ANTIOXIDANTS & REDOX SIGNALING
Volume 21, Number 11, 2014

DOI: 10.1089/ars.2014.5940

Therapeutics

FORUM REVIEW ARTICLE

# Hyperbaric Oxygen, Vasculogenic Stem Cells, and Wound Healing

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## Abstract

***Significance:*** Oxidative stress is recognized as playing a role in stem cell mobilization from peripheral sites and also cell function. ***Recent Advances:*** This review focuses on the impact of hyperoxia on vasculogenic stem cells and elements of wound healing. ***Critical Issues:*** Components of the wound-healing process in which oxidative stress has a positive impact on the various cells involved in wound healing are highlighted. A slightly different view of wound-healing physiology is adopted by departing from the often used notion of sequential stages: hemostatic, inflammatory, proliferative, and remodeling and instead organizes the cascade of wound healing as overlapping events or waves pertaining to reactive oxygen species, lactate, and nitric oxide. This was done because hyperoxia has effects of a number of cell signaling events that converge to influence cell recruitment/chemotaxis and gene regulation/protein synthesis responses which mediate wound healing. ***Future Directions:*** Our alternative perspective of the stages of wound healing eases recognition of the multiple sites where oxidative stress has an impact on wound healing. This aids the focus on mechanistic events and the interplay among various cell types and biochemical processes. It also highlights the areas where additional research is needed. *Antioxid. Redox Signal*. 21, 1634–1647.


## Introduction

WOUND HEALING AFTER an insult or injury is a complex process that involves the coordination of multiple mediators and components. Research continues to expand our understanding of the wound-healing process, which is central to normal physiology and also a growing concern in clinical medicine. For example, more than 6 million individuals in the United States per year are affected by chronic wounds (150). Often, these individuals have other medical disorders, such as diabetes mellitus. As estimated by the Kaiser Foundation in 2012, those with diabetes and lower-extremity wounds in the US Medicare program accounted for $41 billion in cost, which is ~1.6% of all Medicare health care spending. Apart from the costs, wounds that fail to heal may result in limb amputations and death. Lower-extremity amputation in those with diabetes is associated with a risk of mortality of about 20% per year (107, 108, 132).

Attempts to use so-called adjunctive measures to improve the rate of wound healing have added to our understanding of wound physiology. One rather novel approach that has provided an interesting perspective on the role of oxidative stress in wound healing is hyperbaric oxygen ($HBO_2$). $HBO_2$ therapy is a treatment modality in which a person breathes 100% $O_2$ while exposed to increased atmospheric pressure. Treatments are carried out in either a monoplace (single person) or multiplace (typically 2–14 patients) chamber. Pressures applied while in the chamber are usually 2 to 3 atmospheres absolute (ATA), the sum of the atmospheric pressure (1 ATA) plus additional pressure equivalent to 1 or 2 atmospheres (1 atmosphere = a pressure of 14.7 pounds per square inch or 101 kPa). Treatments are usually about 1.5–2 h long and may be performed once or twice daily.

## Results

### *Physiology overview*

During $HBO_2$ treatment, the arterial $O_2$ tension typically exceeds 2000 mmHg, and levels of 200–400 mmHg occur in tissues (167). It is well accepted that an increase in the

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production of reactive oxygen species (ROS) occurs during hyperoxia. While $O_2$ toxicity is a risk, clinical protocols have maintained the incidence of adverse effects very low (27). The beneficial aspect to ROS and also reactive nitrogen species (RNS) is that they serve as signaling molecules in transduction cascades, or pathways, for a variety of growth factors, cytokines, and hormones (5, 23, 113, 176). As such, reactive species can generate either ''positive'' or ''negative'' effects depending on their concentration and intracellular localization. Since exposure to hyperoxia in clinical $HBO_2$ protocols is rather brief, studies show that antioxidant defenses are adequate so that biochemical stresses related to increases in reactive species are reversible (31, 32, 120, 141).

### *Oxygen and wound healing*

Soon after tissue injury, as a part of the repair process, devitalized tissue is removed, keratinocytes migrate and proliferate to the wound edge, and granulation tissue, which is primarily composed of fibroblasts and endothelial cells, begins to form. Granulation tissue contains excessive neovascular proliferation. This process includes the repair, restoration, and regeneration of blood vessels.

Postnatal neovascularization involves two complementary processes. One is the sprouting of the endothelium from preexisting blood vessels (angiogenesis); the second involves endothelial stem/progenitor cells (SPCs) released from the bone marrow as well as peripheral tissue sites that home to foci of ischemia in a process termed vasculogenesis (59, 138, 180). SPCs likely orchestrate vascular repair by differentiating into endothelial cells as well as supporting structures that give rise to repaired and/or regenerated blood vessels. A variety of cell surface markers have been used to identify these cells and since they change as the cells mature and/or assume different functions, we have chosen the moniker SPCs *versus* the term endothelial progenitor cell as used by some investigators because these cells are defined by a narrow range of surface markers and their nature is debated (10, 52, 195).

The notion of redox regulation and varied roles for $O_2$ in wound healing is commonly discussed and has been outlined by many in recent years (126, 146, 152). There is also a burgeoning literature on the role of oxidants in embryonic and hematopoietic stem cells, which is beyond the scope of this review (86, 92, 93, 118, 158, 166, 183). $HBO_2$ has effects on a number of cell types and will influence both angiogenesis and vasculogenesis. In this review, we will frame our discussion around recognized mediators of wound healing to emphasize that $HBO_2$ merely acts by modifying established regulatory pathways. The classical view of wound healing envisages a number of sequential phases (*e.g.*, hemostatic, inflammatory, proliferative, and remodeling), and this perspective has been effective for focusing areas of investigation for $\sim$75 years. We believe that a useful alternative wound-healing paradigm which eases discussion of $HBO_2$—or more generally the availability of $O_2$, ROS, and RNS—involves three overlapping events or ''waves.'' Biochemical energy is generated from $O_2$ for the increased energy demands of repair processes such as cell proliferation, bacterial defense, and collagen synthesis. The second role of $O_2$ is cell signaling that is mediated by the generation of reactive species. A convenient division for cell signaling involves roles for ROS, elevations in wound lactate, and also nitric oxide ($^{\bullet}NO$), as all of them converge to influence cell recruitment/chemotaxis and gene regulation/protein synthesis responses that mediate wound healing.

### *The ROS wave*

$O_2$-derived free radicals as well as $O_2$-derived nonradical species such as $H_2O_2$ and hypochlorous acid are generated as a part of normal metabolism by mitochondria, endoplasmic reticulum (ER), peroxisomes, various oxidase enzymes, and phospholipid metabolism. ROS act in conjunction with several redox systems involving glutathione, thioredoxin, and pyridine nucleotides, and they play central roles in coordinating cell signaling and also antioxidant, protective pathways (26, 75, 190). The main physiological source of extracellular $H_2O_2$ in wounds is considered a family of NADPH oxidases, which transport electrons from cytoplasmic NADPH to generate superoxide radicals ($O_2^{-\bullet}$) or $H_2O_2$ (15). The so-called Nox (NADPH oxidase) group of five genetically distinct enzymes generates superoxide, which can be converted to $H_2O_2$ by superoxide dismutase (SOD); whereas two Duox (dual oxidase) enzymes generate $H_2O_2$ without requiring SOD (7). An overview of components in the ROS wave is shown in Figure 1.

Cell migration/chemotaxis. Acting in a paracrine manner, $H_2O_2$ serves as a chemotactic signal in the first minutes after wounding. Mechanical or chemical stress triggers a burst of $H_2O_2$ from epithelial cell Duox enzyme activity (124). Postwound extracellular $H_2O_2$ can reach concentrations of $\sim$0.5–50 $\mu M$ near the wound margin, with a gradient extending $\sim$200 $\mu$m. $H_2O_2$ diffusion across many cell widths appears to occur *via* aquaporin-like channels (17). It would seem reasonable that there may also be roles for antioxidants such as catalase and peroxidase in this process, but this has not been clearly established. SOD activity decreases in some vascular injury models, and supplementation of SOD either *via* adenoviral vector gene transfer or from SPCs recruitment can improve healing in animal models of diabetes mellitus (91, 101, 109). Neutrophils exhibit a chemotactic response to exogenous $H_2O_2$ (although the molecular details for this response are unknown), and they appear at the wound edge within 10 min after wounding (82, 124). $HBO_2$ will increase production of reactive species within neutrophils (primarily from Nox2, although multiple sources may contribute) and can improve bacteriocidal efficacy (103, 104, 170). It is unclear whether this oxidant source also contributes to cell recruitment.

Platelet aggregation during the early stages postinjury generate ROS, which are derived from Nox as well as from xanthine oxidase (142, 143, 177). Vascular smooth muscle cells synthesize thrombogenic tissue factor in a Nox-dependent fashion, which may perpetuate the thrombogenic process within injured vessels that is initiated by platelets (62). Skin keratinocytes and fibroblasts also use Nox to generate $H_2O_2$, as do recruited leukocytes. In addition to its well-recognized antibacterial function, $H_2O_2$ increases epithelial cell, smooth muscle cell, endothelial cell, and monocyte/macrophage migration (94, 125, 127, 139, 164), and it may increase leukocyte integrin adhesion (100). $HBO_2$ does not alter platelet function and inhibits neutrophil $\beta_2$ integrin adhesion at

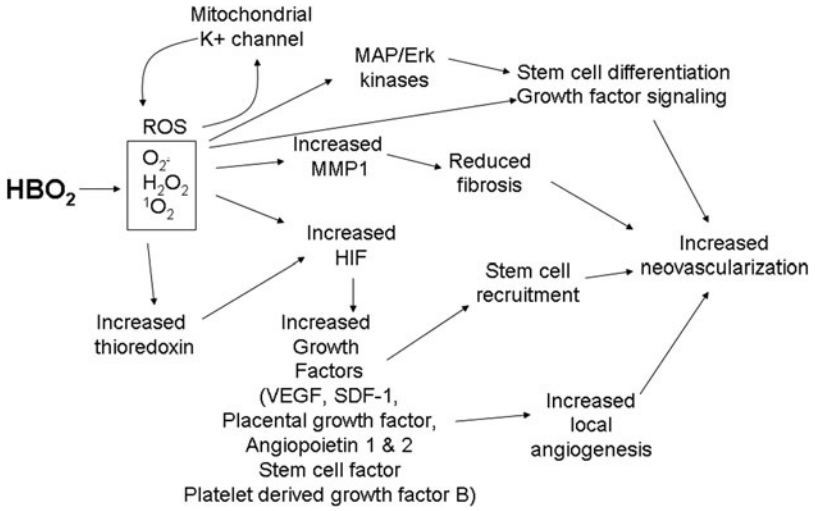


**FIG. 1. ROS wave: Summary of wound-healing events related to reactive oxygen species (ROS).** HIF, hypoxia-inducible factors; MAP, mitogen-activated protein kinase; MMP1, matrix metalloproteinase-1; SDF-1, stromal-derived factor-1; VEGF, vascular endothelial growth factor.

pressures of 2.8 ATA or more, beyond those used in wound-healing protocols (168, 170, 173).

In an *in vivo* Matrigel wound model, $HBO_2$ increases Nox-derived $H_2O_2$ synthesis, which contributes to SPCs recruitment as well as to growth factor synthesis (116). Effects on SPCs and cells that have undergone greater differentiation will be outlined in greater detail in subsequent sections. In an *in vitro* model, transient DNA oxidative stress from short-term $HBO_2$ was also shown to improve endothelial cell tolerance to subsequent oxidant exposure (187). The sources for $H_2O_2$ in wound healing are still not entirely clear, and overlapping roles may exist. Exogenous addition of $H_2O_2$ can activate Nox (140). Although not shown for the endothelium, mitochondrial $H_2O_2$ can regulate Nox activity in smooth muscle cells (*via* protein kinase C) and human 293T cells (*via* phosphoinositide 3 kinase and Rac1), and activated Nox can mediate mitochondrial ATP-sensitive potassium channels and thus mitochondrial $H_2O_2$ production (29, 90, 140).

Gene regulation/protein synthesis. The interruption of blood flow associated with acute injuries rapidly causes wound hypoxia, which contributes to stabilization of hypoxia-inducible factors (HIF) and these transcription factors activate many genes, resulting in the synthesis of a variety of proteins required for wound healing (*e.g.*, vascular endothelial growth factor [VEGF], stromal-derived factor 1 [SDF-1], placental growth factor, angiopoietin 1, angiopoietin 2, platelet-derived growth factor B, and stem cell factor [SCF]) (148, 149). Early elevations in $H_2O_2$ will also stabilize HIF *via* decreased ascorbate availability and secondarily by decreasing prolyl-hydroxylase activity (130).

Among the proteins synthesized in response to ROS is thioredoxin, which not only acts in antioxidant pathways, but also functions as a transcription factor to increase HIF synthesis (193). This pathway is triggered by $HBO_2$ in localized vasculogenic stem cells, which augments VEGF and SDF-1 synthesis, enhancing neovascularization (115, 116). Subsequent signaling between SDF-1 and its cognate cellular receptor, CXCR4, also involves ROS (89). Likely a synergistic process, lactate can also mediate HIF stabilization in endothelial cells by metabolic conversion to pyruvate that inhibits prolyl hydroxylases (160). In ischemic peripheral wounds, placement of SDF-1 into the margins will markedly augment SPCs recruitment associated with $HBO_2$ treatments (48).

ROS (particularly $O_2^{-\bullet}$) generated by platelets and other cells early in the wound process modulate activation of cell surface latent tissue factor (133). Activated tissue factor activates thrombin, which contributes to hemostasis and also activates vascular cell Nox oxidases (thus adding to $H_2O_2$ production in the early wound). ROS production modulates responses of endothelium, lymphoid, and monocytic cells and also smooth muscle cells by influencing NFkB activation (111, 114) and $H_2O_2$ augments macrophage, keratinocyte, and fibroblast-mediated VEGF and VEGF-receptor 2 synthesis (11, 25, 55, 113, 144, 151). Ambient pressure hyperoxia as well as $HBO_2$ increases VEGF synthesis in soft tissue wounds as well as in healing bone (43, 155). As mentioned earlier, enhanced $H_2O_2$ production by $HBO_2$ will result in increases in VEGF and SDF-1 synthesis by SPCs and, as one might expect, supplementing the local environment with catalase will abrogate these responses (115, 116). $HBO_2$ enhances placental growth factor production by bone marrow-derived mesenchymal stem cells through elevations of ROS (156). $H_2O_2$ also increases cellular synthesis of pro-inflammatory tissue necrosis factor in wounds (57, 58).

Singlet oxygen activates plasminogen, which will then activate matrix metalloproteinase 1 (MMP1) to reduce fibrosis during wound remodeling (56). ROS are also involved in cell responses to growth factors. For example, a variety of growth factors influence endothelial cell function by activating protein kinases such as Erk. The pathways by which growth factors activate Erk involve elevations in intracellular ROS, which, in turn, inhibit phosphatase enzymes that impede protein kinase phosphorylation (70). Erk plays a complex role in $HBO_2$-mediated stimulation of SPC-mediated neovascularization (115, 116). $H_2O_2$ as well as other ROS increases signaling by platelet-derived growth factor (PDGF) and transforming growth factor (TGF) beta in several cell types (71). $HBO_2$ was shown to up-regulate PDGF receptors in experimental wounds (19).

A separate and also critically important role for $O_2$ in newly synthesized tissue is collagen cross-linking. $O_2$ is a required co-factor for this process (67), and collagen synthesis by fibroblasts is proportional to local $O_2$ concentration in the range of 0–200 mmHg (80, 155).

ROS play an important role in whether stem cells enter the cell cycle. In embryonic stem cells, p38 inhibition sustains self-renewal; whereas ROS-mediated p38 activation enhances cell turnover (68, 136). Activity of p38 will also increase the transcriptional coactivator peroxisome proliferator-activated receptor $\gamma$ coactivator $1\alpha$, which plays a central role in mitochondrial biogenesis that influences stem cell differentiation (135). ROS also play a role in the stem cell pluripotency, the ability to differentiate into different cell lineage types (73, 157). For example, at progressively higher concentrations of $H_2O_2$, cells exhibited greater differentiation into cardiac myocytes (21). Mesenchymal stem cells transplanted into infarct zones using a rat myocardial infarction model exhibit greater engraftment and improved cardiac function with $HBO_2$ treatment (77, 78). If alternative protein kinase pathways are activated by ROS, stem cell lineage patterns can be modified (69). Recent studies have shown $HBO_2$-mediated enhancement of chondrocyte-specific gene expression and also osteogenesis in differentiating between human and animal adipose-derived stem cells (a peripheral, mesenchymal stem cell type) (24, 33, 46, 153). Neuronal differentiation of mesenchymal stem cells involves up-regulation of Nox and increased ROS synthesis, although whether these changes are cause or effect is unclear (179). Perhaps further insights into the phenomenon can be gleaned by observations that $HBO_2$ also promotes neural progenitor cell neurogenesis *ex vivo,* possibly by modification of the Wnt pathway, and they may promote proliferation of endogenous central nervous system stem cells to form neurons and vascular channels after hypoxic or ischemic insults (185, 188, 191, 192).

### *The •NO wave*

Nitric oxide is synthesized by one of three nitric oxide synthase (NOS) isoforms present in a large variety of cells. All enzyme isoforms use $O_2$, the amino acid arginine, and a variety of cofactors to synthesize •NO. NOS activity increases early after wounding and persists at an elevated level for many days. The primary source of NOS in early wound healing is macrophages, although many other cells (*e.g.*, fibroblasts) contribute to local production (88). Oxygen availability influences activity of NOS enzymes differently, in part because they have different Km values for binding $O_2$ and as the active sites and rates of turnover are different, making them more or less sensitive to oxidation. Reported Km values for type 1 (nNOS) is 350 $\mu M$, type 2 (iNOS) is 135 $\mu M$, and type 3 (eNOS) is 23 $\mu M$ (165). Hence, the rate of •NO formation by nNOS will be much more affected by $O_2$ fluctuations over a greater physiological range than eNOS. Figure 2 summarizes roles for •NO in neovascularization.

$HBO_2$ can augment activity all three NOS isoforms. Activation of nNOS and also eNOS appears to be mediated through enhanced binding of heat shock protein 90 (22, 169). Activation of iNOS, at least in neutrophils, occurs due to an increase in short filamentous actin synthesis and secondary iNOS linkage (171). Consequences linked to NOS activation by $HBO_2$ will be discussed next and in this regard, it is important to remain cognizant that since exposures are transient, enzyme activation is likely relatively brief. In neutrophils, iNOS activity is transient because enzyme association to filamentous actin ceases due to breakdown of a poly-protein complex (171). The notion that transient •NO synthesis is important, because while •NO is required for wound healing to occur, too much hinders healing (14, 81).

Cell migration/chemotaxis. MMPs play critical roles in matrix remodeling and cell migration. ROS and also •NO regulate MMPs at the transcriptional and post-translational levels (121, 128, 159). For example, •NO enhances endothelial cell migration by increasing the local extracellular concentration of MMP-13. MMP-13 activity is usually constrained, because it is bound to membrane caveolae. This linkage is broken by locally synthesized •NO, which increases collagen breakdown (97). Elevated local concentrations of •NO synthesized by iNOS will stimulate keratinocyte migration during re-epithelialization (161). Activating eNOS in bone marrow stroma secondarily nitrosylates MMP-9, which releases the stem cell active cytokine, soluble Kit ligand (SCF) (60). This agent shifts SPCs from a quiescent to the proliferative niche, and stimulates their mobilization to the peripheral blood (2, 3, 60, 61, 119, 137). By directly activating eNOS, $HBO_2$ mobilized bone marrow SPCs in both animal models and humans (48, 54, 172).

Gene regulation/protein synthesis. Nitric oxide plays a central role in synthesizing VEGF (45), cytokines, and growth factors (6, 145, 182). Synthesis of •NO by eNOS (*versus* other isoforms) plays a predominant role in VEGF-mediated angiogenesis (47), possibly by stabilizing HIF-1 (35, 149). Many down-stream effects of VEGF are also stimulated *via* •NO (9, 131). In addition to its local effects within the wound, VEGF gets into the circulation and eventually the bone marrow, where it activates stem cell mobilization *via* NOS activation, which as described earlier, causes S-nitrosylation and activation of MMP-9, release of soluble Kit ligand, and SPCs mobilization (2, 3).

### *The lactate wave*

Wounding impairs blood flow due to damaged vessels along with local consumption of $O_2$ by the varied Nox isoforms, which rapidly establishes hypoxia. An immediate consequence is anaerobic metabolism by local wound cells, which generates lactate. This is only likely transient, and oxygenation improves with any restoration of blood flow. Wound margin lactate concentrations remain elevated, however, because of endothelial cells and recruited leukocytes that preferentially rely on glycolysis, even in an aerobic environment (18, 51, 122). Thus, hyperoxia does not reduce wound lactate concentration (66, 123). In fact, a study of $HBO_2$ metabolic effects in an *ex vivo* blood vessel model suggests that in the hours after hyperoxia (but not during oxygen exposure), lactate levels increase (186). When examining this report, as with all studies of $HBO_2$, readers need to be sensitive to the differences between *ex vivo* and *in vivo* studies. Tissue/cell oxygenation with just normobaric hyperoxia in an *ex vivo* setting equals or exceeds that achieved *in vivo* during hyperoxia. Thus, both normobaric and hyperbaric hyperoxia increased lactate levels in this *ex vivo* tissue model (186). The mechanism for the response was not identified and there are several possibilities. For example, elevated ROS as well as •NO can impede tricarboxylic acid (TCA) cycle metabolism and mitochondrial oxidative

phosphorylation (12, 181, 184). A more complex process could be *via* augmentation of HIF levels, because HIF can suppress metabolism through the TCA cycle and also up-regulate expression of lactate dehydrogenase (LDH) (in fact, this model also reported elevated LDH in the tissue medium, while suggesting there was no oxidative stress-mediated enzyme –''leakage'') (63, 79). In a situation where actively metabolizing tissues are no longer under the influence of hyperoxia, the higher than normal NADH could lead to a reverse LDH effect, catalyzing conversion of pyruvate and NADH to lactate and NAD+ synthesis. Whatever the mechanism(s), there are numerous consequences to lactate elevations and some are synergistic with $HBO_2$. Figure 3 shows an overview of effects.

Cell migration/chemotaxis. Lactate is not itself a chemotactic stimulus but it can influence cell migration secondarily. Lactate stimulates hyaluronic acid synthesis (44, 162). Hyaluronic acid accumulation in the peri-wound extracellular matrix causes expansion of tissue, enabling easy cell movement into damaged tissues and recruitment of new fibroblasts that adhere to matrix *via* the CD44 receptor.

Gene regulation/protein synthesis. Lactate combined with normobaric $O_2$ stimulates angiogenesis (65). Lactate chelates iron in the ER and radicals generated by the concurrent presence of $O_2^{-\bullet}$ and $H_2O_2$ *via* Fenton reaction (while confined to the ER) generates hydroxyl radical ($^{\bullet}OH$) that reduces HIF prolyl hydroxylase activity (4, 98, 99). Similar impairment of prolyl hydroxylase and increased HIF binding to DNA occurs with the glycolytic intermediates pyruvate and oxaloacetate, in addition to lactate (98, 160). Lactate modifies the gene expression pattern of mesenchymal stem cells to one more conducive to wound healing *versus* apoptosis (194). The pro-oxidant action of lactate improves the function of vasculogenic stem cells recruited from bone marrow to peripheral sites as a consequence of $HBO_2$ (117). Lactate *via* elevated ROS production will increase cell content of HIF factors (HIF-1 and HIF-2), resulting in elevated synthesis of VEGF and SDF-1, which then augment local neovascularization as well as recruit additional cells to the healing complex.

The metabolism of lactate by LDH increases intracellular concentration of NADH at the expense of the NAD pool. In addition to feeding reducing equivalents for ROS as mentioned earlier, the altered NAD/NADH ratio reduces the cell content of polyADP-ribose (178). Although unclear whether entirely mediated by an altered oxidation/reduction set point, lactate stimulates collagen mRNA abundance and also collagen promoter activity by fibroblasts (49, 50). In endothelium, lactate-mediated reduction of poly-adenyl ribosylation of VEGF improves VEGF angiogenic potency (85).

### Discussion: Efficacy of $HBO_2$

The outline described earlier highlights the multiple sites where ROS, lactate, and $^{\bullet}NO$ can influence wound healing, with special emphasis on SPCs. We believe this categorization has merit to examine concurrent processes in wound healing and lends itself to highlighting sites where $HBO_2$ has effects. In one regard, this approach may be too simplistic however, because tissues contain a variety of cell types and $HBO_2$ may influence each in different ways. Figure 4 is an attempt to highlight many of these events. $HBO_2$ increases synthesis of many growth factors, although the biochemical mechanisms have not been elucidated in detail. Synthesis of VEGF has been shown to be increased in experimental wounds by $HBO_2$ (41, 154). $HBO_2$ also stimulates synthesis of basic fibroblast growth factor and TGF (1 by human dermal fibroblasts (74), angiopoietin-2 by human umbilical vein endothelial cells (95), and as previously mentioned, it up-regulates PDGF receptor in experimental wounds (19).

$HBO_2$ appears to be a reliable way to mobilize SPCs in humans (102, 172, 174). Animal data indicate that the specific target which initiates this process is NOS-3 in the stromal cell compartment of the bone marrow with subsequent liberation of SCF (54, 172). With regard to this process, in is important to stress that contrary to many of the traditional agents which increase SPCs, $HBO_2$ does not concomitantly elevate the circulating leukocyte count, which may be thrombogenic (102, 134). Newly mobilized SPCs appear to have greater content of HIF-1, HIF-2, and thioredoxin, which in the murine model exhibit improved neovascularization (115, 116, 174). Subsequent to $HBO_2$ treatments of diabetic patients, most wound margin HIFs and thioredoxin appear to be derived from localized SPCs (174). This suggests that SPCs may play an important role in supplying critical factors during wound healing in diabetic patients.

The influence that $HBO_2$ has on HIF isoform expression appears to vary based on chronology (*e.g.*, looking early or late after wounding or an ischemic insult). One recent model showing accelerated wound healing by $HBO_2$ reported lower

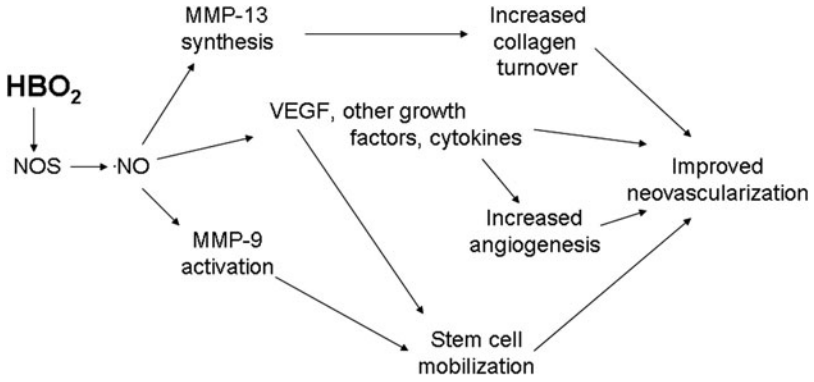


**FIG. 2. $^{\bullet}NO$ wave: Summary of wound-healing events related to elevated synthesis of nitric oxide ($^{\bullet}NO$).** NOS, nitric oxide synthase.

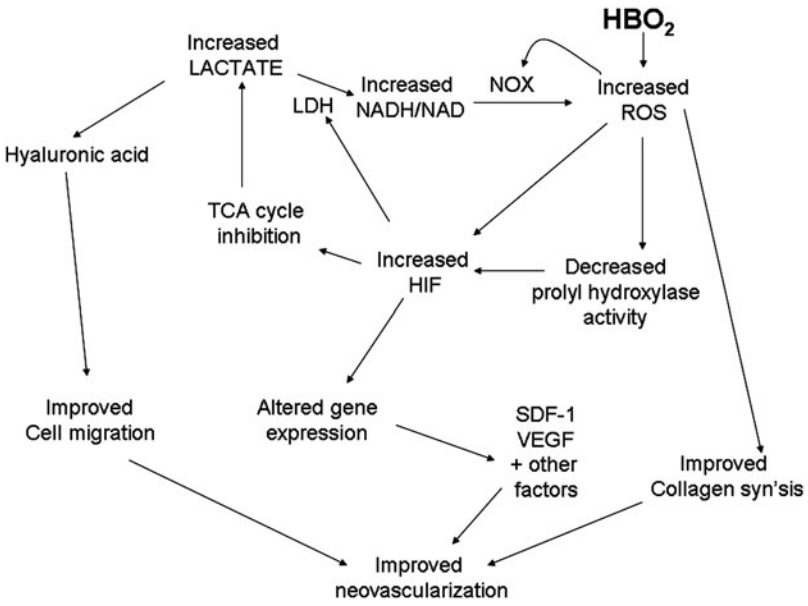


**FIG. 3. Lactate wave: Summary of wound-healing events related to elevated lactate concentrations in the region of a wound.** LDH, lactate dehydrogenase; NAD, nicotine adenine nucleotide; NADH, nicotine adenine nucleotide, reduced; NOX, NAD(P)H oxidase; TCA, tricarboxylic acid.

HIF-1 levels at wound margins with reduced inflammation and fewer apoptotic cells (189). In contrast, higher levels of HIF-1 have been linked to elevated VEGF in wounds in response to hyperoxia (64, 154).

Chronic wounds are said to have stalled in the inflammatory-healing phase, but this characterization does little to address specific flaws that vary depending on the underlying pathophysiology (87). We think this adds to the merit of viewing wound healing as ''waves'' of ROS, lactate, and •NO production. $HBO_2$ in current practice is used to treat refractory diabetic wounds and delayed radiation injuries. The pathophysiology of radiation injury is obviously different than diabetic wounds but the varied tissue abnormalities have been likened to a chronic wound (30). Common elements shared by both disorders include depletion of epithelial and stromal cells, chronic inflammation, fibrosis, an imbalance or abnormalities in extracellular matrix components and remodeling processes, and impaired keratinocyte functions (20, 30, 40, 110, 163, 175). Diabetic wound healing also is impaired by deceased growth factor production, lower •NO production due to low insulin levels, eNOS phosphorylation, and higher levels of asymmetric dimethylarginine and impaired SPCs mobilization; whereas in postradiation tissues, there appears to be an imbalance between factors mediating

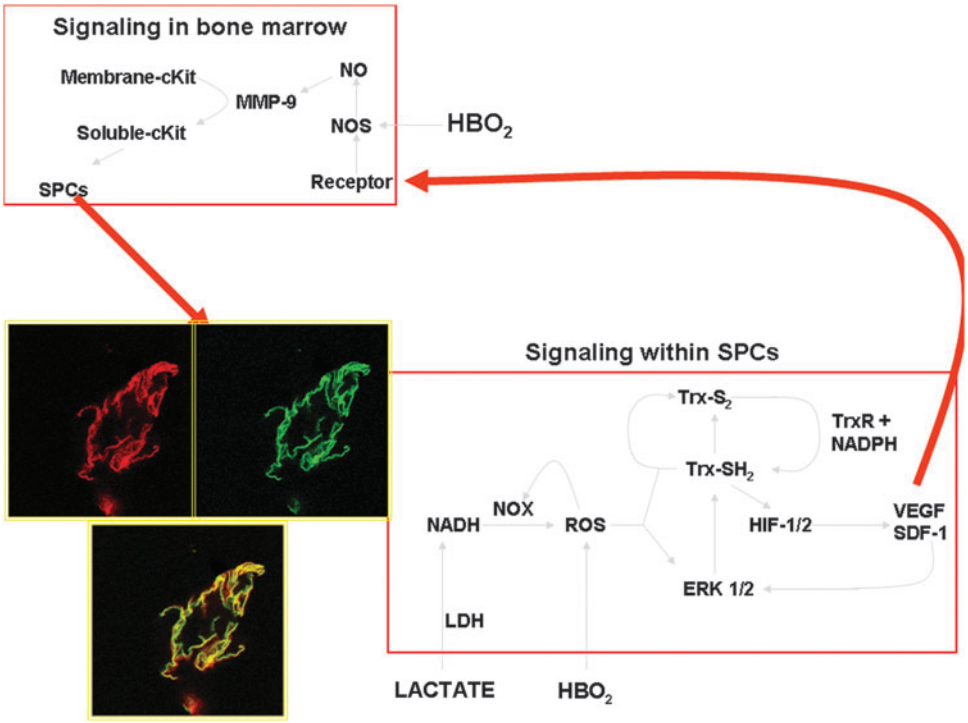


**FIG. 4. Summary of stem cell and peripheral wound site events impacted by $HBO_2$.** Images in *lower left* are confocal microscope images similar to those reported in reference (116). They demonstrate vasculogenesis in a Matrigel implant placed in a mouse that was exposed to $HBO_2$. They show CD34+ SPCs (*green*) and Nile red beads (*red*) injected *via* the heart to demonstrate functional blood vessels. The overlay between CD34+ cells and beads is shown in *yellow*. Trx-$S_2$, oxidized thioredoxin; Trx-$SH_2$, reduced thioredoxin. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

fibrosis and those promoting normal tissue healing (20, 30, 37, 147, 175).

The benefit of $HBO_2$ for radiation injury has been shown in randomized trials and is supported by independent evidence-based reviews (16, 28, 112). With regard to use of $HBO_2$ as a component to refractory diabetic wound management, the most recent meta-analysis involved eight trials and pooled data from three showed an increase in the rate of ulcer healing (odds ratio 5.20, 95% confidence interval [CI] 1.25–21.66; $p = 0.02$) with HBOT at 6 weeks, although benefit did not persist at 1 year follow up (84). Another analysis concluded that adjunctive use of $HBO_2$ as a component to diabetic wound care improves healing with an odds ratio of 11.64 (95% CI 3.457–39.196) (53). This analysis was based on clinical trials conducted through 2007 (1, 13, 34, 38, 39, 42, 72, 76, 129). Another meta-analysis concluded that only four patients needed to be treated with $HBO_2$ to prevent one amputation (83). Since this publication, two additional groups have reported benefits to use of $HBO_2$; one was a double-blinded randomized trial (36, 96). Controlled trials continue to demonstrate that $HBO_2$ improves outcome but there is room for further investigation, as will be emphasized later. The double-blinded trial was a single-center study that enrolled individuals with diabetic foot ulcers. Individuals were randomized to receive either $HBO_2$ (100% oxygen, 2.5 ATA for 85 min 5 days per week for 8 weeks) or control (room air, 2.5 ATA for 85 min 5 days per week for 8 weeks) and standardized wound care. The outcome was a healed wound by 12 months after the commencement of therapy. A total of 94 individuals with wounds present for more than 3 months were evaluated. In the intention-to-treat analysis, complete healing of the index ulcer was achieved in 37 patients at 1 year of follow up: 25 out of 48 (52%) in the $HBO_2$ group and 12 out of 42 (29%) in the placebo group ($p = 0.03$). In a sub-analysis of those patients completing $>35$ $HBO_2$ sessions, healing of the index ulcer occurred in 23 out of 38 (61%) in the $HBO_2$ group and 10 out of 37 (27%) in the placebo group ($p = 0.009$).

It is important to state that for both diabetic wounds and radiation injuries, $HBO_2$ is used in conjunction with standard surgical management. Randomized trials show clinical benefit with $HBO_2$ when attention is paid to potential confounding issues and quality of baseline care. When used by itself or if used only in the postoperative period, however, $HBO_2$ is likely to have no benefit (8, 105). In randomized trials, clinicians are constrained to follow a rigorous wound care plan that may be as important as is $HBO_2$ for improved outcomes. The optimal timing for intervention with $HBO_2$ in relation to more standard forms of therapy, as well as the most appropriate endpoints to be used for evaluating outcomes remains elusive. This can be seen in a ''real world'' comparative effectiveness study involving records review of 6, 259 individuals with foot wounds related to diabetes with adequate lower limb arterial perfusion (106). Individuals receiving $HBO_2$ were less likely to heal their foot ulcer (propensity score odds ratio 0.68 [95% CI: 0.63–0.73]) and more likely to have an amputation (odds ratio 2.37 [95% CI: 1.84–3.04]). However, utilization and how $HBO_2$ was coordinated with other interventions was uncertain. The mean number of treatments was 29 but with a broad range (25th%–75th%: 15–48). If the minimum number of treatments was taken as eight and only this population was studied, the impact of $HBO_2$ was less clear with regard to amputation (odds ratio 2.03 [95% CI: 1.49–2.77]) and with regard to a healed wound (odds ratio 0.73 [95% CI: 0.66–0.81]). These data indicate that basic science and insight into $HBO_2$ is improving, whereas there is still more to learn regarding the coordination of $HBO_2$ with other treatments and there remains a need for further clinical research.

## Innovation

This review highlights the components of wound healing where oxidative stress has a positive impact on the various cells involved in wound healing. It departs from the notion of sequential wound-healing stages by organizing the cascade of wound healing as overlapping events or waves pertaining to reactive oxygen species, lactate, and nitric oxide. This was done because hyperoxia has effects of a number of cell signaling events that converge to influence cell recruitment/chemotaxis and gene regulation/protein synthesis responses which mediate wound healing. This aids the focus on mechanistic events and the interplay among various cell types and biochemical processes.

## Acknowledgments

This work was supported by funds provided by NIH grant R01-DK094260 and the Office of Naval Research N00014-13-10614.

## References

1. Abidia A, Laden G, Kuhan G, Johnson BF, Wilkinson AR, Renwick PM, Masson EA, and McCollum PT. The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial. *Eur J Vasc Endovasc Surg* 25: 513–518, 2003.
2. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, and Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 9: 1370–1376, 2003.
3. Aicher A, Zeiher AM, and Dimmeler S. Mobilizing endothelial progenitor cells. *Hypertension* 45: 321–325, 2005.
4. Ali MA, Yasui F, Matsugo S, and Konishi T. The lactate-dependent enhancement of hydroxyl radical generation by the Fenton reaction. *Free Radic Res* 32: 429–438, 2000.
5. Allen R and Balin A. Oxidative influence on development and differentiation: an overview of a free radical theory of development. *Fr Radic Biol Med* 6: 631–661, 1989.
6. Amadeu TP and Costa AM. Nitric oxide synthesis inhibition alters rat cutaneous wound healing. *J Cutan Pathol* 33: 465–473, 2006.
7. Ameziane-El-Hassani R, Morand S, Boucher JL, Frapart YM, Apostolou D, Agnandji D, Gnidehou S, Ohayon R, Noel-Hudson MS, Francon J, Lalaoui K, Virion A, and Dupuy C. Dual oxidase-2 has an intrinsic Ca2+-dependent $H_2O_2$-generating activity. *J Biol Chem* 280: 30046–30054, 2005.
8. Annane D, Depondt J, Aubert P, Villart M, Gehanno P, Gajdos P, and Chevret S. Hyperbaric oxygen therapy for radionecrosis of the jaw: a randomized, placebo-controlled, double-blind trial from the ORN96 study group. *J Clin Oncol* 22: 4893–4900, 2004.

9. Aramoto H, Breslin JW, Pappas PJ, Hobson RW, 2nd, and Duran WN. Vascular endothelial growth factor stimulates differential signaling pathways in *in vivo* microcirculation. *Am J Physiol Heart Circ Physiol* 287: H1590–H1598, 2004.
10. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, and Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967, 1997.
11. Bae YS, Sung JY, Kim OS, Kim YJ, Hur KC, Kazlauskas A, and Rhee SG. Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. *J Biol Chem* 275: 10527–10531, 2000.
12. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, Yakes FM, Van Houten B, Ballinger CA, Freeman BA, and Runge MS. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res* 86: 960–966, 2000.
13. Baroni G, Porro T, Faglia E, Pizzi G, Mastropasqua A, Oriani G, Pedesini G, and Favales F. Hyperbaric oxygen in diabetic gangrene treatment. *Diabetes Care* 10: 81–86, 1987.
14. Bauer JA, Rao W, and Smith DJ. Evaluation of linear polyethyleneimine/nitric oxide adduct on wound repair: therapy versus toxicity. *Wound Repair Regen* 6: 569–577, 1998.
15. Bedard K and Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87: 245–313, 2007.
16. Bennett M, Feldmeier J, Hampson N, Smee R, and Milross C. Hyperbaric oxygen therapy for late radiation tissue injury (Cochrane review). *Cochrane Database Syst Rev*. 2012 May 16;5:CD005005. DOI: 10.1002/14651858.CD005005.pub3.
17. Bienert GP, Schjoerring JK, and Jahn TP. Membrane transport of hydrogen peroxide. *Biochim Biophys Acta* 1758: 994–1003, 2006.
18. Biswas S, Ray M, Misra S, Dutta D, and Ray S. Is absence of pyruvate dehydrogenase complex in mitochondria a possible explanation of significant aerobic glycolysis by normal human leukocytes? *FEBS Lett* 425: 411–414, 1998.
19. Bonomo SR, Davidson JD, Yu Y, Xia Y, Lin X, and Mustoe TA. Hyperbaric oxygen as a signal transducer: upregulation of platelet derived growth factor-beta receptor in the presence of $HBO_2$ and PDGF. *Undersea Hyperb Med* 25: 211–216, 1998.
20. Brem H and Tomic-Canic M. Cellular and molecular basis of wound healing in diabetics. *J Clin Invest* 117: 1219–1222, 2007.
21. Buggisch M, Ateghang B, Ruhe C, Strobel C, Lange S, Wartenberg M, and Sauer H. Stimulation of ES-cell-derived cardiomyogenesis and neonatal cardiac cell proliferation by reactive oxygen species and NADPH oxidase. *J Cell Sci* 120: 885–894, 2007.
22. Cabigas BP, Su J, Hutchins W, Shi Y, Schaefer RB, Recinos RF, Nilakantan V, Kindwall E, Niezgoda JA, and Baker JE. Hyperoxic and hyperbaric-induced cardioprotection: role of nitric oxide synthase 3. *Cardiovasc Res* 72: 143–151, 2006.
23. Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield D, and Stella A. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nat Rev Neurosci* 8: 766–775, 2007.
24. Cherng JH, Chang SC, Chen SG, Hsu ML, Hong PD, Teng SC, Chan YH, Wang CH, Chen TM, and Dai NT. The effect of hyperbaric oxygen and air on cartilage tissue. *Ann Plast Surg* 69: 650–655, 2012.
25. Cho M, Hunt TK, and Hussain MZ. Hydrogen peroxide stimulates macrophage vascular endothelial growth factor release. *Am J Physiol Heart Circ Physiol* 280: H2357–H2363, 2001.
26. Circu ML and Aw TY. Glutathione and apoptosis. *Free Rad Res* 42: 689–706, 2008.
27. Clark J. Oxygen toxicity. In: *Physiology and Medicine of Hyperbaric Oxygen Therapy,* edited by Neuman TS and Thom SR. Philadelphia, PA: Saunders, 2008, pp. 527–563.
28. Clarke R, Tenorio C, Hussey J, Toklu A, Cone D, Hinojosa J, Desai S, Parra L, Rodrigues S, Long R, and Walker M. Hyperbaric oxygen treatment of chronic radiation proctitis: a randomized and controlled doouble blind crossover trial with long-term follow-up. *Int J Rad Oncol Biol Phys* 72: 134–143, 2008.
29. Daiber A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. *Biochim Biophys Acta* 1797: 897–906, 2010.
30. Denham J and Hauer-Jensen M. The radiotherapeutic injury-a complex 'wound'. *Radiother Oncol* 63: 129–145, 2002.
31. Dennog C, Gedik C, Wood S, and Speit G. Analysis of oxidative DNA damage and HPRT mutations in humans after hyperbaric oxygen treatment. *Mutation Res* 431: 351–359, 1999.
32. Dennog C, Hartmann A, Frey G, and Speit G. Detection of DNA damage after hyperbaric oxygen (HBO) therapy. *Mutagenesis* 11: 605–609, 1996.
33. Dhar M, Neilsen N, Beatty K, Eaker S, Adair H, and Geiser D. Equine peripheral blood-derived mesenchyman stem cells: isolation, identification, trilineage differentiation and effect of hyperbaric oxygen treatment. *Equine Vet J* 44: 600–605, 2012.
34. Doctor N, Pandya S, and Supe A. Hyperbaric oxygen therapy in diabetic foot. *J Postgrad Med* 38: 112–114, 1991.
35. Dulak J and Jozkowicz A. Regulation of vascular endothelial growth factor synthesis by nitric oxide: facts and controversies. *Antioxid Redox Signal* 5: 123–132, 2003.
36. Duzgun AP, Satir AZ, Ozozan O, Saylam B, Kulah B, and Caskun F. Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers. *J. Foot Ankle Surg* 47: 515–519, 2008.
37. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, Grego F, Vigili de Kreutzenberg S, Tiengo A, Agostini C, and Avogaro, A. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arter Thromb Vasc Biol* 26: 2140–2146, 2006.
38. Faglia E, Favales F, Aldeghi A, *et al*. Change in major amputation rate in a center dedicated to diabetic foot care during the 19080s: prognosis determinants for major amputation. *J Diabetes Complications* 12: 96, 1998.
39. Faglia E, Favales F, Aldeghi A, Calia P, Quarantiello A, Oriani G, Michael M, Campagnoli P, and Morabito A. Adjunctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer. *Diabetes Care* 19: 1338–1343, 1996.
40. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet* 366: 1736–1743, 2005.
41. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 25: 581–611, 2004.
42. Fife CE, Buyukcakir C, Otto G, Sheffield P, Love T, and Warriner R, 3rd. Factors influencing the outcome of lower-extremity diabetic ulcers treated with hyperbaric oxygen therapy. *Wound Repair Regen* 15: 322–331, 2007.

43. Fok TC, Jan A, Peel SA, Evans AW, Clokie CM, and Sandor GK. Hyperbaric oxygen results in increased vascular endothelial growth factor (VEGF) protein expression in rabbit calvarial critical-sized defects. *Oral Surg Oral Med Oral Pathol* 105: 417–422, 2008.
44. Formby B and Stern R. Lactate-sensitive response elements in genes involved in hyaluronan catabolism. *Biochem Biophys Res Commun* 305: 203–208, 2003.
45. Frank S, Stallmeyer B, Kampfer H, Kolb N, and Pfeilschifter J. Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes (HaCaT) and during cutaneous wound repair. *Faseb J* 13: 2002–2014, 1999.
46. Fu TS, Ueng SW, Tsai TT, Chen LH, Lin SS, and Chen WJ. Effect of hyperbaric oxygen on mesenchymal stem cells for lumbar fusion *in vivo*. *BMC Musculoskelet Disord* 11: 52, 2010.
47. Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, Buerk DG, Huang PL, and Jain RK. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proc Natl Acad Sci U S A* 98: 2604–2609, 2001.
48. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR, and Velazquez OC. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. *J Clin Invest* 117: 1249–1259, 2007.
49. Ghani Q, Hussain M, Zhang J, and Hunt T. *Control of Procollagen Gene Transcription and Prolyl Hydroxylase Activity by Poly (ADP-Ribose)*. New York: Springer-Verlag, 1992.
50. Gimbel M, Hunt T, and Hussain M. Lactate controls collagen gene promoter activity through poly-ADP-ribosylaton. *Surg Forum* 51: 26–27, 2000.
51. Gladden L. Lactate metabolism: a new paradigm for the third millennium. *J Physiol* 558: 5–30, 2004.
52. Goldie LC and Nix MK. Embryonic vasculogenesis and hematopoietic specification. *Organogenesis* 4: 257–263, 2008.
53. Goldman RJ. Hyperbaric oxygen therapy for wound healing and limb salvage: a systematic review. *Phys Med Rehabil* 1: 471–489, 2009.
54. Goldstein LJ, Gallagher KA, Bauer SM, Bauer RJ, Baireddy V, Liu ZJ, Buerk DG, Thom SR, and Velazquez OC. Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. *Stem Cells* 24: 2309–2318, 2006.
55. Gonzalez-Pacheco FR, Deudero JJ, Castellanos MC, Castilla MA, Alvarez-Arroyo MV, Yague S, and Caramelo C. Mechanisms of endothelial response to oxidative aggression: protective role of autologous VEGF and induction of VEGFR2 by $H_2O_2$. *Am J Physiol Heart Circ Physiol* 291: H1395–H1401, 2006.
56. Grange L, Nguyen MV, Lardy B, Derouazi M, Campion Y, Trocme C, Paclet MH, Gaudin P, and Morel F. NAD(P)H oxidase activity of Nox4 in chondrocytes is both inducible and involved in collagenase expression. *Antioxid Redox Signal* 8: 1485–1496, 2006.
57. Haddad JJ. Redox regulation of pro-inflammatory cytokines and IkappaB-alpha/NF-kappaB nuclear translocation and activation. *Biochem Biophys Res Commun* 296: 847–856, 2002.
58. Haddad JJ, Saade NE, and Safieh-Garabedian B. Redox regulation of TNF-alpha biosynthesis: augmentation by irreversible inhibition of gamma-glutamylcysteine synthetase and the involvement of an IkappaB-alpha/NF-kappaB-independent pathway in alveolar epithelial cells. *Cell Signal* 14: 211–218, 2002.
59. Hanahan D. Signaling vascular morphogenesis and maintenance. *Science* 277: 48–50, 1997.
60. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, and Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 109: 625–637, 2002.
61. Heissig B, Werb Z, Rafii S, and Hattori K. Role of c-kit/Kit ligand signaling in regulating vasculogenesis. *Thromb Haemost* 90: 570–576, 2003.
62. Herkert O, Djordjevic T, BelAiba RS, and Gorlach A. Insights into the redox control of blood coagulation: role of vascular NADPH oxidase-derived reactive oxygen species in the thrombogenic cycle. *Antioxid Redox Signal* 6: 765–776, 2004.
63. Hu C, Wang L, Chodosh L, Keith B, and Simon M. Differential roles of hypoxia-inducible factor 1a (HIF-1a) and HIF-2a in hypoxic gene regulation. *Mol Cell Biol* 23: 9361–9374, 2003.
64. Hunt T, Aslam R, Beckert S, Wagner S, Ghani Q, Hussain M, Roy S, and Sen C. Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. *Antioxid Redox Signal* 9: 1115–1124, 2007.
65. This reference has been deleted.
66. Hunt TK, Conolly WB, Aronson SB, and Goldstein P. Anaerobic metabolism and wound healing: an hypothesis for the initiation and cessation of collagen synthesis in wounds. *Am J Surg* 135: 328–332, 1978.
67. Hunt TK, Zederfeldt B, and Goldstick TK. Oxygen and healing. *Am J Surg* 118: 521–525, 1969.
68. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, and Suda T. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med* 12: 446–451, 2006.
69. Ji AR, Ku SY, Cho MS, Kim YY, Kim YJ, Oh SK, Kim SH, Moon SY, and Choi YM. Reactive oxygen species enhance differentiation of human embryonic stem cells into mesendodermal lineage. *Exp Mol Med* 42: 175–186, 2010.
70. Juarez JC, Manuia M, Burnett ME, Betancourt O, Boivin B, Shaw DE, Tonks NK, Mazar AP, and Donate F. Superoxide dismutase 1 (SOD1) is essential for $H_2O_2$-mediated oxidation and inactivation of phosphatases in growth factor signaling. *Proc Natl Acad Sci U S A* 105: 7147–7152, 2008.
71. Junn E, Lee KN, Ju HR, Han SH, Im JY, Kang HS, Lee TH, Bae YS, Ha KS, Lee ZW, Rhee SG, and Choi I. Requirement of hydrogen peroxide generation in TGF-beta 1 signal transduction in human lung fibroblast cells: involvement of hydrogen peroxide and Ca2+ in TGF-beta 1-induced IL-6 expression. *J Immunol* 165: 2190–2197, 2000.
72. Kalani M, Jorneskog G, Naderi N, Lind F, and Brismar K. Hyperbaric oxygen therapy in treatment of diabetic foot ulcers. *J Diabetes Complications* 16: 153–158, 2002.
73. Kane NM, Xiao Q, Baker AH, Luo Z, Xu Q, and Emanueli C. Pluripotent stem cell differentiation into vascular cells: a novel technology with promises for vascular re(generation). *Pharmacol Ther* 129: 29–49, 2011.

74. Kang TS, Gorti GK, Quan SY, Ho M, and Koch RJ. Effect of hyperbaric oxygen on the growth factor profile of fibroblasts. *Arch Facial Plast Surg* 6: 31–35, 2004.
75. Kemp M, Go YM, and Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems in biology. *Fr Radic Biol Med* 44: 921–937, 2008.
76. Kessler L, Bilbault P, Ortega F, Grasso C, Passemard R, Stephan D, Pinget M, and Schneider F. Hyperbaric oxygenation accelerates the healing rate of nonischemic chronic diabetic foot ulcers: a prospective randomized study. *Diabetes Care* 26: 2378–2382, 2003.
77. Khan M, Meduru S, Gogna R, Madan E, Citro L, Kuppusamy ML, Sayyid M, Mostafa M, Hamlin RL, and Kuppusamy P. Oxygen cycling in conjunction with stem cell transplantation induces NOS3 expression leading to attenuation of fibrosis and improved cardiac function. *Cardiovas Res* 93: 89–99, 2012.
78. Khan M, Meduru S, Mohan IK, Kuppusamy ML, Wisel, Kulkarni A, Rivera BK, Hamlin RL, and Kuppusamy P. Hyperbaric oxygenation enhances transplanted cell graft and functional recovery in the infarct heart. *J Moll Cell Cardiol* 47: 257–287, 2009.
79. Kim JW, Tchernyshyov I, Semenza GL, and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3: 177–185, 2006.
80. Kirkeby L, Ghani QP, Enriquez B, Hussain MZ, and Hunt TK. Stimulation of collagen synthesis in fibroblasts by hydrogen peroxide. *Mol Biol Cell* 6: 44–48, 1995.
81. Kiviluoto T, Watanabe S, Hirose M, Sato N, Mustonen H, Puolakkainen P, Ronty M, Ranta-Knuuttila T, and Kivilaakso E. Nitric oxide donors retard wound healing in cultured rabbit gastric epithelial cell monolayers. *Am J Physiol Gastrointest Liver Physiol* 281: G1151–G1157, 2001.
82. Klyubin IV, Kirpichnikova KM, and Gamaley IA. Hydrogen peroxide-induced chemotaxis of mouse peritoneal neutrophils. *Eur J Cell Biol* 70: 347–351, 1996.
83. Kranke P, Bennett M, Roeckl-Wiedmann I, and Debus S. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev* 2: CD004123, 2004.
84. Kranke P, Bennett MH, Martyn-St James M, Schnabel A, and Debus SE. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev* 4: CD004123, 2012.
85. Kumar VB, Viji RI, Kiran MS, and Sudhakaran PR. Endothelial cell response to lactate: implication of PAR modification of VEGF. *J Cell Physiol* 211: 477–485, 2007.
86. Lam BS and Adams GB. Hematopoietic stem cell lodgment in the adult bone marrow stem cell niche. *Int J Lab Hematol* 32: 551–558, 2010.
87. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, and Rodeheaver G. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol* 130: 489–493, 1994.
88. Lee RH, Efron D, Tantry U, and Barbul A. Nitric oxide in the healing wound: a time-course study. *J Surg Res* 101: 104–108, 2001.
89. Lee RL, Westendorf J, and Gold MR. Differential role of reactive oxygen species in the activation of mitogen-activated protein kinases and Akt by key receptors on B-lymphocytes: CD40, the B cell antigen receptor, and CXCR4. *J Cell Commun Signal* 1: 33–43, 2007.
90. Lee SB, Bae IH, Bae YS, and Um HD. Link between mitochondria and NADPH oxidase 1 isozyme for the sustained production of reactive oxygen species and cell death. *J Biol Chem* 281: 36228–36235, 2006.
91. Leite PF, Danilovic A, Moriel P, Dantas K, Marklund S, Dantas AP, and Laurindo FR. Sustained decrease in asuperoxide dismutase activity underlies constrictive remodeling after balloon injury in rabbits. *Arterioscler Thromb Vasc Biol* 23: 2197–2202, 2003.
92. Lewandowski D, Barroca V, Duconge F, Bayer J, Van Nhieu JT, Pestourie C, Fouchet P, Tavitian B, and Romeo PH. *In vivo* cellular imaging pinpoints the role of reactive oxygen species in the early steps of adult hematopoietic reconstitution. *Blood* 115: 443–452, 2010.
93. Li TS and Marban E. Physiological levels of reactive oxygen species are required to maintain genomic stability in stem cells. *Stem Cells* 28: 1178–1185, 2010.
94. Li W, Liu G, Chou IN, and Kagan HM. Hydrogen peroxide-mediated, lysyl oxidase-dependent chemotaxis of vascular smooth muscle cells. *J Cell Biochem* 78: 550–557, 2000.
95. Lin S, Shyu KG, Lee CC, Wang BW, Chang CC, Liu YC, Huang FY, and Chang H. Hyperbaric oxygen selectively induces angiopoietin-2 in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 296: 710–715, 2002.
96. Londahl M, Katzman P, Nilsson A, and Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diab Care* 33: 998–1003, 2010.
97. Lopez-Rivera E, Lizarbe TR, Martinez-Moreno M, Lopez-Novoa JM, Rodriguez-Barbero A, Rodrigo J, Fernandez AP, Alvarez-Barrientos A, Lamas S, and Zaragoza C. Matrix metalloproteinase 13 mediates nitric oxide activation of endothelial cell migration. *Proc Natl Acad Sci U S A* 102: 3685–3690, 2005.
98. Lu H, Dalgard CL, Mohyeldin A, McFate T, Tait AS, and Verma A. Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. *J Biol Chem* 280: 41928–41939, 2005.
99. Lu H, Forbes R, and Verma A. Hypoxia-inducible factor-1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* 277: 23111–23115, 2002.
100. Lu H, Youker K, Ballantyne C, Entman M, and Smith CW. Hydrogen peroxide induces LFA-1-dependent neutrophil adherence to cardiac myocytes. *Am J Physiol Heart Circ Physiol* 278: H835–H842, 2000.
101. Luo JD, Wang YY, Fu WL, Wu J, and Chen AF. Gene therapy of endothelial nitric oxide synthase and manganese superoxide dismutase restores delayed wound healing n type 1 diabetic mice. *Circulation* 110: 2484–2493, 2004.
102. Ma YH, Lei YH, Zhou M, Li X, and Zhao HY. Effects of hyperbaric oxygen therapy in the management of chronic wounds and its correlation with CD34(+) endothelial progenitor cells. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 91: 3214–3218, 2011.
103. Mader JT, Adams KR, and Sutton TE. Infectious diseases: pathophysiology and mechanisms of hyperbaric oxygen. *J Hyperbaric Med* 2: 133–140, 1987.
104. Mader JT, Brown GL, Guckian JC, Wells CH, and Reinarz JA. A mechanism for the amelioration by hyperbaric oxygen of experimental staphylococcal osteomyelitis in rabbits. *J Infect Dis* 142: 915–922, 1980.

105. Maier A, Gaggl A, Klemen H, Santler G, Anegg U, Fell B, Karcher H, Smolle-Juttner F, and Friehs G. Review of severe osteoradionecrosis treated by surgery alone or surgery with postoperative hyperbaric oxygenation. *Br J Oral Maxillofac Surg* 38: 173–176, 2000.
106. Margolis DJ, Gupta J, Hoffstad O, Papdopoulos M, Thom SR, and Mitra N. Lack of effectiveness of hyperbaric oxygen therapy for the treatment of diabetic foot ulcer and the prevention of amputation. *Diab Care* 36: 1961–1966, 2013.
107. Margolis DJ, Malay DS, Hoffstad OJ, Leonard CE, MaCurdy T, de Nava KL, Tan Y, Molina T, and Siegel KL. Incidence of Diabetic Foot Ulcer and Lower Extremity Amputation Among Medicare Beneficiaries, 2006 to 2008: Data Points #2. 2011.
108. Margolis DJ, Malay DS, Hoffstad OJ, Leonard CE, MaCurdy T, Tan Y, Molina T, de Nava KL, and Siegel KL. Economic Burden of Diabetic Foot Ulcers and Amputations: Data Points #3. 2011.
109. Marrotte EJ, Chen DD, Hakim JS, and Chen AF. Manganese superoxide dismutase expression in endothelial progenitor cells accelerates wound healing in diabetic mice. *J Clin Invest* 120: 4207–4219, 2010.
110. Martin M, Lefaix J, and Delanian S. TGF-beta1 and radiation fibrosis: a master switch and a specific therapeutic target? *Int J Radiat Oncol Biol Phys* 47: 277–290, 2000.
111. Marumo T, Schini-Kerth VB, Fisslthaler B, and Busse R. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kappaB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. *Circulation* 96: 2361–2367, 1997.
112. Marx RE, Johnson RP, and Kline SN. Prevention of osteoradionecrosis: a randomized prospective clinical trial of hyperbaric oxygen versus penicillin. *JADA* 111: 49–54, 1985.
113. Maulik N. Redox signaling and angiogenesis. *Antioxid Redox Signal* 4: 805–815, 2002.
114. Michiels C, Minet E, Mottet D, and Raes M. Regulation of gene expression by oxygen: NF-kappaB and HIF-1, two extremes. *Free Radic Biol Med* 33: 1231–1242, 2002.
115. Milovanova T, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, Velazquez OC, and Thom SR. Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia inducible factor-1. *Mol Biol Cell* 28: 6248–6261, 2008.
116. Milovanova T, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Velazquez OC, and Thom SR. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation *in vivo*. *J Appl Physiol* 106: 711–728, 2009.
117. This reference has been deleted.
118. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, Chen C, Hosokawa K, Nakauchi H, Nakayama K, Harada M, Motoyama N, Suda T, and Hirao A. FoxO3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 1: 101–112, 2007.
119. Nakamura Y, Tajima F, Ishiga K, Yamazaki H, Oshimura M, Shiota G, and Murawaki Y. Soluble c-kit receptor mobilizes hematopoietic stem cells to peripheral blood in mice. *Exp Hematol* 32: 390–396, 2004.
120. Narkowicz CK, Vial JH, and McCartney PW. Hyperbaric oxygen therapy increases free radical levels in the blood of humans. *Free Radic Res Commun* 19: 71–80, 1993.
121. Nelson KK and Melendez JA. Mitochondrial redox control of matrix metalloproteinases. *Free Radic Biol Med* 37: 768–784, 2004.
122. Newsholme EA, Crabtree B, and Ardawi MS. The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. *Biosci Rep* 5: 393–400, 1985.
123. Newsholme P, Costa Rosa L, Newsholme EA, and Curi R. The importance of fuel metabolism to macrophage function. *Cell Biochem Funct* 14: 1–10, 1996.
124. Niethammer P, Grabher C, Look AT, and Mitchison TJ. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature* 459: 996–999, 2009.
125. Nishio E and Watanabe Y. The involvement of reactive oxygen species and arachidonic acid in alpha 1-adrenoceptor-induced smooth muscle cell proliferation and migration. *Br J Pharmacol* 121: 665–670, 1997.
126. Ogasawara MA and Zhang H. Redox regulation and its emerging roles in stem cells and stem-like cancer cells. *Antioxid Redox Signal* 11: 1107–1122, 2009.
127. Ogura M and Kitamura M. Oxidant stress incites spreading of macrophages via extracellular signal-regulated kinases and p38 mitogen-activated protein kinase. *J Immunol* 161: 3569–3574, 1998.
128. Okamoto T, Akuta T, Tamura F, van Der Vliet A, and Akaike T. Molecular mechanism for activation and regulation of matrix metalloproteinases during bacterial infections and respiratory inflammation. *Biol Chem* 385: 997–1006, 2004.
129. Oriani G, Meazza D, Favales F, Pizzi GL, Aldeghi A, and Faglia E. Hyperbaric oxygen therapy in diabetic gangrene. *J Hyper Med* 5: 171–175, 1990.
130. Page EL, Chan DA, Giaccia AJ, Levine M, and Richard DE. Hypoxia-inducible factor-1alpha stabilization in nonhypoxic conditions: role of oxidation and intracellular ascorbate depletion. *Mol Biol Cell* 19: 86–94, 2008.
131. Parenti A, Morbidelli L, Cui XL, Douglas JG, Hood JD, Granger HJ, Ledda F, and Ziche M. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase1/2 activation in postcapillary endothelium. *J Biol Chem* 273: 4220–4226, 1998.
132. Pecoraro RE, Reiber GE, and Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care* 13: 513–521, 1990.
133. Penn MS, Patel CV, Cui MZ, DiCorleto PE, and Chisolm GM. LDL increases inactive tissue factor on vascular smooth muscle cell surfaces: hydrogen peroxide activates latent cell surface tissue factor. *Circulation* 99: 1753–1759, 1999.
134. Powell TM, Paul JD, Hill JM, Thompson M, Benjamin M, Rodrigo M, McCoy JP, Read EJ, Khuu HM, Leitman SF, Finkel T, and Cannon RO, 3rd. Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 25: 296–301, 2005.
135. Puigserver P, Rhee J, Lin J, Wu Z, Yoon CY, Zhang CY, Krauss S, Mootha VK, Lowell BB, and Speigelman BM. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPAR-gamma coactivator-1. *Mol Cell* 8: 971–982, 2001.
136. Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H, Miura S, Mishina Y, and Zhao GQ. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated

protein kinase pathways. *Proc Natl Acad Sci U S A* 101: 6027–6032, 2004.
137. Rafii S, Avecilla S, Shmelkov S, Shido K, Tejada R, Moore MA, Heissig B, and Hattori K. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. *Ann N Y Acad Sci* 996: 49–60, 2003.
138. Rafii S and Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 9: 702–712, 2003.
139. Ranjan P, Anathy V, Burch PM, Weirather K, Lambeth JD, and Heintz NH. Redox-dependent expression of cyclin D1 and cell proliferation by Nox1 in mouse lung epithelial cells. *Antioxid Redox Signal* 8: 1447–1459, 2006.
140. Rathore R, Zheng YM, Niu CF, Liu QH, Korde A, Ho YS, and Wang YX. Hypoxia activates NADPH oxidase to increase [ROS]i and [Ca2+]i through the mitochondrial ROS-PKCepsilon signaling axis in pulmonary artery smooth muscle cells. *Free Radic Biol Med* 45: 1223–1231, 2008.
141. Rothfuss A, Radermacher P, and Speit G. Involvement of heme oxygenase-1 (HO-1) in the adaptive protection of human lymphocytes after hyperbaric oxygen (HBO) treatment. *Carcinogenesis* 22: 1979–1985, 2001.
142. Salvemini D and Botting R. Modulation of platelet function by free radicals and free-radical scavengers. *Trends Pharmacol Sci* 14: 36–42, 1993.
143. Savini I, Arnone R, Rossi A, Catani MV, Del Principe D, and Avigliano L. Redox modulation of Ecto-NOX1 in human platelets. *Mol Membr Biol* 27: 160–169, 2010.
144. Schafer G, Cramer T, Suske G, Kemmner W, Wiedenmann B, and Hocker M. Oxidative stress regulates vascular endothelial growth factor-A gene transcription through Sp1- and Sp3-dependent activation of two proximal GC-rich promoter elements. *J Biol Chem* 278: 8190–8198, 2003.
145. Schaffer MR, Efron PA, Thornton FJ, Klingel K, Gross SS, and Barbul A. Nitric oxide, an autocrine regulator of wound fibroblast synthetic function. *J Immunol* 158: 2375–2381, 1997.
146. Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, and Babilas P. Oxygen in acute and chronic wound healing. *Br J Dermatol* 163: 257–268, 2010.
147. Sciacqua A, Grillo N, Quero M, Sesti G, and Perticone F. Asymetric dimethylarginine plasma levels and endothelial function in newly diagnosed type 2 diabetic patients. *Int J Mol Sci* 13: 13804–13815, 2012.
148. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* 88: 1474–1480, 2000.
149. Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. *Trends Mol Med* 7: 345–350, 2001.
150. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, and Longaker MT. Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen* 17: 763–771, 2009.
151. Sen CK, Khanna S, Babior BM, Hunt TK, Ellison EC, and Roy S. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. *J Biol Chem* 277: 33284–33290, 2002.
152. Sen CK and Roy S. Redox signals in wound healing. *Biochem Biophys Acta* 1780: 1348–1363, 2008.
153. Sever C, Uygur F, Kulahci Y, Torun Kose G, Urhan M, Kucukodaci Z, Uzun G, Ipcioglu O, and Cayci T. Effect of hyperbaric oxygen tehrapy on bone prefabrication in rats. *Acta Orthop Traumatol Turc* 44: 403–409, 2010.
154. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, and Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. *Arch Surg* 135: 1293–1297, 2000.
155. This reference has been deleted.
156. Shyu KG, Hung HF, Wang BW, and Chang H. Hyperbaric oxygen induces placental growth factor expression in bone marrow-derived mesenchymal stem cells. *Life Sci* 83: 65–73, 2008.
157. Simon MC and Keith B. The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol* 9: 285–296, 2008.
158. Simsek T, Kocabas F, Zheng J, Deberardinis RJ, Mahmoud AI, Olson EN, Schneider JW, Zhang CC, and Sadek HA. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. *Cell Stem Cell* 7: 380–390, 2010.
159. Siwik DA and Colucci WS. Regulation of matrix metalloproteinases by cytokines and reactive oxygen/nitrogen species in the myocardium. *Heart Fail Rev* 9: 43–51, 2004.
160. Sonveaux P, Copetti T, DeSaedeleer CJ, Vegran F, Verrax J, Kennedy KM, Moon EJ, Dhup S, Danhier P, Frerart F, Gallez B, Ribeiro A, Michiels C, Dewhirst MW, and Feron O. Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS One* 7: e33418, 2012.
161. Stallmeyer B, Kampfer H, Kolb N, Pfeilschifter J, and Frank S. The function of nitric oxide in wound repair: inhibition of inducible nitric oxide-synthase severely impairs wound reepithelialization. *J Invest Dermatol* 113: 1090–1098, 1999.
162. Stern R, Shuster S, Neudecker BA, and Formby B. Lactate stimulates fibroblast expression of hyaluronan and CD44: the Warburg effect revisited. *Exp Cell Res* 276: 24–31, 2002.
163. Stojadinovic O, Brem H, Vouthounis C, Lee B, Fallon J, Stallcup M, Merchant A, Galiano R, and Tomic-Canic M. Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing. *Am J Pathol* 167: 59–69, 2005.
164. Stone JR and Collins T. The role of hydrogen peroxide in endothelial proliferative responses. *Endothelium* 9: 231–238, 2002.
165. Stuehr DJ, Santolini J, Wang ZQ, Wei CC, and Adak S. Update on mechanism and catalytic regulation in the NO synthases. *J Biol Chem* 279: 36167–36170, 2004.
166. Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, Shima H, Johnson RS, Hirao A, Suematsu M, and Suda T. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell* 7: 391–402, 2010.
167. Thom SR. Hyperbaric oxygen therapy. *J. Intensive Care Med* 4: 58–74, 1989.
168. Thom SR. Platelet function in humans is not altered by hyperbaric oxygen therapy. *Undersea Hyperb Med* 33: 81–83, 2006.
169. Thom SR, Bhopale V, Fisher D, Manevich Y, Huang PL, and Buerk DG. Stimulation of nitric oxide synthase in ce-

rebral cortex due to elevated partial pressures of oxygen: an oxidative stress response. *J Neurobiol* 51: 85–100, 2002.
170. Thom SR, Bhopale VM, Mancini DJ, and Milovanova TN. Actin S-nitrosylation inhibits neutrophil beta2 integrin function. *J Biol Chem* 283: 10822–10834, 2008.
171. Thom SR, Bhopale VM, Milovanova TN, Yang M, Bogush M, and Buerk DG. Nitric oxide synthase-2 linkage to focal adhesion kinase in neutrophils influences enzyme activity and beta-2 integrin function. *J Biol Chem* 288: 4810–4818, 2013.
172. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, and Buerk DG. Stem cell mobilization by hyperbaric oxygen. *Am J Physiol Heart Circ Physiol* 290: H1378–H1386, 2006.
173. Thom SR, Mendiguren I, Hardy K, Bolotin T, Fisher D, Nebolon M, and Kilpatrick L. Inhibition of human neutrophil beta2-integrin-dependent adherence by hyperbaric $O_2$. *Am J Physiol* 272: C770–C777, 1997.
174. Thom SR, Milovanova TN, Yang M, Bhopale VM, Sorokina EM, Uzun G, Malay DS, Troiano MA, Hardy KR, Lambert DS, Logue CJ, and Margolis DJ. Vasculogenic stem cell mobilization and wound recruitment in diabetic patients: Increased cell number and intracellular protein content associated with hyperbaric oxygen therapy. *Wound Rep Reg* 19: 149–161, 2011.
175. Ueno H, Ohya T, Ito H, Kobayashi Y, Yamada K, and Sato M. Chitosan application to X-ray irradiated wound in dogs. *J Plast Reconstr Asthetic Surg* 60: 304–310, 2007.
176. Ushio-Fukai M and Alexander R. Reactive oxygen species as mediators of angiogenesis signaling. *Mol Cell Biochem* 264: 85–97, 2004.
177. Wachowicz B, Olas B, Zbikowska HM, and Buczynski A. Generation of reactive oxygen species in blood platelets. *Platelets* 13: 175–182, 2002.
178. Wagner S, Hussain MZ, Beckert S, Ghani QP, Weinreich J, Hunt TK, Becker HD, and Konigsrainer A. Lactate down-regulates cellular poly(ADP-ribose) formation in cultured human skin fibroblasts. *Eur J Clin Invest* 37: 134–139, 2007.
179. Wang N, Xie K, Huo S, Zhao J, Zhang S, and Miao J. Suppressing phosphatidylcholine-specific phospholipase C and elevating ROS level, NADPH oxidase activity and Rb level induced neuronal differentiation in mesenchymal stem cells. *J Cell Biochem* 100: 1548–1557, 2007.
180. Watt SM, Athanassopoulos A, Harris AL, and Tsaknakis G. Human endothelial stem/progenitor cells, angiogenic factors and vascular repair. *J R Soc Interface* 7 Suppl 6: S731–S751, 2010.
181. Welsh N, Eizirik DL, Bendtzen K, and Sandler S. Interleukin-1 beta-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. *Endocrinology* 129: 3167–3173, 1991.
182. Wetzler C, Kampfer H, Pfeilschifter J, and Frank S. Keratinocyte-derived chemotactic cytokines: expressional modulation by nitric oxide *in vitro* and during cutaneous wound repair *in vivo*. *Biochem Biophys Res Commun* 274: 689–696, 2000.
183. Wilson A and Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol* 6: 93–106, 2006.
184. Yan LJ, Levine RL, and Sohal RS. Oxidative damage during aging targets mitochondrial aconitase. *Proc Natl Acad Sci U S A* 94: 11168–11172, 1997.
185. Yang YJ, Wang XL, Yu XH, Wang X, Xie M, and Liu CT. Hyperbaric oxygen induces endogenous neural stem cells to proliferate and differentiate in hypoxic-ischemic brain damage in neonatal rats. *Undersea Hyperb Med* 35: 113–129, 2008.
186. Yuan J, Handy RD, Moody AJ, and Bryson P. Response of blood vessels *in vitro* to hyperbaric oxygen (HBO): modulation of VEGF and NOx release by external lactate or arginine. *Biochem Biophys Acta* 1787: 828–834, 2009.
187. Yuan J, Handy RD, Moody AJ, Smerdon G, and Bryson P. Limited DNA damage in human endothelial cells after hyperbaric oxygen treatment and protection from subsequent hydrogen peroxide exposure. *Biochim Biophys Acta* 1810: 526–531, 2011.
188. Zadori A, Agoston VA, Demeter K, Hadinger N, Varady L, Kohidi T, Gobl A, Nagy Z, and Madarasz E. Survival and differentiation of neuroectodermal cells with stem cell properties at different oxygen levels. *Exp Neurol* 227: 136–148, 2010.
189. Zhang Q, Chang Q, Cox R, Gong X, and Gould L. Hyperbaric oxygen attenuates apoptosis and decreases inflammation in an ischemic wound model. *J Invest Dermatol* 128: 2102–2112, 2008.
190. Zhang Q, Piston DW, and Goodman RH. Regulation of corepressor function by nuclear NADH. *Science* 295: 1895–1897, 2002.
191. Zhang T, Yang Q, Wang S, Wang J, Wang Q, Wang Y, and Luo Y. Hyperbaric oxygen therapy improves neurogenesis and brain blood supply in piriform cortex in rats with vascular dementia. *Brain Inj* 24: 1350–1357, 2010.
192. Zhang X, Yang Y, Xu P, Zheng C, Wang Q, Chen C, and Yao Y. The role of beta-catenin signaling pathway on proliferation of rats neural stem cells after hyperbaric oxygen therapy *in vitro*. *Cell Mol Neurobiol* 31: 101–109, 2011.
193. Zhou J, Damdimopoulos AE, Spyrou G, and Brune B. Thioredoxin 1 and thioredoxin 2 have opposed regulatory functions on hypoxia-inducible factor-1alpha. *J Biol Chem* 282: 7482–7490, 2007.
194. Zieker D, Schafer R, Glatzle J, Nieselt K, Coerper S, Kluba T, Northoff H, Konigsrainer A, Hunt TK, and Beckert S. Lactate modulates gene expression in human mesenchymal stem cells. *Langenbecks Arch Surg* 393: 297–301, 2008.
195. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, and Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2: 477–488, 2000.

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Date of first submission to ARS Central, April 7, 2014; date of acceptance, April 14, 2014.

**Abbreviations Used**

ATA = atmospheres absolute
Duox = dual oxidase
ER = endoplasmic reticulum
$HBO_2$ = hyperbaric oxygen
HIF = hypoxia-inducible factor
LDH = lactate dehydrogenase
MMP = matrix metalloproteinase
NAD = nicotine adenine nucleotide
NADH = nicotine adenine nucleotide = reduced
NOS = nitric oxide synthase
Nox = NADPH oxidase
PDGF = platelet-derived growth factor
RNS = reactive nitrogen species
ROS = reactive oxygen species
SCF = stem cell factor
SDF-1 = stromal-derived factor-1
SOD = superoxide dismutase
SPCs = stem/progenitor cells
TCA cycle = tricarboxylic acid cycle
TGF = transforming growth factor
TGF-$\beta$ = transforming growth factor-beta
Trx = thioredoxin
TrxR = thioredoxin reductase
VEGF = vascular endothelial growth factor