Ischemia Reperfusion Injury: The New Frontier for Wound Healing & HBOT

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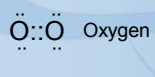
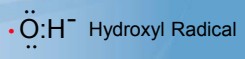
Overview

- Definitions
- Oxygen Free Radicals
- Pathophysiology of ROS
- ROS & Compromised Wound Healing
- Current Management Options

Oxygen Free Radical

- A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence.
- Free radicals are highly reactive due to the presence of an unpaired electron
- Oxygen Free Radical = Reactive Oxygen Species


Oxygen


Hydroxyl Radical

$Fe^{2+} + H_2O_2 \text{ ----> } Fe^{3+} + \cdot OH + OH^-$

Reactive Oxygen Species (ROS)

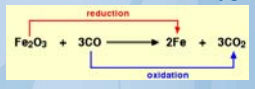
- Hydroxyl radical (OH·)
- Superoxide Anion (O2·)
- Singlet oxygen
- Ozone (O3)
- Hydrogen peroxide (H2O2)
- Nitric Oxide: Peroxynitrite (ONOO-)
- Carbon Based: Peroxyl Radicals (·O2CCl3).
- Thiol compounds (RSO2·)

ROS Production

- Environmental
  - Air Pollution
- External - Exogenous
  - Smoking
- Direct Ionizing Radiation
  - Environmental-Therapeutic
- Cellular Metabolism
- Inflammation

Redox Reactions

- Oxidation is gain of oxygen
- Reduction is loss of oxygen

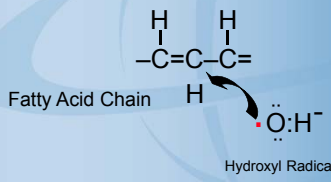


- Oxidation is loss of electrons (Hydrogen)
- Reduction is gain of electrons (Hydrogen)

OIL RIG

ROS Pathophysiology

- Once formed oxygen free radicals seek out electrons to form a stable molecule



ROS Effects & Damage

- **Oxidation Reactions**
  - Lipids (LIPID PEROXIDATION)
  - Amino acids in proteins
  - Enzymes by oxidation of co-factors

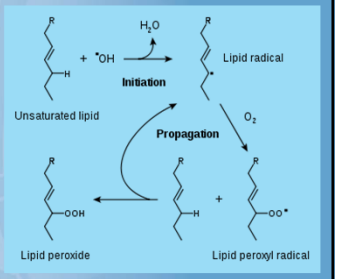
Patel RP, T Cornwell, and VM Darley-USMAR: The biochemistry of nitric oxide and peroxynitrite: implications for mitochondrial function. In: Understanding the process of ageing: The roles of mitochondria, free radicals, and antioxidants. (1999) Eds: E Cadenas and L Packer, Marcel Dekker, Inc. NY. Basel 39-40

Lipid Peroxidation

- Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoproteins.
- PUFAs allow for fluidity and transport across cellular membranes.
- When oxygen free radicals that attack PUFAs the result is damage to cellular membranes
  - LIPID PEROXIDATION

ROS Chain Reactions

- Initiation
- Propagation
- Termination
  - two free radicals combine to form a more stable species



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Antioxidant Defenses

- Antioxidants give up their own electrons to free radicals rendering ROS inactive
- Oxygen Free Radicals are stabilized
  - lipid peroxidation ceases
  - chain reaction of oxidation is broken

Antioxidants

- Vitamin E
- Beta-carotene
- Coenzyme Q
- Intracellular Antioxidant Scavengers
  - vitamin C, superoxide dismutase, catalase

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Oxidative Stress

- **Excessive ROS**
  - Deficient termination reactions
  - Lack of endogenous scavengers / antioxidants
  - Production exceeds reduction reactions

Patel RP, T Cornwell, and VM Darley-USMAR: The biochemistry of nitric oxide and peroxynitrite: implications for mitochondrial function. In: Understanding the process of ageing: The roles of mitochondria, free radicals, and antioxidants. (1999) Eds: E Cadenas and L Packer, Marcel Dekker, Inc. NY. Basel 39-40

Oxidative Stress (Systemic)

- Atherosclerosis
- Parkinson's disease
- Heart Failure
- Myocardial Infarction
- Alzheimer's disease
- Chronic fatigue syndrome
- Aging

Gems D, Partridge L (March 2008). "Stress-response hormesis and aging: "that which does not kill us makes us stronger"". Cell Metab. 7 (3): 200–3.

Oxidative Stress (Cellular)

- Cell Wall Disturbance (PUFAs)
- Enzyme Disruption
- DNA Damage
- Apoptosis (Cellular Death)
- Tissue Necrosis
- COMPROMISED WOUND HEALING

Lennon SV, Martin SJ, Cotter TG (1991). "Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli". Cell Prolif. 24 (2): 203–14.

Sources of Free Radicals Exogenous

Exogenous Sources

- Environmental
- External

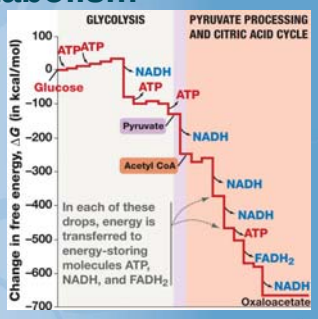
Sources of Free Radicals Endogenous

Endogenous

- **Cellular Metabolism**
  - Glycolysis

Cellular Metabolism

- Glycolysis
- Mitochondria
- Krebs Cycle
- ATP Production



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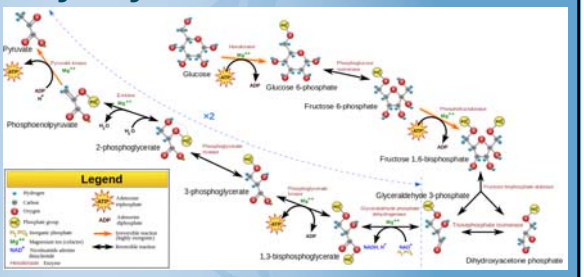
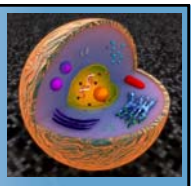
Glycolysis



Mitochondrial ROS



- Submitochondrial localization of ROS generating sites
  - Cytochrome b5 reductase
  - Monoamine oxidases
  - Dihydroorotate dehydrogenase
  - Dehydrogenase a-glycerophosphate
  - Succinate dehydrogenase
  - Aconitase
  - a-Ketoglutarate dehydrogenase complex

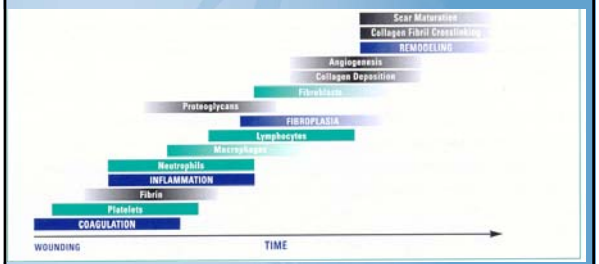
A. Yu. Andreyev, Yu. E. Kushnareva, A. A. Starkov. Mitochondrial Metabolism of Reactive Oxygen Species. Biochemistry, Vol. 70, No. 2, 2005, pp. 200-214.

Sources of Free Radicals Endogenous

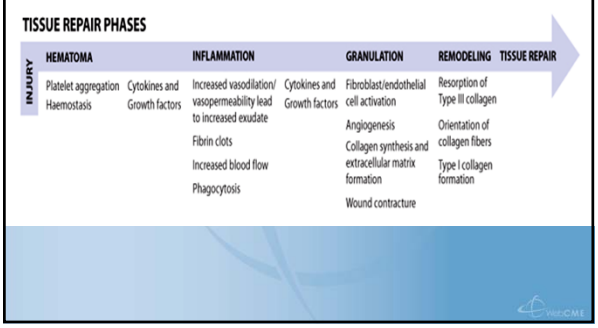
Endogenous

- Cellular Metabolism
  - Glycolysis
- **Inflammation**
  - Phagocytosis – Respiratory Oxidation
  - Protease Induction

Wound Healing Physiology



Wound Healing Physiology



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Inflammatory Phase

- Polymorphonuclear Neutrophils (PMN)
  - White Blood Cells
- Action
  - Cleanse the wound by secreting proteases
  - Phagocytize debris and bacteria
  - Kill bacteria
    - free radicals
    - **respiratory or oxidative burst**

de la Torre J., Sholar A. (2006). Wound healing: Chronic wounds. Emedicine.com. Accessed January 20, 2008

Respiratory (Oxidative) Burst

- PMNs and Macrophages
- Degradation of internalized particles
- **Formation free radicals**
- Rapid release of reactive oxygen species
  - ROS
  - superoxide radical and hydrogen peroxide
- **BACTERIAL KILLING**

Greenhalgh D.G. (1998). The role of apoptosis in wound healing. The International Journal of Biochemistry & Cell Biology 30 (9): 1019–1030.
Muller M.J. , Hollyoak M.A., Moaveni Z., La T., Brown H., Herndon D.N., Heggers J.P. 2003. Retardation of wound healing by silver sulfadiazine is reversed by Aloe vera and nystatin. Burns 29 (8): 834-836.

ROS and Proteases

- Proteolytic enzymes are the second line of defense against the ROS in that they degrade and eliminate the damaged molecules.
- Proteolytic process preferentially degrade oxidatively modified and damaged proteins
- ROS may activate cellular proteases or damage protease inhibitors and promote indiscriminate proteolysis
- Studies have suggested an increased activity of erythrocyte proteolytic enzymes in degrading oxidant damaged hemoglobin in diabetes mellitus

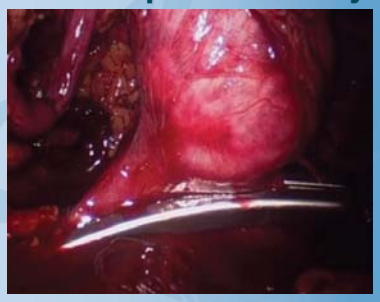
Varashree BS, Bhat PGK. A Study on Proteolytic Enzyme Activity in the Erythrocytes of Diabetic Patients. Online J Health Allied Scs. 2010;9(4):13URL: http://www.ojhas.org/issue36/2010-4-13.htm

Sources of Free Radicals Endogenous

Endogenous

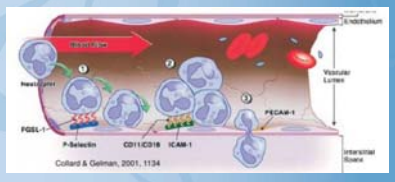
- Cellular Metabolism
  - Glycolysis
- Inflammation
  - Phagocytosis – Respiratory Oxidation
  - Protease Induction
- **Ischemia Reperfusion Injury**

Ischemia Reperfusion Injury



Ischemia Reperfusion Injury

- Ischemic Event
- Reperfusion of Tissue



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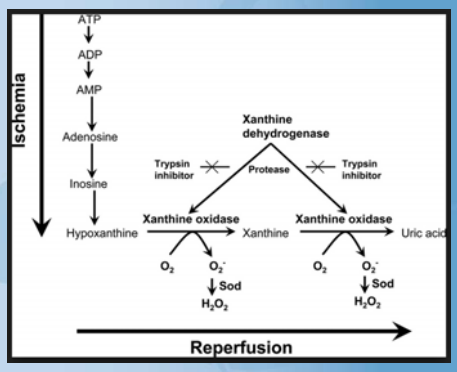
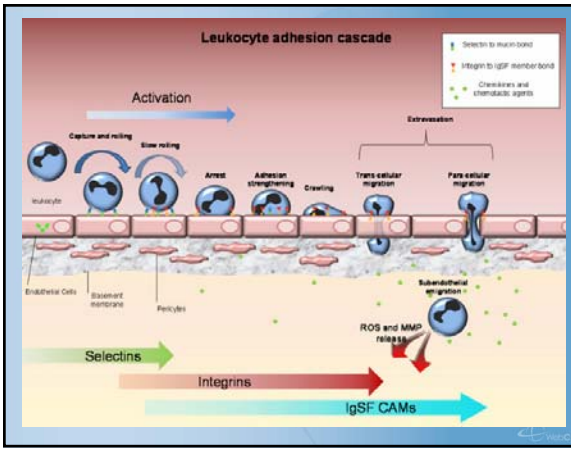
Ischemia Reperfusion Injury

- **Ischemic Event**
  - Direct cellular injury due tissue hypoxia
  - ROS production via Xanthine Oxidase system

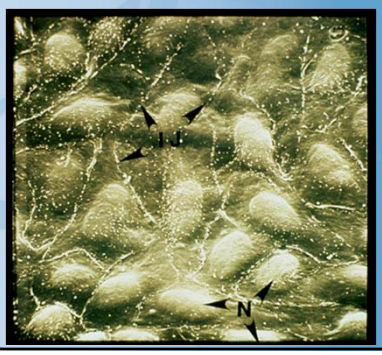


Ischemia Reperfusion Injury

- Ischemic Event
  - Direct cellular injury due tissue hypoxia
  - ROS production via Xanthine Oxidase system
- **Reperfusion of Tissue**
  - Neutrophil Activation
    - ✓ Binding and Vasculature Adhesion
    - ✓ ROS Production
  - Growing evidence that neutrophil mediated free radical production may be more important than xanthine oxidase in ischemia-reperfusion injury



Neutrophil Adhesion



Neutrophil Adhesion

- Following 4 hours of ischemia, there is an increase in the number of neutrophils that adhere to post-capillary venules
- This was maintained throughout a 3-hour reperfusion observation period
- Venule walls became ill defined and disruption of endothelial basement membranes adjacent to adherent neutrophils was observed

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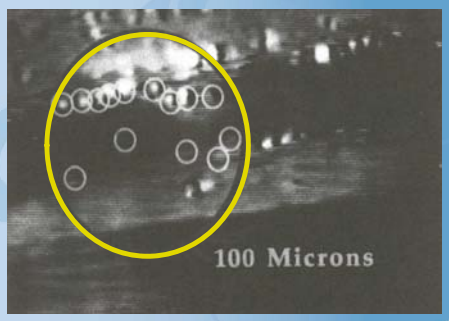
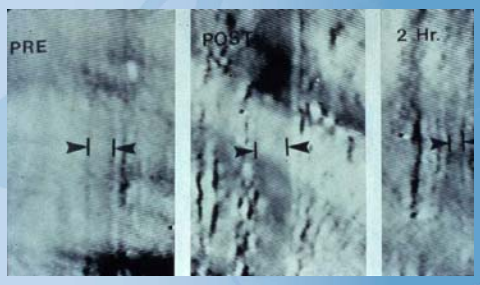
Neutrophil Adhesion



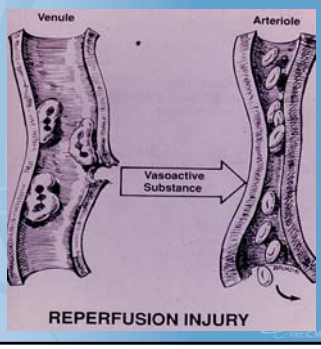
PMN Adhesion & Vascular Insult

- Vasoconstriction



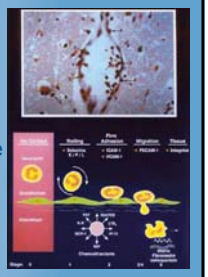
PMN Adhesion & Vascular Insult

- Vasoconstriction
- Endothelial damage



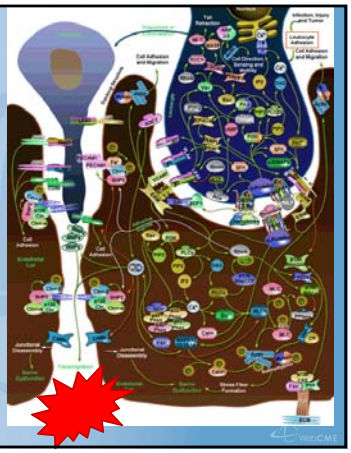
PMN Adhesion & Vascular Insult

- Vasoconstriction
- Endothelial damage
- Vessel leakage and tissue edema
- Vascular sludging
- ROS damage to perivascular tissue
- ADDITIONAL
  - Tissue ischemia and hypoxia
  - ROS Cascade



Terminal Result

Immune Response
Free Radical Production
Tissue Destruction



IRI and Anaerobic Respiration

- Aerobic Conditions
  - ROS due to glycolysis
- Anaerobic Conditions
  - Exercise induces increased formation ROS
  - Additional increase in post-exercise ROS free radical production mediated by xanthine oxidase

Groussard C, Rannou-Bekono F, Machefer G, et al. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. Eur J Appl Physiol 2003; 89 (1):14-20

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Flaps & Grafts

Transplantation

Reimplantation

Surgical Bypass

Fasciotomy

Crush Injury

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Endovascular

Acute Hypotensive Episodes

Wounds

Burn Physiology

Evolutionary process over 3-5 days

- Central area zone of coagulation
- Surrounding zone of stasis
- Outer zone of hyperemia

Zone of coagulation
- Can increase 10x in 1st 48hrs
Hemo-concentration
Platelet microthrombi
Edema formation in area of injury & distant areas as well
Larger "transition area" than mechanical trauma

Zone of Stasis

- Vasogenic and Cytogenic Edema
- Platelet microthrombi and hemoconcentration in postcapillary venules
- RBC and WBC adhesion and activation
- Inflammatory mediators liberated
- Sludging and loss of integrity of microvasculature
- Tissue desiccation and thrombosis of capillaries
- Tissue death and progression of Zone of Necrosis/Coagulation

IRI and Intravascular Gas

- Acute Gas Embolism
- Decompression Illness
- Blast Injury

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Therapeutic Target & Goals

- Oxidative Stress
- Excessive Oxygen Free Radicals
- Ischemia Reperfusion
- Endogenous Imbalances

ROS Management Strategies

- Endogenous Antioxidant Pathways
  - Nutritional Support
  - Antioxidant Therapy ???? (caution)
    - Beta-carotene, Vitamin C, and Vitamin E
    - Zinc, Selenium
    - Phytochemicals
    - Glutathione
    - Melatonin

ROS Management Strategies

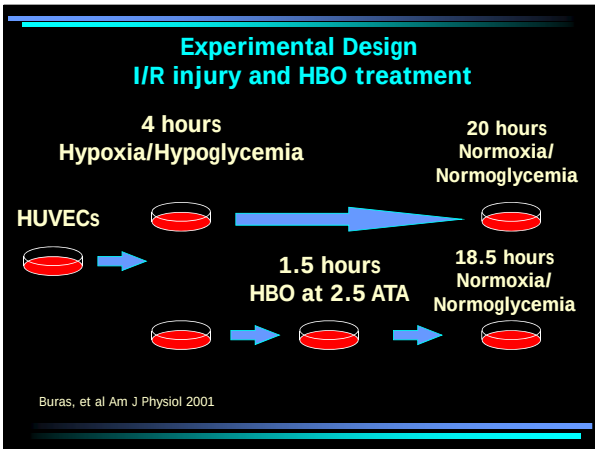
- Endogenous Antioxidant Pathways
- Hyperbaric Oxygen Therapy

HBOT Down-regulates Endothelial Cell Adhesion Molecules in IRI

- In vitro endothelial cell model
- HBO 2.5 ATA prevented IRI by decreasing or preventing induced E-selectin, ICAM-1 and VCAM-1
- Conclusions:
  - Demonstrates positive role of HBO on endothelium
  - Demonstrates WBC anti-adhesive effect of HBO

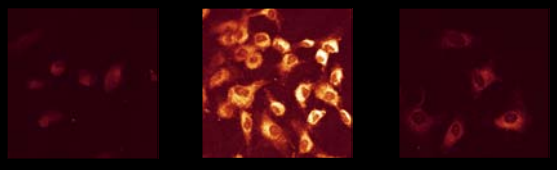
Buras J: Basic Mechanisms of hyperbaric oxygen in the treatment of ischemia-reperfusion injury. International Anesthesiology Clinics 2000; 38 (1): 91-109.
Buras J, Stahl G, Svoboda KH, et al: Hyperbaric oxygen down regulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. The American Journal of Cell Physiology 2000; 278: 292-302.

Experimental Design
I/R injury and HBO treatment



Buras, et al Am J Physiol 2001

HBO down-regulates hypoxia/hypoglycemia-induced VCAM-1 expression



control | hypoxia/hypoglycemia | hypoxia/hypoglycemia HBO 2.5 ATA 1.5 h

Buras, et al Am J Physiol 2001

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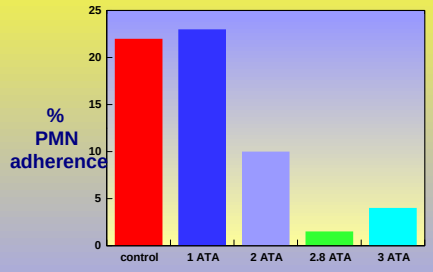
Ischemia Reperfusion Injury:
The New Frontier for Wound Healing & HBOT

Effect of HBO on PMN Adherence



Thom, SR AM J Physiol 272: C770-C777, 1997

HBOT Inhibits WBC Adherence

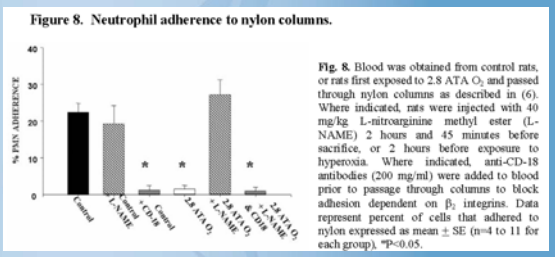
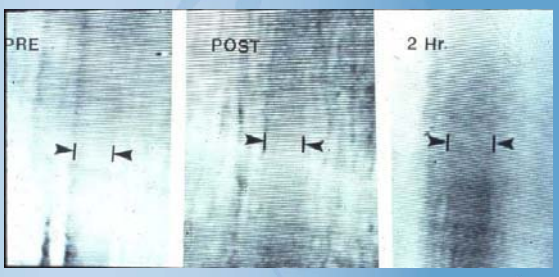


Fig. 8. Blood was obtained from control rats, or rats first exposed to 2.8 ATA O_2 and passed through nylon columns as described in (6). Where indicated, rats were injected with 40 mg/kg L-nitroarginine methyl ester (L-NAME) 2 hours and 45 minutes before sacrifice, or 2 hours before exposure to hyperoxia. Where indicated, anti-CD-18 antibodies (200 mg/ml) were added to blood prior to passage through columns to block adhesion dependent on β_2 integrins. Data represent percent of cells that adhered to nylon expressed as mean ± SE (n=4 to 11 for each group). *P<0.05.

THOM SR. Effects of hyperoxia on neutrophil adhesion. UHM 2004, Vol. 31, No. 1

HBOT Prevents Vasoconstriction Post Ischemia

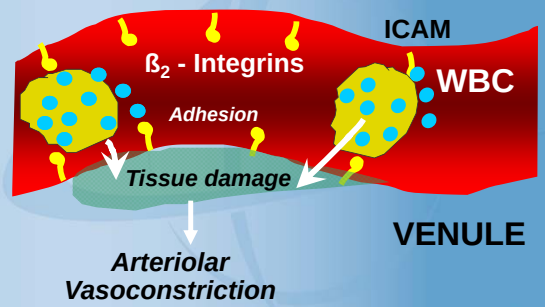


Zamboni WA. "The microcirculation and ischemia-reperfusion: basic mechanisms of hyperbaric oxygen." Hyperbaric Medicine Practice, 1999; Best Publishing, Flagstaff AZ, pages 779-794

HBOT in Ischemia Reperfusion

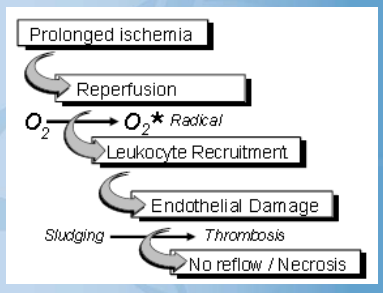
- HBO decreases WBC adherence following ischemia.
- The leukocyte adhesion molecule is Beta-2-integrin.
- Hypoxia and sepsis can cause membrane guanylate cyclase to trigger the neutrophil β2-integrin.
- HBO appears to act via NO to inhibit neutrophil β2 integrin function.
- HBO does not induce vasoconstriction in post ischemic muscle.

HBOT in Ischemia Reperfusion



Zamboni. Post Ischaemic Leucocyte sequestration ('93) Plas Recon Surg; 91(6) 1112

Oxygen Paradox



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IRI / HBOT Indications

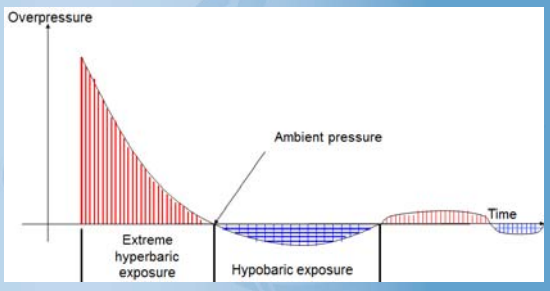
- AGE, DCS, Blast Injury
- Carbon Monoxide Poisoning
- Thermal Burns
- Compromised Flaps & Grafts
- Acute Ischemia
- Compartment Syndrome
- CRAO
- ISSHL

Emboli in Blast Injury

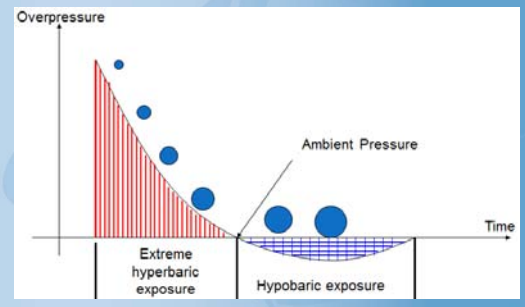
- Over pressurization and over distention
- Evolved gas
  - related to duration & pressure
- Compressible turbulence of multiple hypersonic waves
- Nano cavitation
  - pressure reductions associated with shock waves can produce cavitation nuclei and bubble excitation*

Wienke, B.R., Hyperbaric Physics with Bubble Mechanics and Decompression Theory in Depth, 2008

Pressure-time Curve
Air Blast Overpressurization

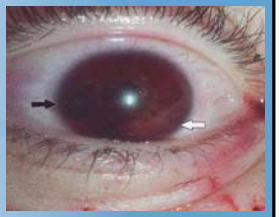


Bubble Evolution



Bubble Embolization

Decompression Event **Blast Event**



Historical "Silent Wounding"

- DaCosta's syndrome*
- Effort syndrome
- Irritable Heart*
- Battle Fatigue
- Tristesse Sombre*
- Soldier's Heart*
- Shell Shocked
- Combat Fatigue
- PTSD
- Mild TBI

Silent Wounding has been reported since the beginning of the use of gunpowder… 1768

Since that time it has perplexed those seeking to care for the wounded or explain deaths with no evidence of external wounds*

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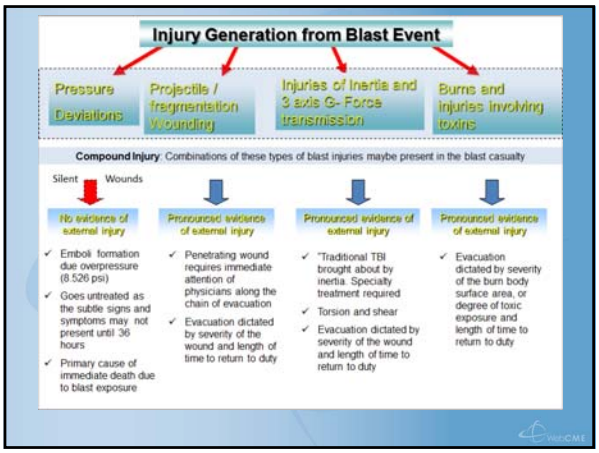
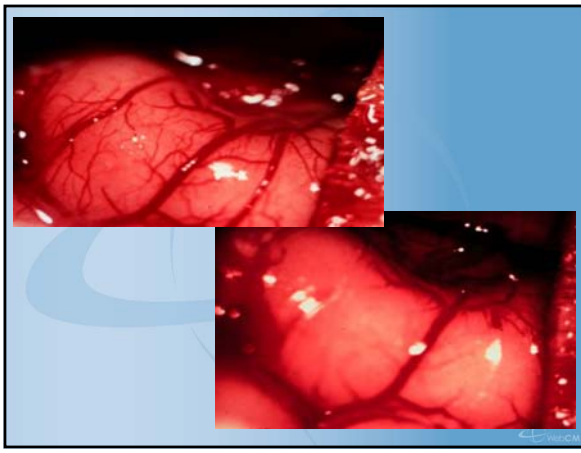


Do "Bubbles" cause Ischemia Reperfusion Injury?

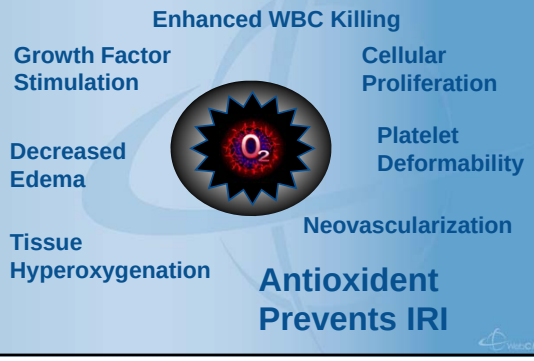
YES!

- **Complete Arterial Occlusion**
  - Early
  - Large
- **Compliment Activation**
  - Sludging
  - Lo-Flow/No-Flow

Do we use HBOT to treat "Bubbles"?



Summary of HBO Mechanisms



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ROS Management Strategies

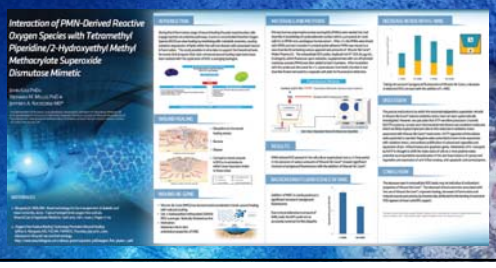
- Endogenous Antioxidant Pathways
- Hyperbaric Oxygen Therapy
- **Steric Hindrance**

Steric Hindrance

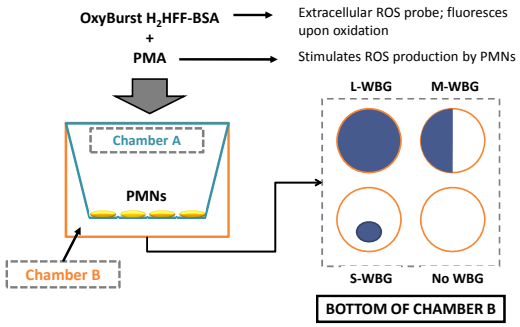
- Large Molecules
- Free Radical Traps
- End ROS chain reactions via termination

In Vitro Evidence

- John Kao, PhD
  - Professor & Chairman, Department of Department Biomaterials University of Wisconsin



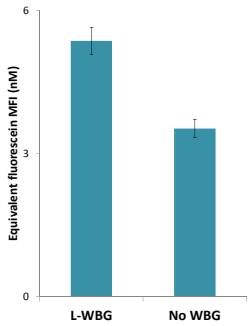
Experimental Methods



- After 2 h, supernatant removed & fluorescence quantified

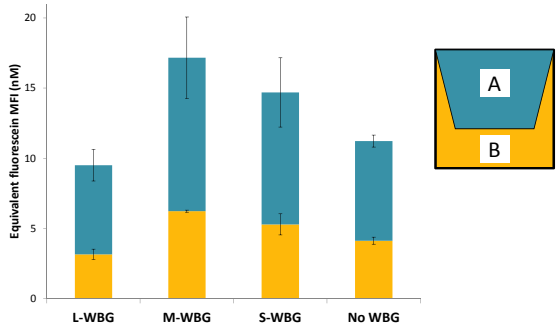
82

Background Fluorescence of WBG



- Addition of WBG to media produced a significant increase in background fluorescence

83



- Taking into account background fluorescence of Wound-Be-Gone, a decrease in detected ROS can be seen with the addition of L-WBG

84

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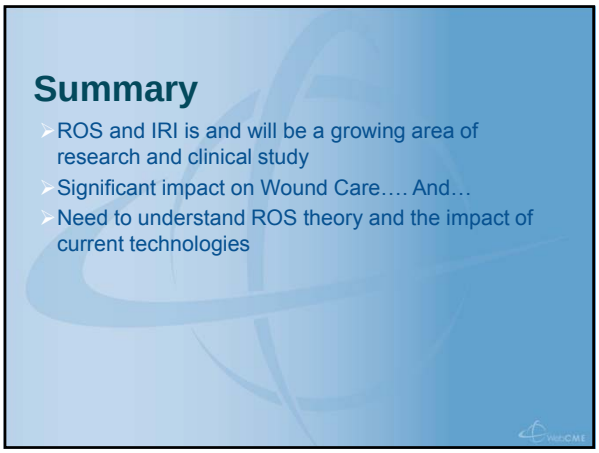

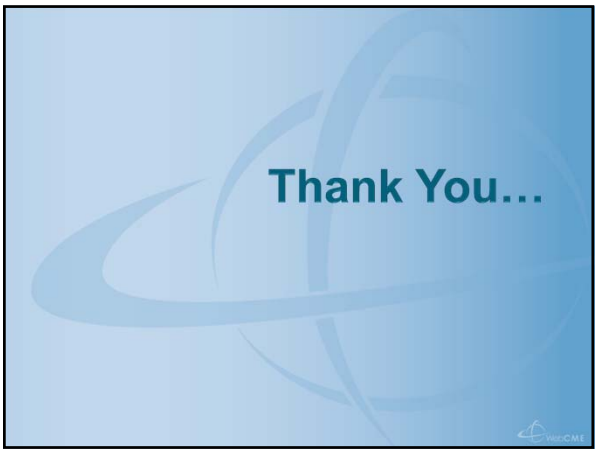

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