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

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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 HBO, 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 endothelial 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 prostanoi

doids, 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-eicosatetraenoic 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 F2α 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 eicosatetraenoic 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 PO₂ [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].

Unfrer 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 epoxygenase 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-epoxygenase 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 (O2⁻), hydroxyl radical (OH), alkoxyl radical (RO), and peroxyl radical (ROO). Oxygen-centered nonradicals are
hydrogen peroxide (H$_2$O$_2$), 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 HBO$_2$ treatment was noted—this might be due to activation of compensatory mechanisms by HBO$_2$ [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 HBO$_2$ such as antioxidative and anti-inflammatory effects, have been attributed to the enhanced ROS production induced by the HBO$_2$ 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-κB 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-κB 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 HBO$_2$, which is the most specific growth factor for neovascularization [92]. It is controversial that HBO$_2$-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 β2-integrin-dependent adhesion of neutrophils to the endothelium by blocking CD18 surface polarization and through S-nitrosation of β2-integrin, with no effect on the cell-surface expression of β2-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 HBO$_2$ [1, 94].

Studies on ApoE KO mice that exhibit accelerated atherosclerosis and related complications showed that HBO$_2$ 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. HBO 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
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.

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<table>
<thead>
<tr>
<th>Target group of mechanisms or single mechanism</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial dysfunction</td>
<td>↑ NO bioavailability</td>
<td>[20–23]</td>
</tr>
<tr>
<td>Arachidonic acid metabolites</td>
<td>↑ EETs synthesis, CYP epoxygenase expression, vascular sensitivity to EETs (?)</td>
<td>[2, 3, 11, 13, 47]</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>↑ ROS</td>
<td>[2, 70–74]</td>
</tr>
<tr>
<td>Inflammation</td>
<td>↑ Proinflammatory mediators</td>
<td>[1, 2, 90–94]</td>
</tr>
<tr>
<td>Renin-angiotensin system</td>
<td>↑ Vascular reactivity to ANG-(1–7)</td>
<td>[2, 3, 11]</td>
</tr>
<tr>
<td>Physical effects</td>
<td>↑ Dissolved oxygen in plasma and tissues</td>
<td>[104–106]</td>
</tr>
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Table 1. Major potential mechanisms of HBO-induced vascular functional changes in diabetic animal models.
References


[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...


[34] Imig JD. Epoxides and soluble epoxide hydrolase in cardiovascular physiology. Physiological Reviews. 2012 January;92(1):101-130


[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


[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


