therapy presents medical and experimental administration of 100% oxygen (O_2) at pressures above 1 atm [1, 2]. HBO is widely used for the treatment of various clinical diseases, but numerous studies indicate its benefit in conditions of vascular pathology [2]. The exact mechanisms that are involved in the actions of therapy with HBO₂ are largely unknown, although its effects have been documented clinically and in experimental models [2, 3]. Investigations focusing on physiological effects of hyperbaric oxygen on vascular function

still do not provide a clear mechanism of its action. They focus on endothelial function and dysfunction, as well as HBO-induced changes in concentrations and actions of physiological mediators of vascular function, such as nitric oxide (NO), acetylcholine, metabolites of arachidonic acids, and others. Some works also suggest that HBO might cause changes in conducted vasomotor responses and in that way influences vascular sensitivity and reactivity to vasodilators and vasoconstrictors [4].

2. Endothelial function and dysfunction

Endothelial cells are responsible for vascular tone, supply the thromboresistance, and determine the extent to which the vasculature is permeable to cells and molecules through the synthesis and release of a wide variety of substances [5]. The pathogenetic concept of micro- and macroangiopathy, which are well-known vascular complications of diabetes mellitus (DM) [6], is based on an endothelial lesion that is a result of parameters specific for diabetes, which damage the endothelium [6]. Although basal tone and myogenic reactivity are intrinsic to vascular smooth muscle, the ambient level of tone is modulated by various vasoconstricting and vasodilating mediators released by the endothelium. It is generally accepted that long-term diabetes is associated with endothelial dysfunction and reduced endothelium-dependent vasodilation [7, 8]. The main endothelium-dependent vasodilatory mediator is NO, but various metabolites of arachidonic acid such as prostaglandins, epoxyeicosatrienoic acids (EETs), and hydroxyeicosatetraenoic acids (HETEs) also contribute to vascular responses to different stimuli [9, 10] and may be essential for vascular response in various physiologic and pathological conditions such as diabetes mellitus [11–13].

Hyperbaric oxygen therapy affects the function and structure of cerebral resistant arteries, which is impaired in DM and will have beneficiary effect on vascular function by modulating mechanisms of vascular responses to various dilator and constrictor agonists, leading to restored vascular reactivity. It has been demonstrated that hyperglycemia, acute or chronic, may cause several changes in vascular function, including a decrease in endothelium-dependent vasodilation and an increase in contractile response of vascular smooth muscle [14]. Impaired endothelium-dependent relaxation has been shown in various vascular beds of different animal models [15]. The mechanisms associated with these observations may include changes in synthesis, release, and degradation of various factors that are produced by endothelium. The most notable characteristic of endothelium dysfunction in DM is the vascular NO reduction. Various multiple mechanisms are involved in this effect, but it seems that increased level of oxidative stress is the first alteration that triggers several others. Furthermore, the vascular smooth muscle sensitivity may be reduced, which certifies the vascular studies in human and animal models of DM that showed reduced sensitivity of vascular smooth muscle to NO donors [16].

On the other side, endothelial dysfunction may also be related to the release of vasoconstrictor factors. In vessels of diabetics, there is an increase in endothelium-dependent vasoconstrictor mechanisms, mostly mediated by prostanoids, which play an important role in endothelium dysfunction. TxA₂ plays a role in the reduced endothelium response in type 1 DM, but it may also be involved in the enhanced contractile response to vasoconstrictor stimuli [17]. Furthermore, hyperglycemia increases the COX-2 expression, causing enhanced release of

vasoconstrictor and prostanoids [18]. Hyperglycemia not only modifies the profile of prostanoids, leading to alteration of vasomotor tone, but also increases the release of arachidonic acid by vascular cells [19].

An increasing number of evidence proposes that HBO induces neuronal nitric oxide (NO) synthase (NOS) activity, while the influence on endothelial NOS (eNOS) activity and vascular NO bioavailability remains unclear [20]. Thom et al. reported that NO bioavailability in rat and mouse cerebral cortex was increased during HBO exposure, and cerebral NO production was enlarged much more in knockout mice lacking genes for eNOS than in those lacking genes for nNOS [21]. Studies on conscious rats with inhibition of NOS were used to assess the dynamics of cerebral blood flow during hyperbaric oxygenation and had shown that hyperbaric oxygen changes cerebral blood flow and modulates oxygen neurotoxicity via eNOS and nNOS [22]. eNOS- and nNOS-deficient mice were used to study the contributive roles of the NOS isoforms in mediating changes in cerebral vascular tone in response to hyperoxia, and results demonstrate that under HBO, eNOS-derived NO is responsible for the early vasoconstriction, whereas late HBO-induced vasodilation depends upon both eNOS and nNOS [23].

3. Influence on arachidonic acid metabolites and the renin-angiotensin system

HBO should be viewed as a factor for increased availability of oxygen as an active molecule in changing vascular function. HBO, CYP450 activity alternations, and arachidonic acid (AA) metabolism are connected in many different pathways. Besides vascular reactivity changes due to epoxidation reactions, Hjelde et al. showed that anti-inflammatory effect of HBO is mediated by reducing expression of cyclooxygenase-2 and reducing the number of intercellular adhesion molecules and therefore reducing adhesion and infiltration of leucocytes [24].

In various aspects of metabolic diseases, evidence from different studies suggests a role for enzymes involved in arachidonic acid (AA) metabolism, including cytochrome P450 (CYP) epoxygenases and soluble epoxide hydrolase (sEH), and their eicosanoid metabolites (epoxyeicosatrienoic acids (EETs)) [25–27]. EETs have been shown to exert beneficial effects on diabetes-related endothelial dysfunction, enhanced cardio protection, and alleviation of diabetic nephropathy. In contrast, CYP4A proteins were upregulated in the livers of mice with genetically induced and diet-induced diabetes [28].

Arachidonic acid in endothelial cell can be metabolized in three different pathways: CYP450 enzymes (omega-hydroxylase and epoxygenase), cyclooxygenase and lipoxygenase, and non-enzymatic degradation of arachidonic acid in the presence of free radicals to isoprostane [29]. Epoxygenase is a cytochrome P450 family of enzymes (primarily CYP2C and CYP2J families), which in the endothelial cell produces 4 epoxyeicosatrienoic acid (EETs) isomers (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET), of which 14,15-EETs and 11,12-EETs are the most active metabolites [30]. In most cell types and organs, EETs can be present as dihydroxyeicosatrienoic acids (DHETs) [31], which are more stable and less bioactive than EETs. DHETs are produced by sEH hydrolysis of EETs [32]. There is no evidence of EET production in a smooth muscle cell. In a smooth muscle cell, cytochrome P450 ω -hydroxylase promotes the production of

20-hydroxy-eicosatrienoic acid (20-HETE), which is a vasoconstrictor. Cyclooxygenase (COX) is an enzyme existing in two isoforms, COX-1 and COX-2, involved in the synthesis of prostanoid from arachidonic acid (AA). The resulting prostanoids act in contradiction, causing vasodilation (prostaglandin D₂, prostaglandin E₂, and prostacyclin I₂) and vasoconstriction (prostaglandin F₂ α and thromboxane A₂). Hypoxia activates the COX pathway, where mostly prostacyclin, PGI₂, is generated. It diffuses into the smooth muscle cell in which it activates the enzyme adenylate cyclase and increases the amount of cyclic adenosine monophosphate (cAMP). cAMP promotes the opening of several types of potassium channels, resulting in hyperpolarization of the smooth muscle membrane with consequent vasodilation [33]. Lipoxygenase is an enzyme that from AA generates 12- and 15-hydroxy eicosatrienoic acids (HETEs) as the major active metabolites in the endothelial cell [29, 34].

Streptozocin-induced diabetes in rats (a model for type 1 diabetes mellitus) reduces the levels of protective EETs, and the reduced EET levels lead to exacerbation of stroke [35]. Tsai et al. showed impaired endothelium-dependent vasodilation of coronary arterioles caused by reduced CYP activity and EET production due to increased glucose-induced superoxide levels in coronary endothelial cells [36]. EETs might constitute a key link between insulin resistance and endothelial dysfunction [37]. Endothelial dysfunction in diabetes could also be related to the release of vasoconstrictor mediators, e.g., increased production of 20-HETE leading to activation of ROS through an NAD(P)H-dependent pathway. Diabetes alters CYP expression and 20-HETE formation, leading to upregulation of CYP4A isoforms and to elevated levels of 20-HETE [37]. Li et al. also suggested contribution of 20-HETE to endothelial dysfunction in diabetes and other insulin-resistant conditions showing the attenuation of diabetes-induced vascular dysfunction by using the 20-HETE inhibitor HET0016 [38]. Insulin-stimulated vasodilation mediated by the IRS-1/PI3K/AKT/eNOS pathway can be impaired by 20-HETE [39]. Issan et al. associated dysfunction of circulating endothelial progenitor cells and angiogenic capacity with increased levels of CYP-derived 20-HETE in diabetic patients with cardiac ischemia [39]. P450 4A metabolite 20-HETE by vascular tissue is directly dependent on the concentration of oxygen within the normal physiological range of blood and tissue PO₂ [40]. It is known that various arachidonic acid metabolites (prostaglandins, EETs, HETEs) and NO are of utmost importance in the mediation of vascular reactions to vasodilators and vasoconstrictors [41–46], including hypoxia and hyperoxia stimuli [46]. In conditions of reduced blood flow, the use of HBO can significantly increase tissue oxygenation. Although all P450 enzymes require molecular oxygen, the majority of them (such as those found in the liver) require only very low PO₂ levels for normal activity. Results from our previous study suggest that hyperbaric oxygen increases vascular sensitivity to EETs, instead of significantly increasing EET synthesis [3]. Our studies also show that HBO is a highly effective treatment for stroke even in the presence of long-term untreated diabetes, by inhibition of 20-HETE production [47]. Unfirer et al.'s study showed changes in the dilatation mechanisms in diabetic rats under the influence of hyperbaric oxygenation. It has been shown that hyperbaric oxygenation causes activation of the CYP450 epoxygenase pathway and increased EET production in diabetic animals exposed to HBO [13]. Furthermore, Kibel et al. showed a changed relaxation response to ANG-(1–7) influenced by HBO in healthy and diabetic animals, where they also linked to a changed mechanism and improved relaxation after HBO with CYP450 activation and EET synthesis [3, 11]. HBO was shown to increase relaxation responses to ANG-(1–7) in rat aortic rings of diabetic animals, and this effect was eliminated with the addition of an EET

synthesis inhibitor. There was no effect of HBO on ANGII reactivity of these aortic ring preparations nor was there a difference in serum concentrations of ANG-(1–7) [3]. mRNA and protein expression of several CYP isoforms that are involved in EET synthesis were also shown to be upregulated in aortic samples of animals, where DM was caused by streptozocin [3].

Both HBO as a treatment and in vitro hyperbaric oxygenation have been shown to change reactivity of rat thoracic aortic ring preparations to certain compounds [20, 48]. It is well known that changes in oxygen availability are crucial in the control of vascular tone, leading to changes in production of, or vessel sensitivity to, vasoconstrictor and vasodilator metabolites of arachidonic acid and nitric oxide (NO) [40, 49, 50]. The production of EETs is known to be reduced with a decrease in PO_2 [42]. EETs have been recognized to induce vasorelaxation and enhance K^+ current in smooth muscle cells, in addition to others (including pro-angiogenic, anti-inflammatory, and pro-fibrinolytic effects) [51–54].

CYP P450 3A13 was found to be involved in oxygen sensing, mediating ductus arteriosus constriction to oxygen, together with endothelin-1 [55]. Considering this, along with the interaction of arachidonic acid pathways with nitric oxide pathways in oxygen sensitivity [49], regional differences of arachidonic acid metabolite roles, and various conflicting evidence [49], it is clear that role of CYP450 enzymes in oxygen homeostasis is very complex and may be significant factor mediating the responses to HBO.

4. Changes in acetylcholine pathways

In the literature, there are a lot of studies on animal models of diabetes mellitus that confirmed impaired mechanisms of vasodilation and vasoconstriction. Streptozotocin-induced diabetes mellitus in rats demonstrates attenuated vasodilation response to acetylcholine [56, 57]. Experiments on healthy mouse coronary arteries demonstrate that vasodilation to acetylcholine is accomplished 50% by NO and 50% by EDHF. In spontaneously diabetic mouse type II (db/db), that ratio is 81% to production of EDHF [12].

Unfirer et al. [13] first investigated mechanisms of vasorelaxation in diabetic animal models after HBO exposure. Thoracic aortal rings from SD rats were used to evaluate vasorelaxation responses to acetylcholine after preconstruction with noradrenalin. With NG-nitro-L-arginine methyl ester (L-NAME)-(NOS inhibitor), indomethacin-(COX inhibitor), and N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH)-(CYP 450-epoxygenase inhibitor), they investigated which pathway is involved in enhanced vasorelaxation responses in diabetic and healthy rats after HBO exposure. HBO exposure protocol was performed in therapeutic range [58]. DM duration of 6 weeks did not change vasorelaxation response in diabetic group, and after application of inhibitors, results showed that the NO pathway is dominant in macrocirculation. In the diabetic and healthy groups, after HBO exposure, there was partial inhibition of vasorelaxation after NOS inhibition, which indicates that other pathways were included in vasorelaxation mechanisms. MS-PPOH partially blocked vasorelaxation in both HBO groups, which indicates that HBO changes vasorelaxation mechanisms to alternative pathways—enhanced production or sensitivity to EETs. Indomethacin did not inhibit vasorelaxation in any group, so COX pathway did not have influence. These findings were verified with upregulation of eNOS and COX-1 enzymes in the diabetic HBO

group and higher protein expression of CYP450-4A1/A2/A3 in both HBO groups when compared with their respective controls. Also in this study, there was not oxidative stress caused by HBO because thiobarbituric acid-reactive substances (TBARSs) were elevated in DM group but were normal in the healthy HBO group. This difference between studies is probably a result of different experimental protocols (intermittent hyperbaric oxygenation—2 hours, 4 days at 2.0 atm abs vs. 90 minutes, 7 days at 2.4 atm abs in Matsunami study [59]).

Same authors investigate HBO effect on microcirculation (middle cerebral arteries) in diabetic animal model, 6-week duration of DM. Preliminary results shown impaired vasodilation response in diabetic rats and restored vasodilation after HBO exposure. Using inhibitors such as indomethacin (COX), NG-monomethyl-L-arginine (L-NMMA) (NOS), and clotrimazole (nonselective CYP 450 inhibitor), they notice shift in vasodilation mechanisms from mainly NO pathway toward two other pathways COX/CYP 450 because in both HBO groups, L-NMMA did not blocked vasodilation to acetylcholine. Further investigation is necessary [60].

In normal condition, vasodilation response to hypoxia is made by activating cyclooxygenase (COX) and production of prostacyclin (PGI₂) [61]. There is evidence that CYP 450-epoxigenase enzyme in minor part causes vasodilation in healthy vessels [62]. Experiments on middle cerebral arteries (MCAs) of 6 weeks diabetic rats that underwent HBO exposure were used to evaluate the effect of HBO in acute hypoxia. They used COX inhibitor indomethacin and selective CYP 450 epoxigenase inhibitor MS-PPOH. COX inhibition partially preserved vasodilation in HBO groups, and eliminated vasodilation in response to hypoxia in the presence of MS-PPOH in both HBO groups suggests that HBO activates CYP450-epoxigenase in MCAs of healthy and DM rats and shifts vasodilation mechanisms in response to acute hypoxia [63].

5. Effects on oxidative stress [reactive oxygen species (ROS)]

Life on Earth is impossible without oxygen that is in our atmosphere, which consists of 21% oxygen. Paradoxically, oxygen can also potentially be very toxic for organisms that use it. Free radical formation occurs continuously in cells as a consequence of both enzymatic and nonenzymatic reactions [64]. The main compartments of these kinds of reactions in cells are mitochondria. Mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, mitochondria are the site of significant reactive oxygen species (ROS) production [65]. The term “ROS” is generally used to describe reactive molecules containing oxygen. Such molecules have many common and similar characteristics; they also exhibit very different features, resulting in potentially beneficial or even toxic effects [66]. On the other hand, the term reactive oxygen species (ROS) can be defined as highly reactive oxygen-centered chemical species containing one or two unpaired electrons, where an unpaired electron is one that exists in an atomic or molecular orbital alone. The unpaired electron containing chemical species can also be called “free radicals.” Furthermore, the term “ROS” can also be used as a “collective term” to include both radicals and nonradicals, the latter being devoid of unpaired electrons. So, ROS is classified into two categories: (1) oxygen-centered radicals and (2) oxygen-centered nonradicals. Oxygen-centered radicals include superoxide anion (O_2^-), hydroxyl radical (OH), alkoxyl radical ($RO\cdot$), and peroxy radical ($ROO\cdot$). Oxygen-centered nonradicals are

hydrogen peroxide (H_2O_2), singlet oxygen (O_2 , high-energy form of oxygen), and hypochlorous acids (HOCl) [67]. Sometimes when ROSs break the upper concentration limit of cellular antioxidant defense system capacity, based on high ROS intracellular concentration or low cellular antioxidant defense system, oxidative stress will show up and manifest with nucleic acids, proteins, and lipids damage, leading to carcinogenesis, neurodegenerative disorders, atherosclerosis, diabetes, and aging [68]. Under normal physiological conditions, ROS and the peroxidized molecules are neutralized by a powerful antioxidant system involving superoxide dismutases, catalases, glutathione S-transferases, and thioredoxins [69].

In diabetes and hyperglycemia in general, NADPH oxidase represents the principal source of ROS production in different organs [67]. The most acceptable thesis is that oxidative stress, as a main result of HBO, is a major trigger of most of its effects, but the exact mechanisms are not completely clear. It could be confusing to understand different consequences of HBO depending on protocol type that was used. For example, the duration of exposure, the used oxygen pressure, the subject species, and the underlying disease are factors that may play a role in changes of blood pressure levels [70], and changes of specific oxidative parameters depend on lapsed time after exposure or on the number of repeated exposures (analyzing rat lung tissue) [71, 72]. Although increased superoxide dismutase and glutathione peroxidase activity and increased thiobarbituric acid-reactive substance levels are documented, after some hyperbaric protocols, there is no change in aforementioned enzyme concentrations in red blood cells. On the other hand, a significant induction of heat shock protein HSP70 in lymphocytes after even a single HBO₂ treatment was noted—this might be due to activation of compensatory mechanisms by HBO₂ [70]. After hyperbaric treatment with high oxygen concentration, an increased ROS production is noticed, but paradoxically, HBO induces an antioxidant environment in plasma by increasing the plasma catalase activity. Different studies have documented increases in the total plasma antioxidant capacity determined after a session with HBO [73]. The therapeutic use of HBO can give positive results by activation of ROS resulting in increased perfusion, reduced edema, decreased inflammatory cytokines, increased fibroblast proliferation, increased collagen production, and angiogenesis promotion. Finally, increase of ROS may improve the regulation of antioxidant enzyme activity of tissues [74].

6. Inflammation

Pathological effects of DM on the vascular wall include enhanced ROS production and endothelial activation leading to inflammation, atherogenesis, and vascular dysfunction, which further results in clinical impairment of the micro- and macrocirculation. Interestingly, positive therapeutic effects of HBO₂, such as antioxidative and anti-inflammatory effects, have been attributed to the enhanced ROS production induced by the HBO₂ treatment [1].

Numerous studies on experimental DM animal models revealed ongoing vascular inflammation under diabetic/hyperglycemic conditions, characterized by (a) increased proinflammatory cytokine levels, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α); (b) endothelial activation followed by increased expression of vascular cellular adhesion molecule-1 (VCAM-1);

and (c) increased leukocyte homing to the vessels and tissues induced by excessive secretion of chemokines like monocyte chemoattractant protein (MCP-1) [75–77]. In addition to that, same noxa that lead to inflammation also precipitate development of vascular dysfunction, marked by substantial decrease in NO bioavailability, which is discussed in more detail elsewhere in this chapter [78]. Studies on diabetic (db/db) and control (db/+) mice have shown that DM prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation, particularly the TNF- α [79]. Similar results were also obtained from experiments using type 1 DM animal model (mice receiving multiple low-dose streptozotocin treatments), suggesting that the observed proinflammatory status of diabetic mice is predominately linked to hyperglycemia rather than pathomechanism involved in the development of a specific type of DM [80]. Additionally, impaired function of macrophages, including reduced efferocytosis and anti-inflammatory cytokine expression, has been attributed to the prolonged and ineffective resolution of inflammation in the wounds of diabetic mice, which is a leading complication in diabetic humans [81]. This was further confirmed by intravital microscopy that allowed researchers to real-time follow-up leukocytes in live diabetic and healthy control mice, which was followed by leukocyte isolation and functional tests that all together revealed enhanced recruitment but defective function of leukocytes during the inflammation in mouse models of type 1 and type 2 DM resulting in defective bacterial clearance [82]. Studies have also shown that hyperglycemia changes the intrinsic TCR-induced naïve T activation to increased T cell responsiveness in diabetes [83]. In the kidneys, the observed proinflammatory condition in DM animals has been linked to oxidative stress-induced JNK activation [84]. It has also been shown that diabetic condition facilitates binding of monocytes to vascular smooth muscle cells and their subsequent differentiation through induction of key chemokines in the vasculature, which can lead to enhanced atherogenesis [85]. In addition, endothelial cells (EC) express pattern-recognition receptors including Toll-like receptors (TLR) that have a central role in recognizing pathogens and damage signals and initiating immune responses [86]. It seems that in the vessels of diabetic animals/individuals, increased oxidative stress, free fatty acids, and hyperglycemia are directly involved in the pathogenesis of vascular inflammation via several cellular mechanisms, including TLR-mediated activation of protein kinase C (PKC) and NF- κ B pathways resulting in increased expression of the proinflammatory molecules such as IL-6 and TNF- α . In turn, secretion of cytokines IL-1 and TNF- α increases NF- κ B activity and production of cellular adhesion molecules by endothelial cells, further aggravating the inflammation [87].

Some of the beneficial anti-inflammatory effects of HBO include reduced proinflammatory cytokine expression, suppressed development of T helper cells, shrinking of spleen and lymph nodes, decreased responses to antigens, recruitment and differentiation of circulating stem cells, and reduced frequencies of circulating leukocytes [88, 89]. However, these effects were mainly observed in studies exploring experimental animal models of colitis, while in the particular case of DM, data on the effects of HBO on the vascular inflammation are scarce. This is in contrast to our knowledge about the effects of the HBO on the wound-healing mechanisms that have been subjects of intensive investigations for many years, which lead to profound understanding of the clinically observed positive effects of HBO [90].

Beneficial effects of HBO on the wound-healing processes include facilitation of the neovascularization through enhanced regional angiogenic stimuli and increased recruitment and differentiation of circulating stem cells from the bone marrow [1]. Under ischemic and hyperglycemic conditions, HBO further promotes wound repair by increasing tissue perfusion

and collagen deposition [91]. A study on an experimental wound model revealed increased synthesis of vascular endothelial growth factor (VEGF) in damaged tissue during HBO₂, which is the most specific growth factor for neovascularization [92]. It is controversial that HBO₂-induced oxidative stress leads to hypoxia-inducible factor (HIF)-1 and 2 mediated transcriptions of many genes involved with neovascularization, including stromal-derived factor-1 (SDF-1) and its counterpart ligand, CXCR4, as well as VEGF [1]. These effects could be especially beneficial for DM individuals whose stem cell mobilization is compromised by impaired NOS activity in the bone marrow [1].

It has been shown that HBO inhibits ischemia reperfusion induced β 2-integrin-dependent adhesion of neutrophils to the endothelium by blocking CD18 surface polarization and through S-nitrosation of β 2-integrin, with no effect on the cell-surface expression of β 2-integrins [93]. Studies on monocyte-macrophages retrieved from healthy humans and animals exposed to HBO in vivo or cells exposed to HBO under in vitro condition revealed lower stimulus-induced proinflammatory cytokine production upon exposure to HBO₂ [1, 94].

Studies on ApoE KO mice that exhibit accelerated atherosclerosis and related complications showed that HBO₂ reduces the circulating levels of antibodies to _{MDA}LDL and dampens delayed hypersensitivity response to oxLDL challenge. The same studies demonstrated significant reduction in the production of proinflammatory cytokines, along with marked increase in the constitutive production of the anti-inflammatory cytokine IL-10 in splenocytes stimulated by LPS [95]. This effect was independent of antigen specificity, as indicated by polyclonal activation of T cells.

7. The role of HBO in stroke

Approximately 25% of all stroke patients have DM and 40% have hyperglycemia, which is associated with worse neurologic outcome as well as higher risk of recurrence of stroke [96, 97]. Diabetic patients, compared to nondiabetics, are known to be more sensitive to cerebral ischemia. Thus, the same duration of ischemia results in more severe neurologic deficits and larger brain infarcts in diabetic patients. Female patients with DM have 4.8-fold higher risk for developing ischemic stroke than the general population (compared to 3.7-fold for men) and more often suffer fatal strokes (standardized mortality ratios of 3.1 for males and 4.4 for females) [98–100]. The outcome is frequently lethal, regardless of any therapy undertaken, including recombinant tissue plasminogen activator (rtPA) and mechanical thrombectomy. Possible underlying causes are chronic hyperglycemia, which leads to free oxygen radicals and cytokines production and increases ischemic brain cells predisposition to apoptosis [101]. In addition, the intimal artery thickening and arteriolar occlusion occur in diabetes, contributing by impaired vascular function to inadequate tissue perfusion. Moreover, DM is, in some cases, such as treatment of recurrent stroke with thrombolysis, one of the exclusion criteria [102].

A total of 90–95% diabetic patients are type 2 DM of noninsulin dependence and 5–10% are type 1 DM of insulin dependence. Type 2 DM patients have asymptomatic period of hyperglycemia for about 4–7 years that leads to most important problems—chronic complications of diabetes, leading to disability and premature death [103]. First diabetic complications are associated with

microangiopathy of retina, kidney, and peripheral neuropathy and next with macroangiopathy causing myocardial infarction, stroke, hypertension, and peripheral artery lesion. Patients with DM have progressive cerebrovascular atherosclerosis and increased cerebral vascular reaction to vascular constrictors, a deregulated reaction to vascular dilators and damaged automatic regulation of brain-blood stream. Damaged endothelium and vascular motor function of small arteries can lead to hypoperfusion of certain areas of the brain in diabetic patients.

The principles of HBO are based on physical laws and mechanisms of oxygen transport in human body. At sea level (1 ATA), almost all hemoglobin is saturated with oxygen, and HBO can increase its saturation only slightly. However, HBO increases the amount of oxygen dissolved in plasma from 0.3 to 5.6% at 2.5 ATA, and due to this mechanism, it increases tissue oxygenation even in areas where erythrocytes cannot pass [104]. Due to oxygen pressure gradient, HBO promotes diffusion of oxygen to longer distances in ischemic region. HBO₂ raises oxygenation of ischemic penumbra by 20% and improves mitochondrial function [105, 106]. Single or multiple exposures to HBO create environment of intermittent relative hypoxia that can not only prepare tissue for longer hypoxia but also save tissue until other salvation strategies (such as thrombolysis, mechanical thrombectomy, stenting, and endarterectomy) take effect [47, 107]. Not only oxygen in ischemic core and penumbra itself plays a vital role in surviving tissues; HBO also influences on many different pathophysiological mechanisms. HBO improves oxygen delivery to ischemic brain tissue due to the higher arterial blood-brain oxygen gradient.

In animal models, it stabilizes blood-brain barrier (BBB) and therefore reduces brain edema formation. It improves brain microcirculation and brain metabolism, creating sufficient energy and ion homeostasis needed for survival of cells until reperfusion or collateral circulation creation. Some concern was about vasoconstriction of arteries under HBO. This can be applied to normal, but not ischemic vessels, where secondary vasodilatation is salvation mechanism and vasoconstriction does not appear. HBO actually improves microcirculation in ischemic areas [108, 109]. HBO reduces poststroke inflammation by various mechanisms, reduces the number of brain cells undergoing apoptotic pathways and necrotic death, and if applied early, it can reduce ischemia-reperfusion injury and reduce oxidative stress. These combined effects reduce brain edema and modulate cerebral vascular flow resulting in reduced intracranial pressure. Longer effects of HBO include promotion of angiogenesis and neurogenesis in ischemic tissues with positive effect on neurorehabilitation. In numerous animal experimental models, HBO was effective in reducing brain infarction after stroke. However, few human studies were so successful.

HBO has been used in humans in many different stroke types (hemorrhagic, ischemic, large and small artery stroke, global ischemia, etc.) using different pressures, protocols of application (single or multiple) and in different poststroke time windows. Due to these inconsistent standards, some studies showed lack of effect and other benefits. Another point of concern is that only the small number of these studies were well-designed randomized controlled trials and that their limitations include the small number of patients, which means that precise conclusions cannot be drawn. Some cautious conclusions could be suggested. HBO is so far the only effective early treatment of air embolism (mostly after surgery). HBO early after stroke improves recovery after stroke, but this effect progressively decreases if treatment is applied later. The most significant results are achieved in first 3 hours after stroke (similar to thrombolysis and other revascularization trials). Time window for HBO is 3–6 hours in

acute ischemic stroke. The question of later and repetitive administration of HBO shows some promising results; however, they are still based on a few clinical cases and lack scientific proof and larger number of cases. Multiple repetitive HBO has positive effect on cognitive recovery after stroke and metabolism of temporal lobe. In one clinical trial, HBO combined with antidepressants showed better results than any of these therapies alone. HBO reduces cerebrovascular vasospasm and secondary brain infarctions after aneurismal subarachnoid hemorrhage (SAH). In intracerebral hemorrhage patients, HBO also provided improvement if started early, and the patient is stable [110].

When one thinks about treating acute stroke in diabetic patients with HBO, a few still unanswered questions arise, mostly due to the paucity of experiments in these settings. There are a few experiments conducted in animal models, but they vary in criteria for its use. In humans, we can rely only on a small number of cases with very diverse inclusion criteria and different results. Therefore, we can only draw some direct and more indirect conclusions about it from experiments on nondiabetic stroke experiments.

There is a question of optimal model of animal stroke in diabetic animals. The most commonly used experimental model of stroke in rats is a model of middle cerebral artery occlusion (MCAO) by intra-luminal suture. There are variations of this model in terms of use of permanent or transitory MCA occlusion-induced ischemia. The duration of occlusion varies in models from permanent MCAO to transitory MCAO (t-MCAO) of 180, 120, 105, or 60 minutes [111]. Taking into account the observed differences in clinical presentation of diabetic vs. nondiabetic patients with stroke, there are few issues that variations in experimental approach to stroke study are brought to light. For example, in diabetic rat stroke models, the same duration of MCAO as in nondiabetic rat models is used.

The usual duration of t-MCAO used in non-diabetic rats was 60-120 minute [112]. In diabetic rats the same duration of t-MCAO produced massive stroke with malignant brain edema, devastating neurological deficits (such as inability to move, eat and drink) that become worse over time, leading to unconsciousness and death of animals within the first 24 hours (mostly due to massive edema and a rise in intracranial pressure). If ischemia lasts too long, laser Doppler flowmetry (LDF) finds lesser than expected reperfusional values. This brain vascular sign could be a marker of point of no return in stroke treatment [111]. Therefore (to develop the adequate diabetic female rat model, using transitory middle cerebral artery occlusion (t-MCAO) that would produce treatable stroke conditions in rats with diabetes), one has to significantly shorten the duration of t-MCAO to avoid already-irreversible brain infarct with brain vascular derangement. One study suggests that 30-minute t-MCAO could be a more appropriate stroke model than the usual 60-120 minute t-MCAO models, consistently producing medium-sized stroke, which affects 30–50% of ischemic hemisphere [111] (865443). Similarly, patients with the most severe strokes of the whole MCA territory and high National Institute of Health Stroke Score (NIHSS) not only are poor candidates for treatment with thrombolysis and mostly die due to brain edema and complications of dysphagia and immobility, but also have higher risk of secondary hemorrhage.

In conclusion, it is questionable to compare results of artery occlusion for rats with and without diabetes, even if the duration of t-MCAO is equal.

The only effective pharmacological therapy of acute ischemic stroke in humans is thrombolysis with recombinant tissue plasminogen activator, but DM is sometimes an exclusion criterion in recurrent stroke treatment. The time window for the therapy is narrow, and no other pharmacological agents have demonstrated efficacy in improving outcomes after ischemic stroke [1–4, 100, 102]. Thus, the searches for alternative approaches are welcomed. HBO [113] improves oxygen delivery and postischemic metabolism, restores ion pump function, and allows time for collateral circulation to develop [107]. In normal tissue, it causes vasoconstriction, but in ischemic brain tissue, it increases microvascular flow and improves oxygen dissolution and transport [109]. Time window for HBO application may be up to 6 hours [108], which is longer than the time window for thrombolytic therapy. HBO raises oxygenation of ischemic penumbra by 20% and improves mitochondrial function [107, 108]. It has anti-inflammatory effect by reducing expression of cyclooxygenase-2 and reduces the number of intercellular adhesion molecules and therefore reduces adhesion and infiltration of leukocytes [24]. However, guidelines do not recommend HBO treatment for acute ischemic stroke due to somewhat inconclusive data [102]. Some data imply that the intervention may be harmful causing middle ear trauma, epileptic seizures, and claustrophobia, while others found no firm evidence that HBO improves clinical outcomes for acute stroke. However, the main disadvantage of these trials used in meta-analysis was delay from stroke onset to initiation of HBO and the need for care delivery in a specialized chamber [114].

To conclude, HBO is currently not recommended for patients with acute ischemic stroke outside of clinical trials (except caused by air embolism).

On the other hand, some preclinical experiments suggest that if administered shortly after the stroke, HBO is highly effective treatment of stroke in diabetic female rats, even in the presence of long-term untreated DM [109]. Experiments that did not show effectiveness of HBO were possibly unsuccessful due to the unrecognizing the vulnerability of neurons. They used prolonged ischemia and applied HBO treatment too late after stroke.

8. Conclusion

The mechanisms by which HBO exerts its potentially beneficial effects are not completely clear. They cannot be simply explained as a consequence of supplementation of the oxygen deficit in certain conditions where oxygen is lacking, but it was demonstrated that HBO affects signaling cascades in cells and has multiple interacting complex mechanisms that might contribute to functional changes of blood vessels. Interactions of mechanisms affecting endothelial dysfunction, NO synthesis, EETs formation, CYP expression changes, oxidative stress and antioxidant defense system changes, and multiple effects on inflammation take place that might be considered as mediating factors for the observed positive (or negative) clinical effects in diabetes mellitus (for instance in chronic diabetic wounds). Studies on vasculature in diabetic animal models can provide us with more information that can help us understand its effects on blood vessel function, and **Table 1** summarizes the most relevant mechanisms that have been described in this text regarding functional vascular changes in

Target group of mechanisms or single mechanism	Effect	References
Endothelial dysfunction	↑ NO bioavailability	[20–23]
Arachidonic acid metabolites	↑ EETs synthesis, CYP epoxygenase expression, vascular sensitivity to EETs (?) ↓ 20-HETE	[2, 3, 11, 13, 47]
Oxidative stress	↑ ROS ↑ Antioxidant defense systems (?)	[2, 70–74]
Inflammation	↓ Proinflammatory mediators ↑ Angiogenic mediators	[1, 2, 90–94]
Renin-angiotensin system	↑ Vascular reactivity to ANG-(1–7)	[2, 3, 11]
Physical effects	↑ Dissolved oxygen in plasma and tissues	[104–106]

Table 1. Major potential mechanisms of HBO-induced vascular functional changes in diabetic animal models.

animal experimental models of diabetes. However, this represents only a part of the complete picture, and further studies are necessary to completely elucidate all the mechanisms involved in the effects of HBO on blood vessels.

Conflict of interest

The authors have no conflict of interest to declare.

Author details

Ivana Jukic¹, Mihael Mišir^{1,2}, Martina Mihalj¹, Zrinka Mihaljevic¹, Sanela Unfirer^{1,3}, Dijana Kibel^{1,4} and Aleksandar Kibel^{1,5*}

*Address all correspondence to: aleksandar_mf@yahoo.com

1 Department of Physiology and Immunology, Faculty of Medicine, University of Osijek, Osijek, Croatia

2 Neurological Clinic, Osijek University Hospital, Osijek, Croatia

3 Emergency Department, Osijek University Hospital, Osijek, Croatia

4 Department of Diagnostic and Interventional Radiology, Osijek University Hospital, Osijek, Croatia

5 Department for Heart and Vascular Diseases, Osijek University Hospital, Osijek, Croatia

References

- [1] Thom SR. Oxidative stress is fundamental to hyperbaric oxygen therapy. *Journal of Applied Physiology* (1985). Mar 2009;**106**(3):988-995
- [2] Drenjancevic I, Kibel A. Restoring vascular function with hyperbaric oxygen treatment: Recovery mechanisms. *Journal of Vascular Research*. 2014;**51**:1-13
- [3] Kibel A, Novak S, Cosic A, Mihaljevic Z, Falck JR, Drenjancevic I. Hyperbaric oxygenation modulates vascular reactivity to angiotensin-(1-7) in diabetic rats: Potential role of epoxyeicosatrienoic acids. *Diabetes & Vascular Disease Research*. Jan 2015;**12**(1):33-45
- [4] Drenjancević-Perić I, Gros M, Kibel A. Influence of hyperbaric oxygen on blood vessel reactivity: Concept of changes in conducted vasomotor response. *Collegium Antropologicum*. Jun 2009;**33**(2):681-685
- [5] Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. *Open Cardiovascular Medicine Journal*. Dec 23, 2010;**4**:302-312
- [6] Gärtner V, Eigentler TK. Pathogenesis of diabetic macroand microangiopathy. *Clinical Nephrology*. 2008;**70**(1):1-9
- [7] Fujii KD, Heistad D, Faraci FM. Effect of diabetes mellitus on flow-mediated and endothelium-dependent dilation of the rat basilar artery. *Stroke*. 1992;**23**:1494-1498
- [8] Mayhan WG, Simmons LK, Sharpe GM. Mechanism of impaired responses of cerebral arterioles during diabetes mellitus. *The American Journal of Physiology*. 1991;**260**: H319-H326
- [9] Armitage ME, Wingler K, Schmidt HH, La M. Translating the oxidative stress hypothesis into the clinic: NOX versus NOS. *Journal of Molecular Medicine*. 2009;**87**(11):1071-1076
- [10] Vallance PJT, Webb DJ, eds. Chapter 12: The eicosanoids: Cyclooxygenase, lipoxygenase and epoxygenase pathways. In: *Vascular Endothelium in Human Physiology and Pathophysiology*. 2008. pp. 332-361
- [11] Kibel A, Cavka A, Cosic A, Falck JR, Drenjancevic I. Effects of hyperbaric oxygenation on vascular reactivity to angiotensin II and angiotensin-[1-7] in rats. *Undersea & Hyperbaric Medicine*. 2012;**39**(6):1053-1066
- [12] Park Y, Capobianco S, Gao X, Falck JR, Dellsperger KC, Zhang C. Role of EDHF in type 2 diabetes-induced endothelial dysfunction. *American Journal of Physiology. Heart and Circulatory Physiology*. 2008;**295**(5):H1982-H1988
- [13] Unfirer S, Mihalj M, Novak S, Kibel A, Cavka A, Mijalevic Z, Gros M, Brizic I, Budimir D, Cosic A, Boban M, Drenjancevic I. Hyperbaric oxygenation affects the mechanisms of acetylcholine-induced relaxation in diabetic rats. *Undersea & Hyperbaric Medicine*. 2016 Nov-Dec;**43**(7):787-803
- [14] Davel AP, Wenceslau CF, Akamine EH, Xavier FE, Couto GK, Oliveira HT, Rossoni LV. Endothelial dysfunction in cardiovascular and endocrine-metabolic diseases: An update. *Brazilian Journal of Medical and Biological Research*. 2011 Sep;**44**(9):920-932

- [15] Pieper GM. Enhanced, unaltered and impaired nitric oxide-mediated endothelium-dependent relaxation in experimental diabetes mellitus: Importance of disease duration. *Diabetologia*. 1999;**42**:204-213
- [16] Chan NN, Vallance P, Colhoun HM. Endothelium-dependent and -independent vascular dysfunction in type 1 diabetes: Role of conventional risk factors, sex, and glycemic control. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003;**23**:1048-1054
- [17] Xavier FE, Davel AP, Rossoni LV, Vassallo DV. Time-dependent hyperreactivity to phenylephrine in aorta from untreated diabetic rats: Role of prostanoids and calcium mobilization. *Vascular Pharmacology*. 2003;**40**:67-76
- [18] Bagi Z, Erdei N, Papp Z, Edes I, Koller A. Up-regulation of vascular cyclooxygenase-2 in diabetes mellitus. *Pharmacological Reports*. 2006;**58**(Suppl):52-56
- [19] Tesfamariam B, Brown ML, Deykin D, Cohen RA. Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *The Journal of Clinical Investigation*. 1990;**85**:929-932
- [20] Hink J, Thom SR, Simonsen U, Rubin I, Jansen E. Vascular reactivity and endothelial NOS activity in rat thoracic aorta during and after hyperbaric oxygen exposure. *American Journal of Physiology. Heart and Circulatory Physiology*. 2006 Oct;**291**(4):H1988-H1998
- [21] Thom SR, Bhopale V, Fisher D, Manevich Y, Huang PL, Buerk DG. Stimulation of nitric oxide synthase in cerebral cortex due to elevated partial pressures of oxygen: An oxidative stress response. *Journal of Neurobiology*. 2002;**51**:85-100
- [22] Moskvina AN, Zhilyaev SY, Sharapov OI, Platonova TF, Gutsaeva DR, Kostkin VB, Demchenko IT. Brain blood flow modulates the neurotoxic action of hyperbaric oxygen via neuronal and endothelial nitric oxide. *Neuroscience and Behavioral Physiology*. 2003;**33**(9):883-888
- [23] Atochin DN, Demchenko IT, Astern J, Boso AE, Piantadosi CA, Huang PL. Contributions of endothelial and neuronal nitric oxide synthases to cerebrovascular responses to hyperoxia. *Journal of Cerebral Blood Flow and Metabolism*. 2003;**23**(10):1219-1226
- [24] Hjelde A, Hjelstuen M, Haraldseth O, Martin D, Thom R, Brubakk O. Hyperbaric oxygen and neutrophil accumulation/tissue damage during permanent focal cerebral ischemia in rats. *European Journal of Applied Physiology*. 2002;**86**:401-405
- [25] Luria A, Bettaieb A, Xi Y, Shieh GJ, Liu HC, Inoue H, Tsai HJ, Imig JD, Haj FG, Hammock BD. Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America*. 2011 May 31;**108**(22):9038-9043
- [26] Xu X, Zhao CX, Wang L, Tu L, Fang X, Zheng C, Edin ML, Zeldin DC, Wang DW. Increased CYP2J3 expression reduces insulin resistance in fructose-treated rats and db/db mice. *Diabetes*. Apr 2010;**59**(4):997-1005
- [27] Xu X, Li R, Chen G, Hoopes SL, Zeldin DC, Wang DW. The role of cytochrome P450 epoxygenases, soluble epoxide hydrolase, and epoxyeicosatrienoic acids in metabolic

- diseases. *Advances in Nutrition*. Nov 2016;**7**(6):1122-1128. Published online: Nov 10, 2016. DOI: 10.3945/an.116.012245
- [28] Park EC, Kim SI, Hong Y, Hwang JW, Cho GS, Cha HN, Han JK, Yun CH, Park SY, Jang IS, Lee ZW, Choi JS, Kim S, Kim GH. Inhibition of CYP4A reduces hepatic endoplasmic reticulum stress and features of diabetes in mice. *Gastroenterology*. Oct 2014;**147**(4):860-869
- [29] Drenjančević I, Jukić I, Mihaljević Z, Ćosić A, Kibel A. The metabolites of arachidonic acid in microvascular function. In: Lenasi H, editor. *Microcirculation Revisited – From Molecules to Clinical Practice*. Rijeka: InTech; 2016. pp. 101-133
- [30] Bellien J, Joannides R. Epoxyeicosatrienoic acid pathway in human health and diseases. *Journal of Cardiovascular Pharmacology*. Mar 2013;**61**(3):188-196
- [31] Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. *Nature Reviews. Drug Discovery*. Oct 2009;**8**(10):794-805
- [32] Capdevila J, Wang W. Role of cytochrome P450 epoxygenase in regulating renal membrane transport and hypertension. *Current Opinion in Nephrology and Hypertension*. 2013 Mar;**22**(2):163-169
- [33] Félétou M, Huang Y, Vanhoutte PM. Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. *British Journal of Pharmacology*. 2011;**164**:894-912
- [34] Imig JD. Epoxides and soluble epoxide hydrolase in cardiovascular physiology. *Physiological Reviews*. 2012 January;**92**(1):101-130
- [35] Jouihan SA, Zuloaga KL, Zhang W, Shangraw RE, Krasnow SM, Marks DL, et al. Role of soluble epoxide hydrolase in exacerbation of stroke by streptozotocin-induced type 1 diabetes mellitus. *Journal of Cerebral Blood Flow & Metabolism*. 2013;**33**:1650-1656
- [36] Tsai SH, Hein TW, Kuo L, Yang VC. High glucose impairs EDHF-mediated dilation of coronary arterioles via reduced cytochrome P450 activity. *Microvascular Research*. 2011;**82**(3):356-363
- [37] Mustafa S, Sharma V, McNeill JH. Insulin resistance and endothelial dysfunction: Are epoxyeicosatrienoic acids the link? *Experimental & Clinical Cardiology*. 2009;**14**:e41-e50
- [38] Li X, Zhao G, Ma B, Li R, Hong J, Liu S, Wang DW. 20-Hydroxyeicosatetraenoic acid impairs endothelial insulin signaling by inducing phosphorylation of the insulin receptor substrate-1 at Ser616. *PLoS One*. 2014;**9**(4):e95841
- [39] Issan Y, Hochhauser E, Guo A, Gotlinger KH, Kornowski R, Leshem-Lev D, Lev E, Porat E, Snir E, Thompson CI, Abraham NG, Laniado-Schwartzman M. Elevated level of pro-inflammatory eicosanoids and EPC dysfunction in diabetic patients with cardiac ischemia. *Prostaglandins & Other Lipid Mediators*. 2013;**100-101**:15-21
- [40] Harder DR, Narayanan J, Birks EK, et al. Identification of a putative microvascular oxygen sensor. *Circulation Research*. 1996;**79**:54-61

- [41] Katusic ZS. Vascular endothelial dysfunction: Does tetrahydrobiopterin play a role? *The American Journal of Physiology*. 2001;**281**:H981-H986
- [42] Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RAK, Warnholtz A, Meinerz T, Griendling K, Harrison DG, Forstermann U, Munzer T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circulation Research*. 2001;**88**:E14-E22
- [43] Zenere BM, Arcaro G, Saggiani F, Rossi L, Muggeo M, Lechi A. Noninvasive detection of functional alterations of the arterial wall in IDDM patients with and without microalbuminuria. *Diabetes Care*. 1995;**18**:975-982
- [44] Gazis A, White DJ, Page SR, Cockcroft JR. Effect of oral vitamin E (alphatocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. *Diabetic Medicine*. 1999;**16**:304-311
- [45] Bagi Z, Koller A. Lack of nitric oxide mediation of flow-dependent arteriolar dilation in type I diabetes is restored by sepiapterin. *Journal of Vascular Research*. 2003;**40**:47-57
- [46] Drenjancevic-Peric I, Phillips SA, Falck JR, Lombard JH. Restoration of normal vascular relaxation mechanisms in cerebral arteries by chromosomal substitution in consomic SS.13BN rats. *The American Journal of Physiology*. 2005;**289**(1):H188-H195
- [47] Misir M, Renic M, Novak S, Mihalj M, Cosic A, Vesel M, Drenjancevic I. Hyperbaric oxygenation and 20-hydroxyeicosatetraenoic acid inhibition reduce stroke volume in female diabetic Sprague-Dawley rats. *Re-Experimental Physiology*. 2017;**102**(12):1596-1606
- [48] Imperatore F, Cuzzocrea S, Luongo C, et al. Hyperbaric oxygen therapy prevents vascular derangement during zymosan-induced multiple-organ-failure syndrome. *Intensive Care Medicine*. 2004;**30**(6):1175-1181
- [49] Kerkhof CJ, Bakker EN, Sipkema P. Role of cytochrome P-450 4A in oxygen sensing and NO production in rat cremaster resistance arteries. *The American Journal of Physiology*. 1999;**277**(4 Pt 2):H1546-H1552
- [50] Phillips SA, Drenjancevic-Peric I, Frisbee JC, Lombard JH. Chronic AT(1) receptor blockade alters mechanisms mediating responses to hypoxia in rat skeletal muscle resistance arteries. *American Journal of Physiology. Heart and Circulatory Physiology*. 2004;**287**(2):H545-H552
- [51] Alkayed NJ, Birks EK, Hudetz AG, Roman RJ, Henderson L, Harder DR. Inhibition of brain P-450 arachidonic acid epoxygenase decreases baseline cerebral blood flow. *The American Journal of Physiology*. 1996;**271**(4 Pt 2):H1541-H1546
- [52] Liclican EL, Doumad AB, Wang J, et al. Inhibition of the adenosine2A receptor-epoxyeicosatrienoic acid pathway renders Dahl salt-resistant rats hypertensive. *Hypertension*. 2009;**54**:1284-1290
- [53] Pozzi A, Macias-Perez I, Abair T, et al. Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. *The Journal of Biological Chemistry*. 2005;**280**(29):27138-27146

- [54] Xu X, Zhang XA, Wang DW. The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. *Advanced Drug Delivery Reviews*. 2011. Ahead of print. DOI: 10.1016/j.addr.2011.03.006
- [55] Baragatti B, Ciofini E, Scebba F, et al. Cytochrome – P450 3A13 and endothelin jointly mediate ductus arteriosus constriction to oxygen in mice. *American Journal of Physiology. Heart and Circulatory Physiology*. 2011;**300**(3):H892-H901
- [56] Lash JM, Bohlen HG. Structural and functional origins of suppressed acetylcholine vasodilation in diabetic rat intestinal arterioles. *Circulation Research*. 1991;**69**:1259-1268
- [57] Sartoretto JL et al. Metformin treatment restores the altered microvascular reactivity in neonatal streptozotocin-induced diabetic rats increasing NOS activity, but not NOS expression. *Life Sciences*. 2005;**77**:2676-2689
- [58] Thom SR. Hyperbaric oxygen: Its mechanisms and efficacy. *Plastic and Reconstructive Surgery*. 2011;**127**(Suppl 1):131S-141S
- [59] Matsunami T, Sato Y, Sato T, Ariga S, Shimomura T, Yukawa M. Oxidative stress and gene expression of antioxidant enzymes in the streptozotocin-induced diabetic rats under hyperbaric oxygen exposure. *International Journal of Clinical and Experimental Pathology*. 2010;**3**(2):177-188
- [60] Unfirer S, Drenjančević I. The mechanisms of vascular reactivity to ACh and serotonin are modulated by hyperbaric oxygen treatment in cerebral resistance arteries of diabetic rats (Pohl U, Sperandio M, editors). *Journal of Vascular Research*. 2011;**48**(S1):276
- [61] Lombard JH, Liu Y, Fredricks KT, Bizub DM, Roman RJ, Rusch NJ. Electrical and mechanical responses of rat middle cerebral arteries to reduced PO₂ and prostacyclin. *The American Journal of Physiology*. 1999;**276**(2):H509-H516
- [62] Feletou M, Vanhoutte PM. Chapter 4: EDHF and the physiological control of blood flow. In: *EDHF: The Complete Story*. Boca Raton, FL: CRC Press; 2006. pp. 133-144
- [63] Unfirer S, Falck JR, Drenjancevic I. Cytochrome P450-epoxygenase metabolites play role in vasodilation of middle cerebral arteries in response to reduced pO₂ in healthy and diabetic rats that underwent hyperbaric oxygenation. In: *International Union of Physiological Sciences; Birmingham, UK. July 21-26, 2013. Abstract Book*. 950 p
- [64] Bachi K, Puri S. Free radicals and antioxidants in health and disease. *La revue de santé de la Méditerranée orientale*. 1998;**4**(2):350
- [65] Matsunami T, Sato Y, Hasegawa Y, Ariga S, Kashimura H, Sato T, Yukawa M. Enhancement of reactive oxygen species and induction of apoptosis in streptozotocin-induced diabetic rats under hyperbaric oxygen exposure. *International Journal of Clinical and Experimental Pathology*. Mar 31, 2011;**4**(3):255-266
- [66] Gerber PA, Rutter GA. The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. *Antioxidants & Redox Signaling*. Apr 1, 2017;**26**(10):501-518
- [67] Selim F, Wael A, Jackson KE. Diabetes-induced reactive oxygen species: Mechanism of their generation and role in renal injury. *Journal of Diabetes Research*. 2017;**2017**:8379327

- [68] Ray PD, Huang B-W, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular Signalling*. May 2012;**24**(5):981-990
- [69] Kirill K, Shmuel M. ROS and intracellular ion channels. *Cell Calcium*. Aug 2016;**60**(2):108-114
- [70] Drenjancevic I, Kibel A, Kibel D, Seric V, Cosic A. Blood pressure, acid-base and blood gas status and indicators of oxidative stress in healthy male rats exposed to acute hyperbaric oxygenation. *UHM Journal*. 2013;**40**(4):319-328
- [71] Ay H, Topal T, Uysal B, et al. Time-dependent course of hyperbaric oxygen-induced oxidative effects in rat lung and erythrocytes. *Clinical and Experimental Pharmacology & Physiology*. 2007;**34**:787-791
- [72] Simsek K, Ay H, Topal T, et al. Long-term exposure to repetitive hyperbaric oxygen results in cumulative oxidative stress in rat lung tissue. *Inhalation Toxicology*. 2011;**23**:166-172
- [73] Sureda A, Batle JM, Martorell M, Capó X, Tejada S, Tur JA, Pons A. Antioxidant response of chronic wounds to hyperbaric oxygen therapy. *PLoS One*. 2016;**11**(9):e0163371
- [74] Nyoman S, Hendry I. Blood glucose and lipid profile in patients with diabetic foot ulcer that underwent hyperbaric oxygen therapy. *Bali Medical Journal*. 2017;**6**(2):405-408
- [75] Hartge MM, Unger T, Kintscher U. The endothelium and vascular inflammation in diabetes. *Diabetes & Vascular Disease Research*. Jun 2007;**4**(2):84-88. PubMed PMID: 17654441
- [76] Lee J, Lee S, Zhang H, Hill MA, Zhang C, Park Y. Interaction of IL-6 and TNF-alpha contributes to endothelial dysfunction in type 2 diabetic mouse hearts. *PLoS One*. 2017;**12**(11):e0187189. PubMed PMID: 29095915. Pubmed Central PMCID: 5667841
- [77] Gustavsson C, Agardh CD, Zetterqvist AV, Nilsson J, Agardh E, Gomez MF. Vascular cellular adhesion molecule-1 (VCAM-1) expression in mice retinal vessels is affected by both hyperglycemia and hyperlipidemia. *PLoS One*. Sep 13, 2010;**5**(9):e12699. PubMed PMID: 20856927. Pubmed Central PMCID: 2938334
- [78] Chandra SB, Mohan S, Ford BM, Huang L, Janardhanan P, Deo KS, et al. Targeted over-expression of endothelial nitric oxide synthase in endothelial cells improves cerebrovascular reactivity in Ins2Akita-type-1 diabetic mice. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*. Jun 2016;**36**(6):1135-1142. PubMed PMID: 26661212. Pubmed Central PMCID: 4908624
- [79] Naguib G, Al-Mashat H, Desta T, Graves DT. Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. *The Journal of Investigative Dermatology*. Jul 2004;**123**(1):87-92. PubMed PMID: 15191547
- [80] Graves DT, Naguib G, Lu H, Leone C, Hsue H, Krall E. Inflammation is more persistent in type 1 diabetic mice. *Journal of Dental Research*. Apr 2005;**84**(4):324-328. PubMed PMID: 15790737

- [81] Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. *PLoS One*. Mar 4, 2010;**5**(3):e9539. PubMed PMID: 20209061. Pubmed Central PMCID: 2832020
- [82] Pettersson US, Christoffersson G, Massena S, Ahl D, Jansson L, Henriksnas J, et al. Increased recruitment but impaired function of leukocytes during inflammation in mouse models of type 1 and type 2 diabetes. *PLoS One*. 2011;**6**(7):e22480. PubMed PMID: 21799868. Pubmed Central PMCID: 3143146
- [83] Martinez N, Vallerskog T, West K, Nunes-Alves C, Lee J, Martens GW, et al. Chromatin decondensation and T cell hyperresponsiveness in diabetes-associated hyperglycemia. *Journal of Immunology*. Nov 1, 2014;**193**(9):4457-4468. PubMed PMID: 25246495. Pubmed Central PMCID: 4242014
- [84] Wu J, Mei C, Vlassara H, Striker GE, Zheng F. Oxidative stress-induced JNK activation contributes to proinflammatory phenotype of aging diabetic mesangial cells. *American Journal of Physiology Renal Physiology*. Dec 2009;**297**(6):F1622-F1631. PubMed PMID: 19776174. Pubmed Central PMCID: 2801342
- [85] Meng L, Park J, Cai Q, Lanting L, Reddy MA, Natarajan R. Diabetic conditions promote binding of monocytes to vascular smooth muscle cells and their subsequent differentiation. *American Journal of Physiology Heart and Circulatory Physiology*. Mar 2010;**298**(3):H736-H745. PubMed PMID: 20008269. Pubmed Central PMCID: 2838549
- [86] Salvador B, Arranz A, Francisco S, Cordoba L, Punzon C, Llamas MA, et al. Modulation of endothelial function by Toll like receptors. *Pharmacological Research*. Jun 2016;**108**:46-56. PubMed PMID: 27073018
- [87] Pechlivani N, Ajjan RA. Thrombosis and vascular inflammation in diabetes: Mechanisms and potential therapeutic targets. *Frontiers in Cardiovascular Medicine*. 2018;**5**:1. PubMed PMID: 29404341. Pubmed Central PMCID: 5780411
- [88] Milovanova TN, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, et al. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. *Journal of Applied Physiology*. Feb 2009;**106**(2):711-728. PubMed PMID: 19023021. Pubmed Central PMCID: 2644249
- [89] Novak S, Drenjancevic I, Vukovic R, Kellermayer Z, Cosic A, Tolusic Levak M, et al. Anti-inflammatory effects of hyperbaric oxygenation during DSS-induced colitis in BALB/c mice include changes in gene expression of HIF-1alpha, proinflammatory cytokines, and antioxidative enzymes. *Mediators of Inflammation*. 2016;**2016**:7141430. PubMed PMID: 27656047. Pubmed Central PMCID: 5021505
- [90] Kendall AC, Whatmore JL, Winyard PG, Smerdon GR, Eggleton P. Hyperbaric oxygen treatment reduces neutrophil-endothelial adhesion in chronic wound conditions through S-nitrosation. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] The European Tissue Repair Society*. Nov-Dec 2013;**21**(6):860-868. PubMed PMID: 24134224

- [91] Andre-Levigne D, Modarressi A, Pignel R, Bochaton-Piallat ML, Pittet-Cuenod B. Hyperbaric oxygen therapy promotes wound repair in ischemic and hyperglycemic conditions, increasing tissue perfusion and collagen deposition. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] The European Tissue Repair Society*. Nov 2016;**24**(6):954-965. PubMed PMID: 27684570
- [92] Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. *Archives of Surgery*.. Nov 2000;**135**(11):1293-1297. PubMed PMID: 11074883
- [93] Thom SR, Mendiguren I, Hardy K, Bolotin T, Fisher D, Nebolon M, et al. Inhibition of human neutrophil beta2-integrin-dependent adherence by hyperbaric O₂. *The American Journal of Physiology*. Mar 1997;**272**(3 Pt 1):C770-C777. PubMed PMID: 9124510
- [94] Benson RM, Minter LM, Osborne BA, Granowitz EV. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. *Clinical and Experimental Immunology*. Oct 2003;**134**(1):57-62. PubMed PMID: 12974755. Pubmed Central PMCID: 1808843
- [95] Kudchodkar B, Jones H, Simecka J, Dory L. Hyperbaric oxygen treatment attenuates the pro-inflammatory and immune responses in apolipoprotein E knockout mice. *Clinical Immunology*. Sep 2008;**128**(3):435-441. PubMed PMID: 18595776. Pubmed Central PMCID: 2562533
- [96] Williams LS, Rotich J, Qi R, Fineberg N, Espay A, Bruno A, Fineberg SE, Tierney WR. Effects of admission hyperglycemia on mortality and costs in acute ischemic stroke. *Neurology*. 2002;**59**:67-71
- [97] Kaarisalo MM, Raiha I, Sivenius J, Immonen-Raiha P, Lehtonen A, Sarti C, Mähönen M, Torppa J, Tuomilehto J, Salomaa V. Diabetes worsens the outcome of acute ischemic stroke. *Diabetes Research and Clinical Practice*. 2005;**69**:293-298
- [98] Janghorbani M, Hu FB, Willett WC, Li TY, Manson JE, Logroscino G, Rexrode KM. Prospective study of type 1 and type 2 diabetes and risk of stroke subtypes: The nurses' health study. *Diabetes Care*. 2007;**30**:1730-1735
- [99] Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: A cohort study using the general practice research database. *Diabetes Care*. 2006;**29**:798-804
- [100] Secrest AM, Prince CT, Costacou T, Miller RG, Orchard TJ. Predictors of and survival after incident stroke in type 1 diabetes. *Diabetes & Vascular Disease Research*. 2013;**1**:3-10
- [101] Tsuruta R, Fujita M, Ono T, Koda Y, Koga Y, Yamamoto T, Nanba M, Shitara M, Kasaoka S, Maruyama I, et al. Hyperglycemia enhances excessive superoxide anion radical generation, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats. *Brain Research*. 2010;**1309**:155-163

- [102] Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC, Kidwell CS, Leslie-Mazwi TM, Ovbiagele B, Scott PA, Sheth KN, Southerland AM, Summers DV, Tirschwell DL; American Heart Association Stroke Council. Guidelines for the Early Management of Patients with Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke* 2018;**49**:46-110
- [103] Xu RS. Pathogenesis of diabetic cerebral vascular disease complication. *World Journal of Diabetes*. 2015;**6**(1):54-66
- [104] Michalski D, Hartig W, Schneider D, Hobohm C. Use of normobaric and hyperbaric oxygen in acute focal cerebral ischemia-a preclinical and clinical review. *Acta Neurologica Scandinavica*. 2011;**123**:85-97
- [105] Sunami K, Takeda Y, Hashimoto M, et al. Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. *Critical Care Medicine*. 2000;**28**:2831-2836
- [106] Liu S, Liu W, Ding W, et al. Electron paramagnetic resonance-guided normobarichyperoxia treatment protects the brain by maintaining penumbral oxygenation in a rat model of transient focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 2006;**26**:1274-1284
- [107] Singhal AB. A review of oxygen therapy in ischemic stroke. *Neurological Research*. 2007;**29**:173-183
- [108] Lou M, Eschenfelder CC, Herdegen T, Brecht S, Deuschl G. Therapeutic window for use of hyperbaric oxygenation in focal transient ischemia in rats. *Stroke*. 2004;**35**:578-583
- [109] Singhal AB, Lo EH, Dalkara T, et al. Advances in stroke neuroprotection: Hyperoxia and beyond. *Neuroimaging Clinics of North America*. 2005;**15**:697-720
- [110] Zhai WW, Sun L, Yu ZQ, Chen G. Hyperbaric oxygen therapy in experimental and clinical stroke. *Medical Gas Research*. 2016;**6**:111-118
- [111] Mišir M, Renić M, Mihalj M, Novak S, Drenjančević I. Is shorter transient middle cerebral artery occlusion (t-MCAO) duration better in stroke experiments on diabetic female Sprague Dawely rats? *Brain Injury*. 2016;**30**:1390-1396
- [112] Nagasawa H, Kogure K. Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. *Stroke*. 1989;**20**:1037-1043
- [113] Xu Y, Ji R, Wei R, et al. The efficacy of hyperbaric oxygen therapy on middle cerebral artery occlusion in animal studies: A meta-analysis (Pignataro G, editor.) *PLoS One*. 2016;**11**:e0148324
- [114] Bennett MH, Wasiak J, Schnabel A, et al. Hyperbaric oxygen therapy for acute ischaemic stroke. *Cochrane Database of Systematic Reviews*. 2005;**3**:CD004954