HBOT: An Essential Component for the Regenerative Treatment of Pain from Sports Injuries, Chronic Inflammation and Infection

Dr. John Hughes, DO

Advancing Hyperbaric Medicine Globally in the 21st Century

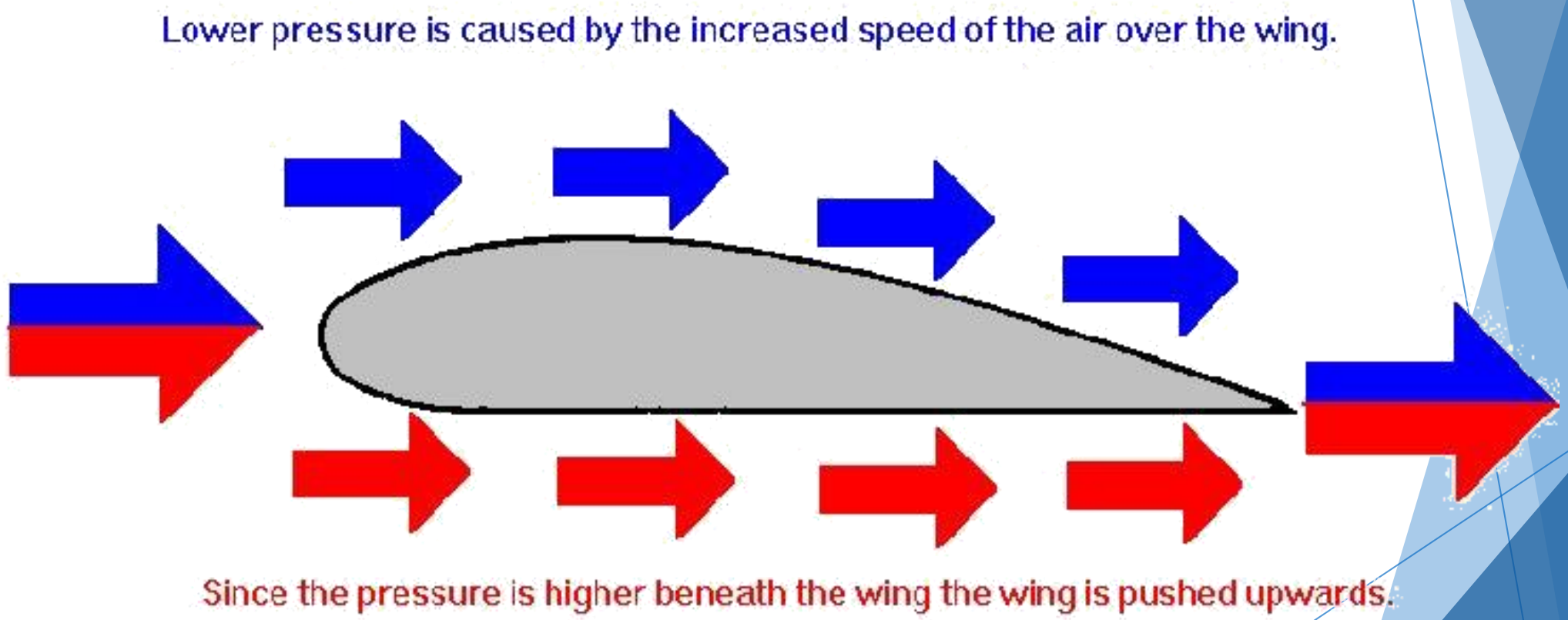
International Hyperbaric Medical Association

August 12th, 2018

Preface: Law of Gravity

- 1686: Sir Isaac Newton first published: mutual attraction of bodies in nature
- 1798: First test of Newton's theory of gravitation between masses in the laboratory - Cavendish experiment
- 1915: Einstein’s general **theory** of relativity: **gravity** as a distortion of spacetime caused by the presence of matter or energy

Preface: Bernoulli Principle



Disclaimer

- I have no relevant financial relationships with any commercial interests to disclose.

- The content of this presentation has been peer reviewed for fair balance and evidence based medicine.

Advanced Evidence Based Medicine = Creative Expertise

*Advanced evidence based medicine is not **rule following**.*
There are five levels of learning:

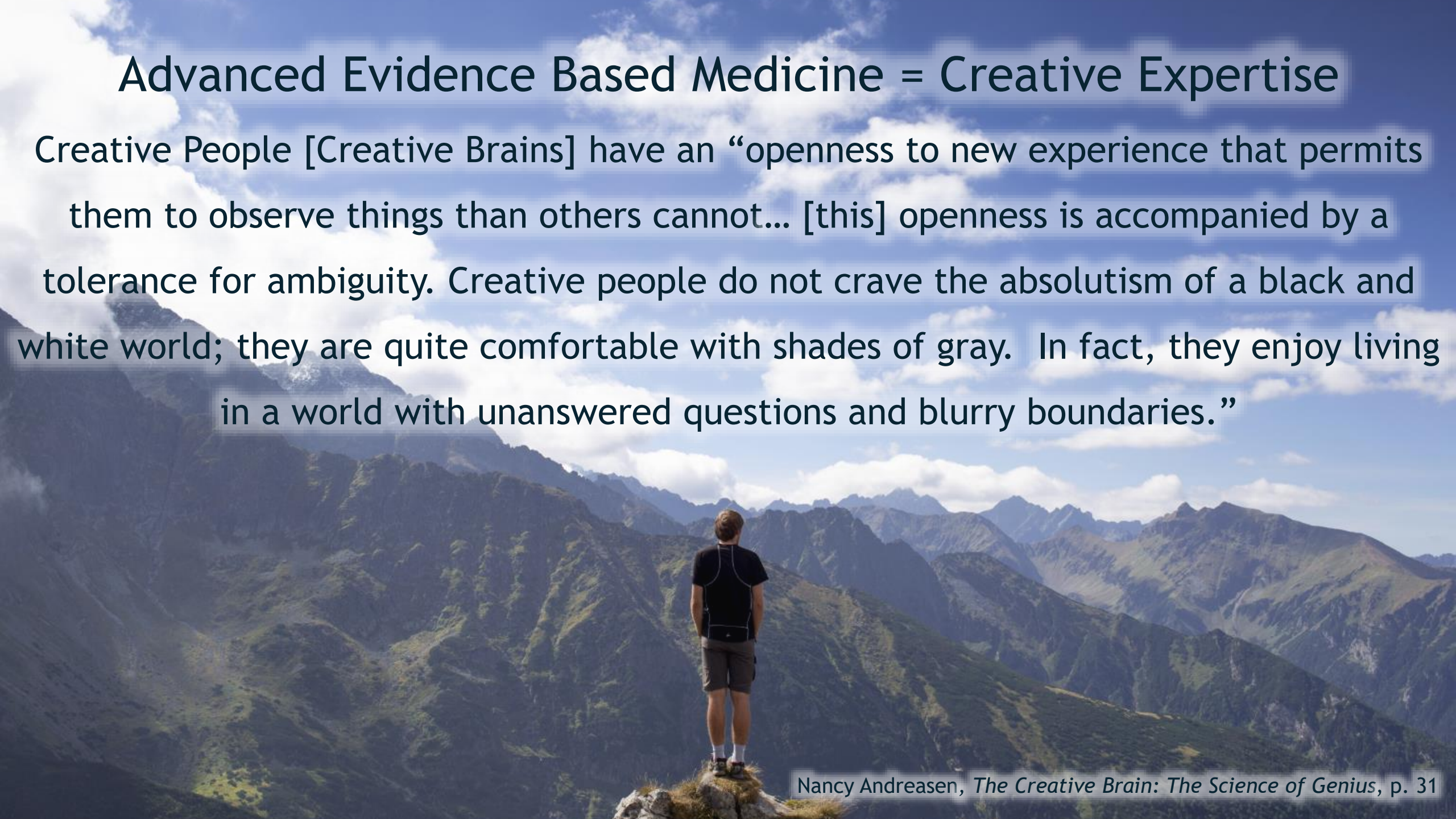
The Novice Stage: Learns the basic rules and applies them mechanically with no attention to context.

Second and Third Stages: Increasing depth of knowledge and sensitivity to context when applying rules.

Fourth and Fifth Stages: Rule following gives way to expert judgments - characterized by rapid, intuitive reasoning informed by imagination, common sense, and judiciously selected research evidence.

Advanced Evidence Based Medicine = Creative Expertise

Creative People [Creative Brains] have an “openness to new experience that permits them to observe things than others cannot… [this] openness is accompanied by a tolerance for ambiguity. Creative people do not crave the absolutism of a black and white world; they are quite comfortable with shades of gray. In fact, they enjoy living in a world with unanswered questions and blurry boundaries.”



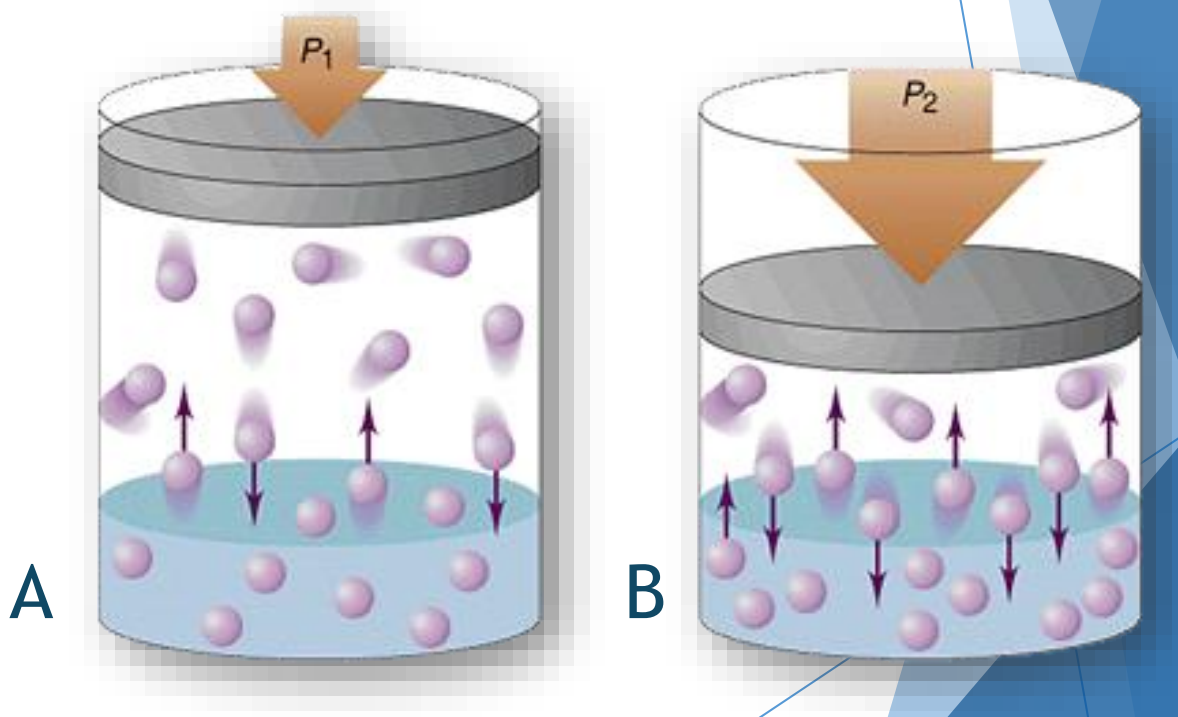
Nancy Andreasen, *The Creative Brain: The Science of Genius*, p. 31

HBOT: An Essential Component for the Regenerative Treatment of Pain from Sports Injuries, Chronic Inflammation and Infection

I. Introduction to HBOT
II. HBOT: Mechanisms for Addressing Chronic Pain
III. HBOT: Treatment for Sports Injuries
IV. HBOT: Upregulates Pluripotent Adult Stem Cells (aka VSELs – Very Small Embryonic-Like Stem Cells) in the Blood
V. VSELs over MSCS: Regenerative Treatments with Pluripotent Stem Cells for Sports Injuries and Arthritis
VI. HBOT: Adjunctive to IV Therapies for Chronic Infection

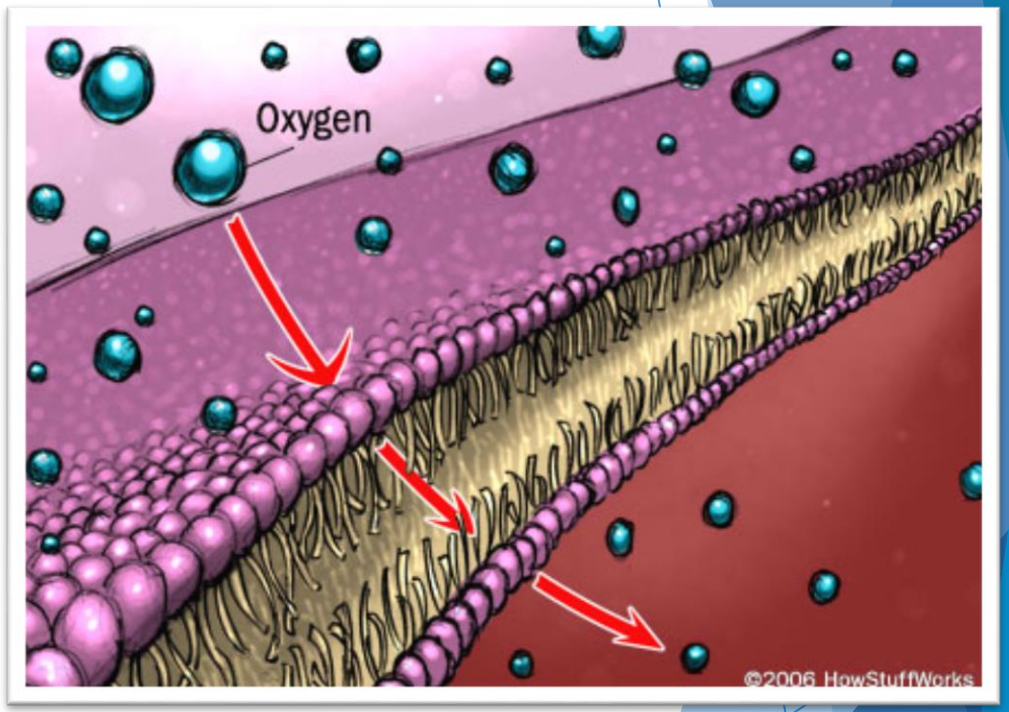
Introduction to HBOT: Physics

- Henry's Law of Gas Solubility: The solubility of a gas in a liquid is directly proportional to the partial pressure of the gas above the liquid.
- Increasing the atmospheric pressure increases the amount of gas that is dissolved into a fluid.
- Oxygen → Blood Plasma



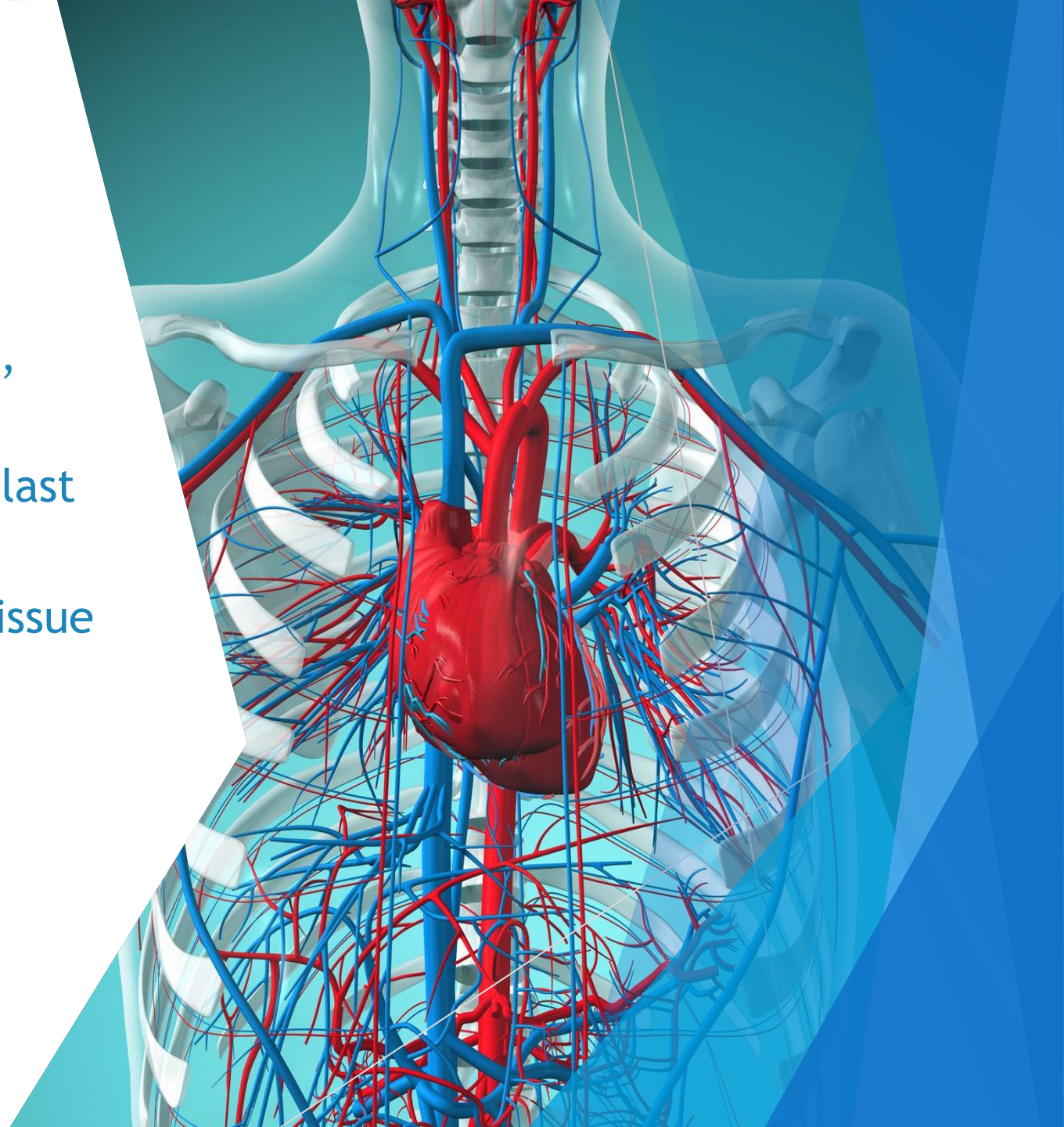
Introduction to HBOT: Physiology

- What Gets Hyper-Oxygenated?
  - Blood Plasma
  - Cerebrospinal Fluid
  - Lymph Fluid
- Clinical Hyperbaric Pressures
  - 7 – 22 psi
  - 10 – 15 normal amount of oxygen
  - Bypasses body's normal system of transporting oxygen



Introduction to HBOT: Mechanism of Action

- Limits ischemic damage, cell death, inflammation
- Promotes collagen synthesis (fibroblast stimulation)
- Decreases lactate production and tissue acidosis
- Aids in oxygen dependent killing of bacteria – WBC
- Limits leukocyte adhesion and degranulation
- Decreases tissue edema

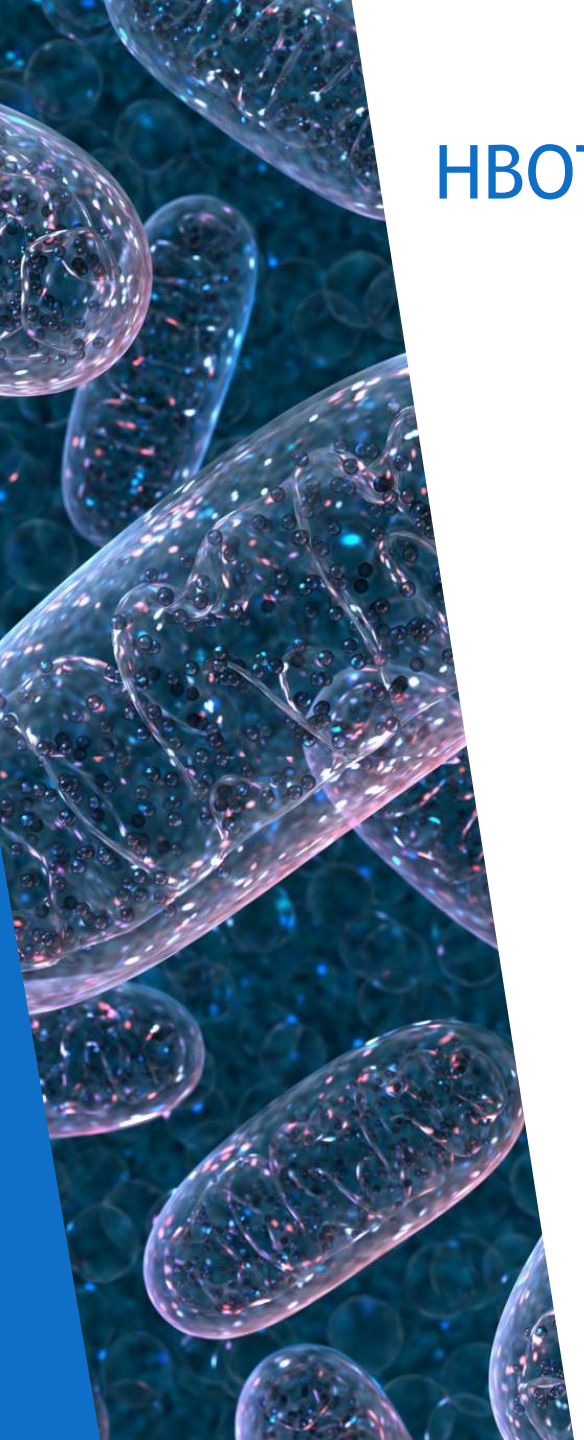


HBOT: Mechanisms for Addressing Chronic Pain

HBOT: Mechanisms for Addressing Chronic Pain

- Decreases inflammation, reduces hypoxia, and improves microcirculation
- For neuropathic pain, analgesic and antinociceptive effects are due to cellular modulation
  - Autophagy in the mitochondria of microglia (mitophagy)

    (Han et al., 2017)



HBOT: Mechanisms for Addressing Chronic Pain

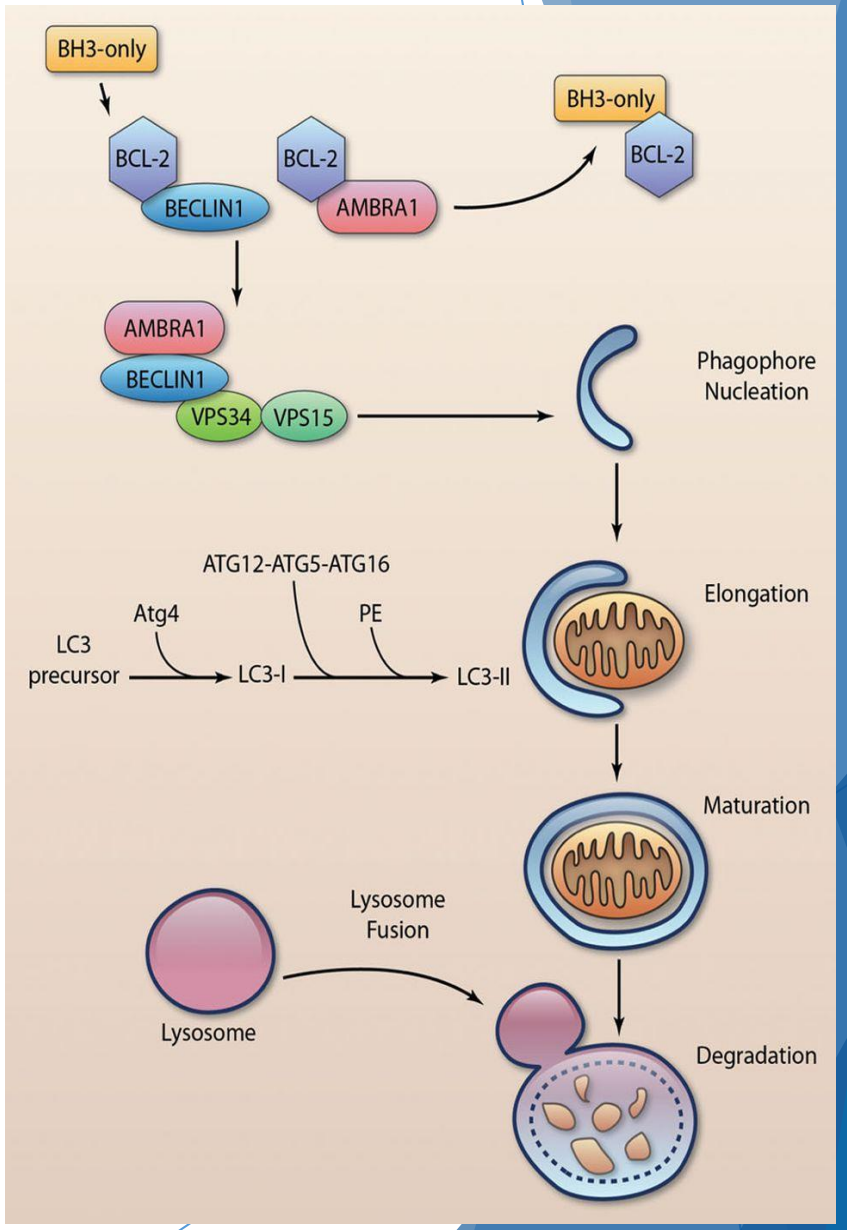
- Mitochondria are the primary source of ROS
- ROS can:
  - Induce mutations in mtDNA causing protein deficiencies
  - Restrict ability to self-repair, leaving cells more vulnerable to ROS attack
  - Damage mitochondrial proteins and lipids by inducing oxidative stress

(Nie et al., 2015; Koirala et al., 2013; Lupfer et al., 2013)

HBOT: Mechanisms for Pain
Latent mitochondria are like campfires left burning all night

HBOT: Addressing Chronic Pain with Mitophagy

- HBOT modulates cellular autophagy (mitochondria of microglia) and directly reduces pain
- Appropriate clearance of mitochondria is important for maintaining homeostasis in cells



HBOT: Addressing Chronic Pain with Mitophagy

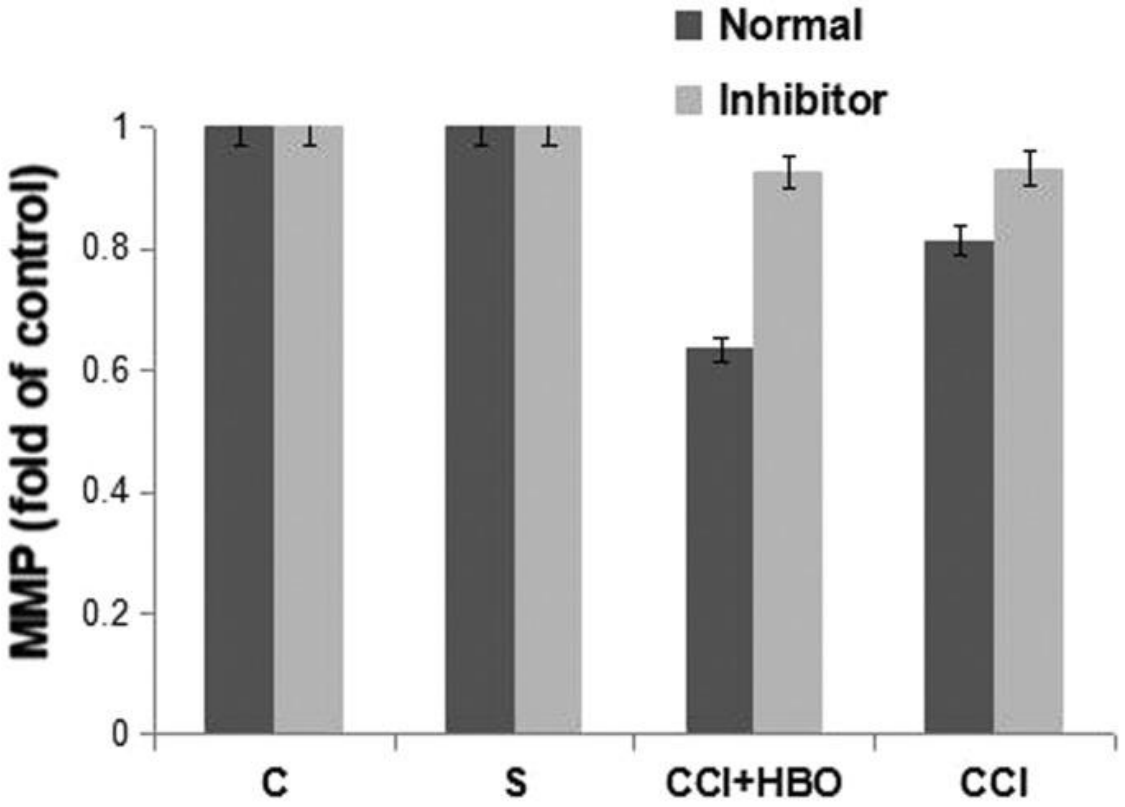
Mitophagy study with 80 rats (Han et al., 2017)

- 20 rats were given a CCI (chronic constriction injury); 20 rats got CCI+ HBOT
- 20 rats were sham CCI and 20 rats were controls
- All 80 rats were given CSI (a mitophagy) before testing
- MMP was used to measure mitophagy (lower MMP observed with more mitophagy)

HBOT: Addressing Chronic Pain with Mitophagy

Mitophagy study with 80 rats (Han et al., 2017)

- HBOT improved mitochondrial permeability via transitive pores on the mitochondrial membrane
- More permeability results in more mitophagy (see as lowered MMP) which reduces ROS calming neuro-inflammation and pain

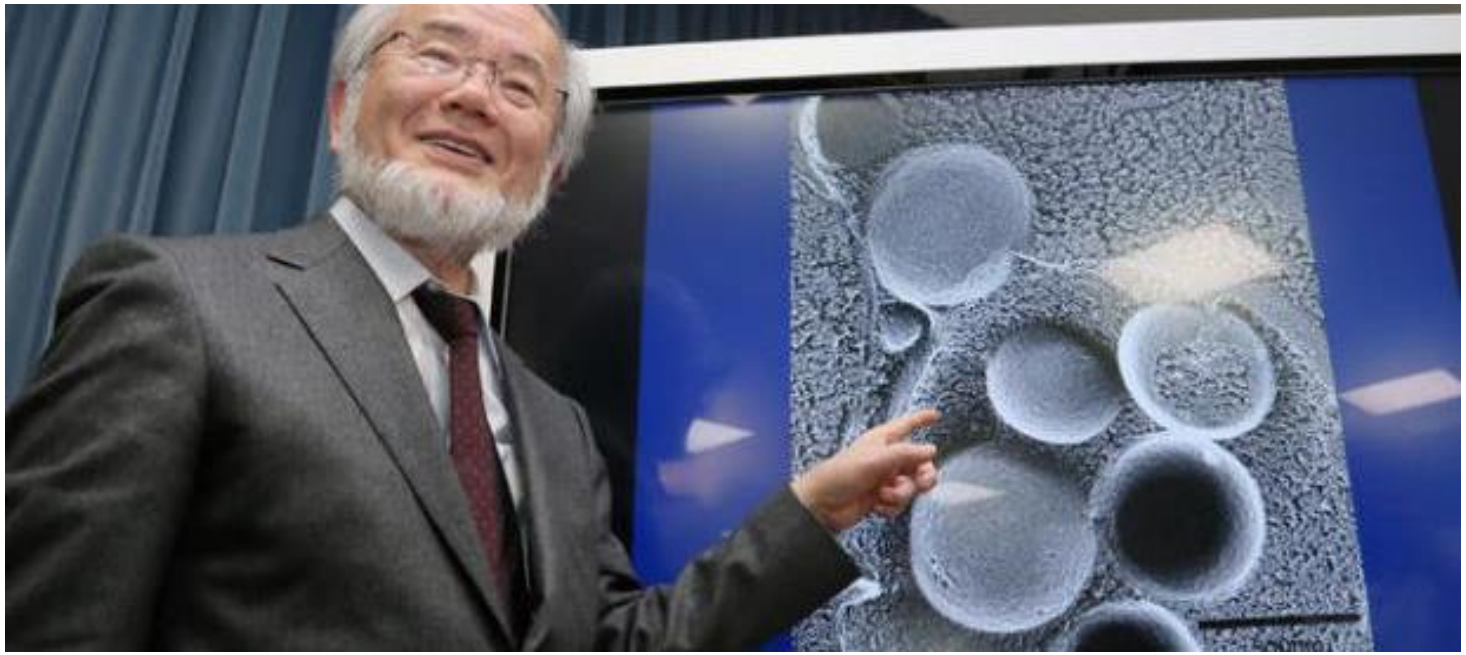


Control & Sham – minimal to no mitophagy (no change in MMP)
MMP: Mitochondrial membrane potential
CCI: Chronic constriction injury

Mitophagy is putting the mitochondrial fires out by involuting the ashes and soil upon the remaining embers. Without mitophagy, wildfires (of pain) get out of control.

July 4th, 2018 Basalt, CO (Courtesy of Pete McBride)

Fun Fact #1: What else encourages cellular autophagy (including neuronal autophagy)?



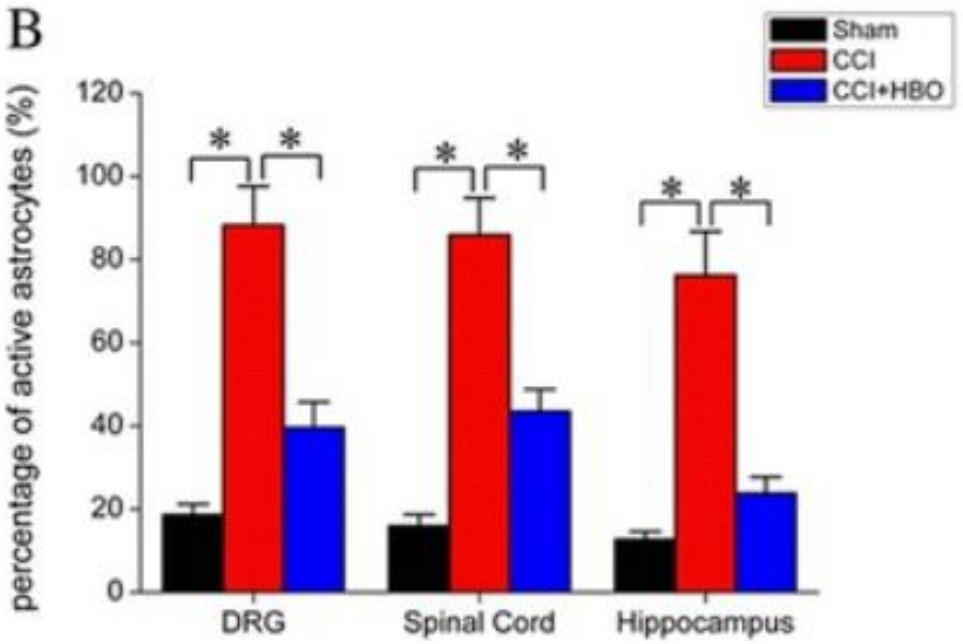
Intermittent Fasting!

Dr. Yoshinori Ohsumi Wins Nobel Prize for this discovery)

https://www.garmaonhealth.com/intermittent-fasting-cellular-autophagy/

HBOT: Other Mechanisms for Addressing Chronic Pain
(Zhao, B., Pan, Y., Xu, H., & Song, X., 2017)

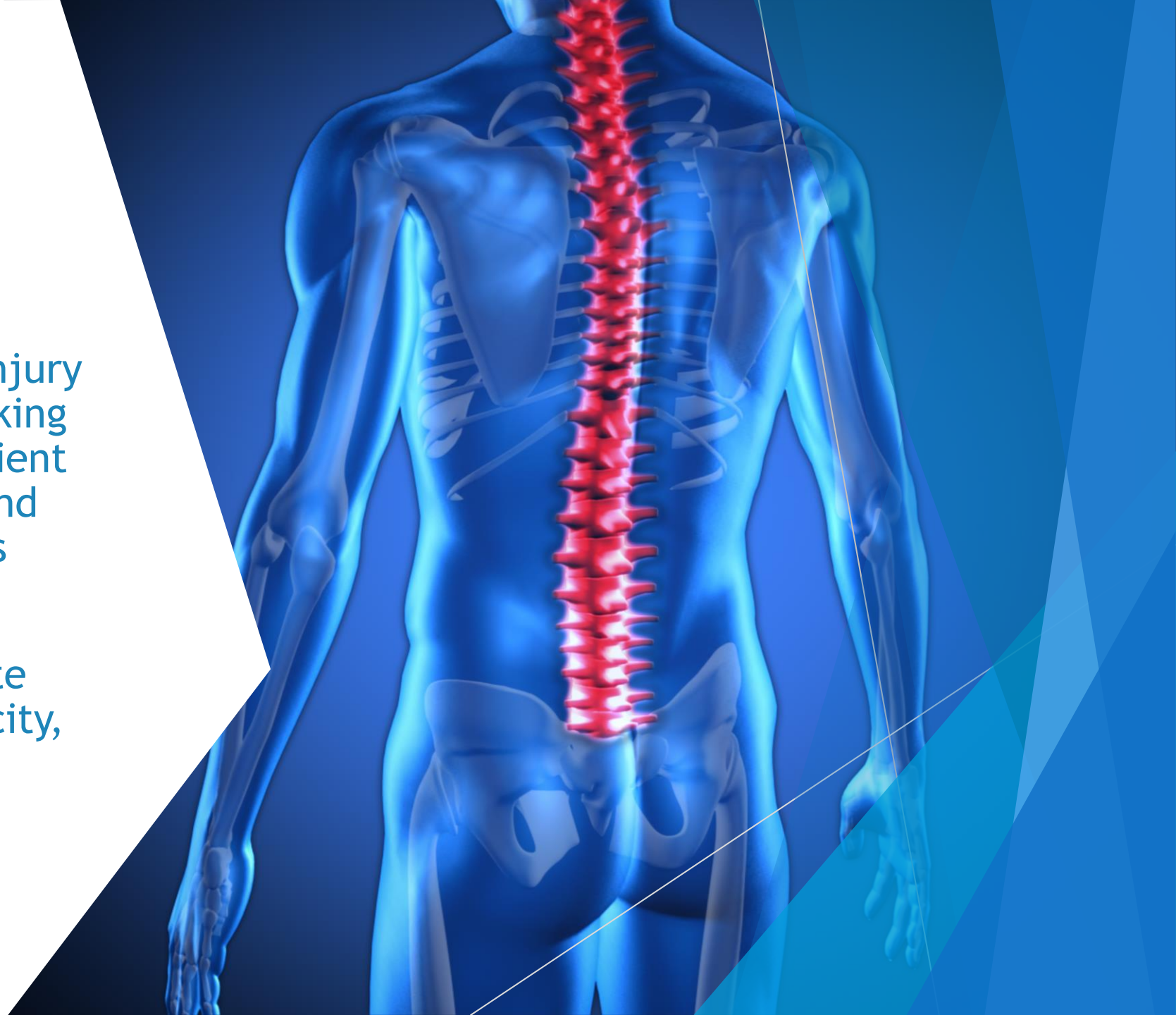
- Suppresses pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-alpha and simultaneous releases anti-cytokines
- Suppresses astrocyte activation and inflammatory responses (stopping gliosis) by:
  - Increasing TNF-α
  - Decreasing Kindlin-1 and Wnt-10a in the dorsal root ganglia (DRG), spinal cord, and hippocampus of rats

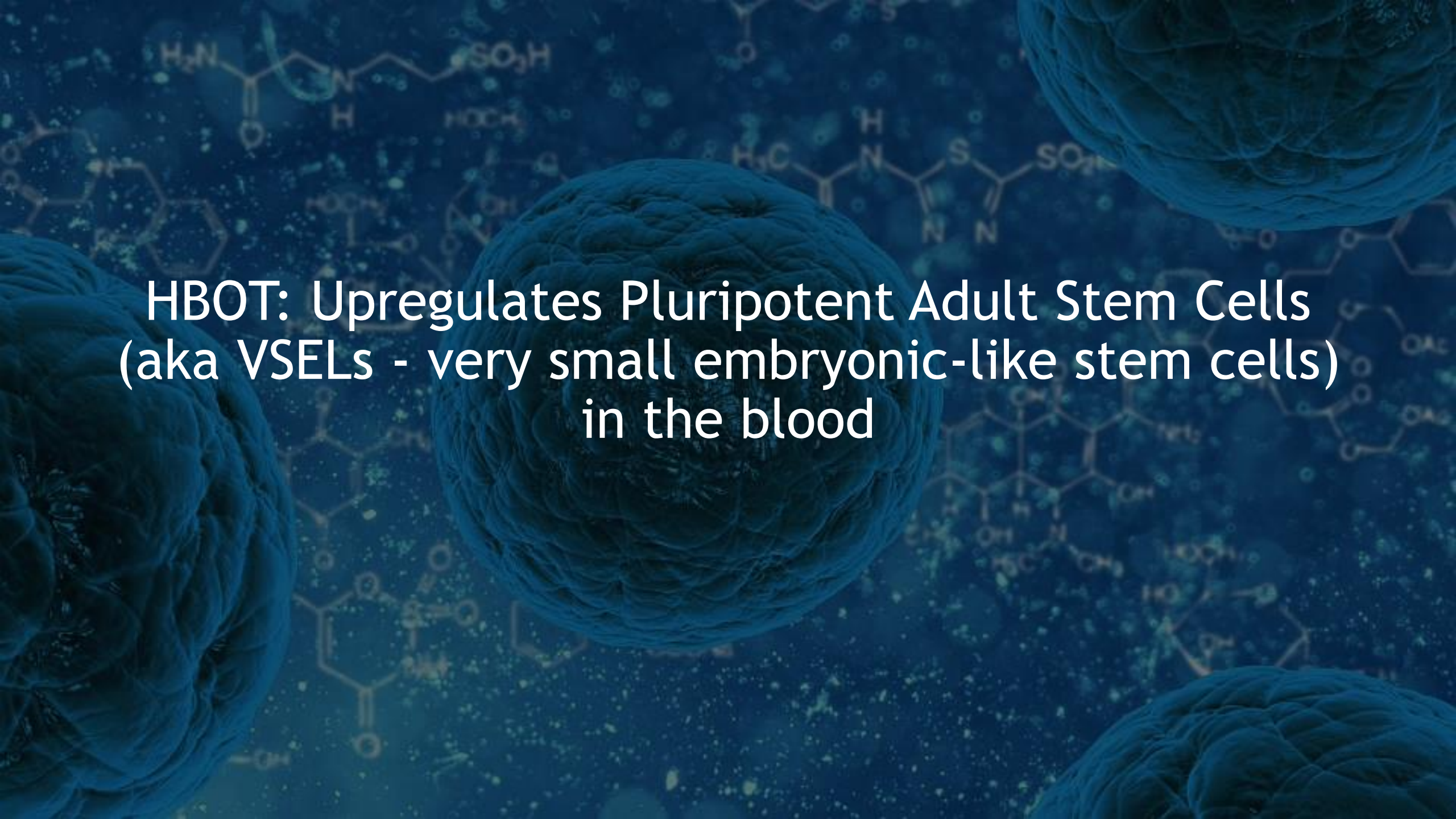


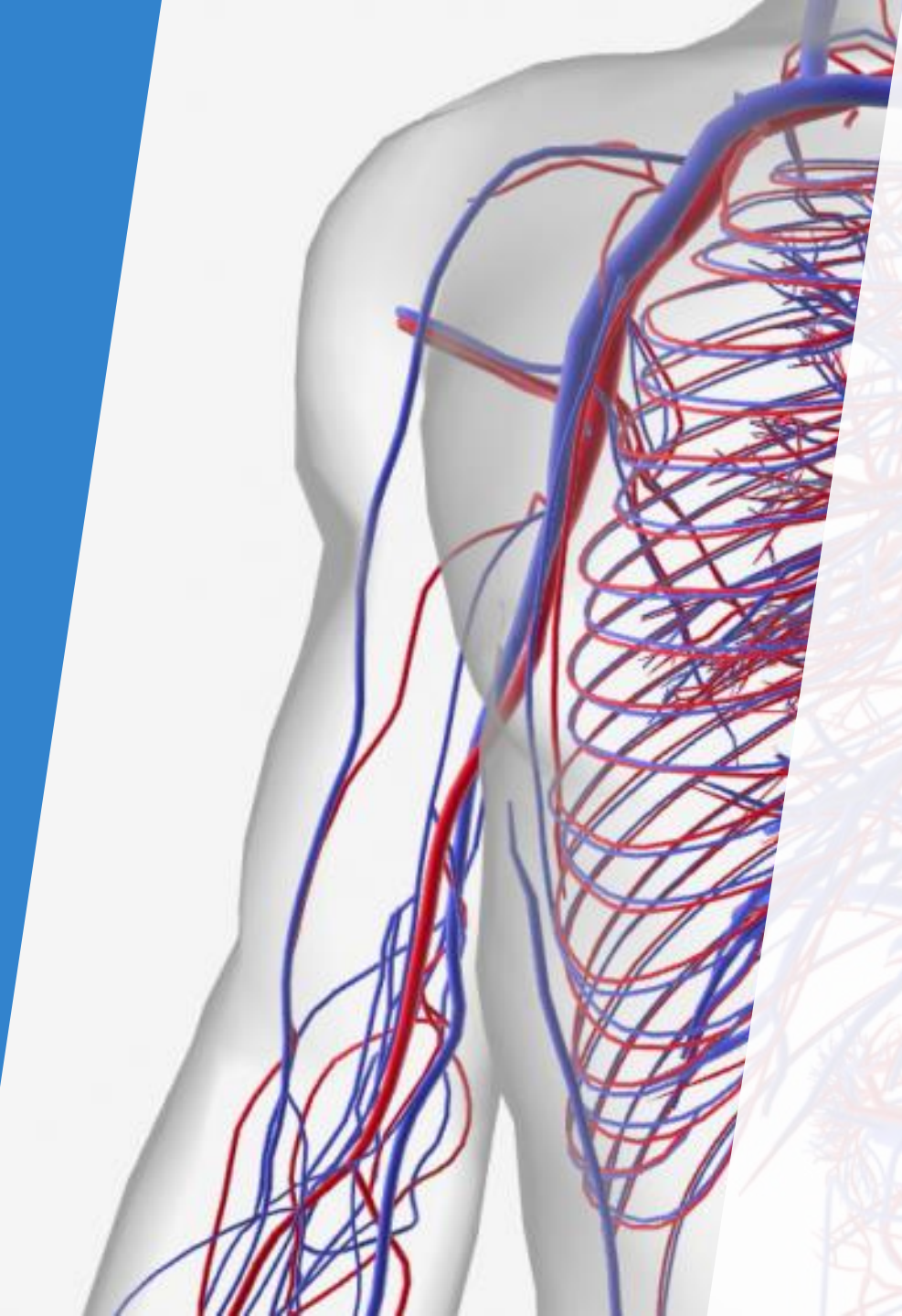
HBOT: Mechanisms for Chronic Pain: Case Study

- 40 year old spinal cord injury (C4 burst fx from mtn biking accident) paraplegic patient with chronic spasticity and pain in lower extremities

- Reports almost immediate reduction in neuroplasticity, inflammation, and pain when treated in a HBOT chamber at 2.4 ATA




HBOT: Upregulates Pluripotent Adult Stem Cells
(aka VSELs - very small embryonic-like stem cells)
in the blood

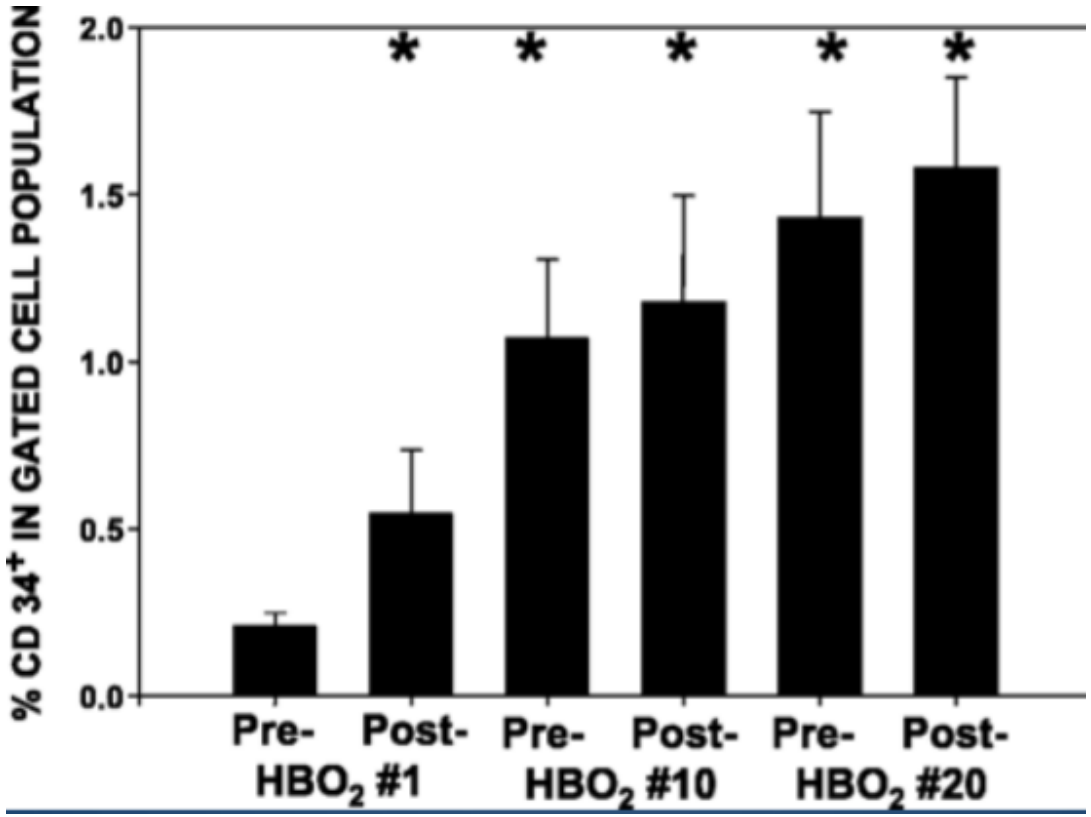


"[Hyperbaric oxygen therapy] is the safest way clinically to increase stem cell circulation, far safer than any of the pharmaceutical options."

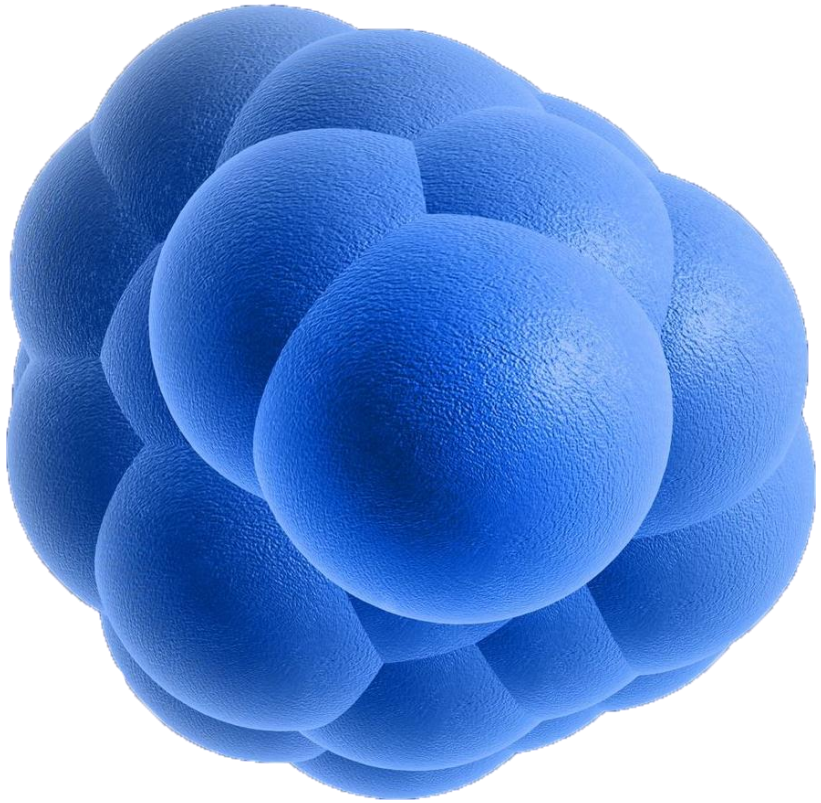
Stephen Thom, MD, Ph.D. (2005)

HBOT: Upregulates Pluripotent Stem Cells in the Blood

- Mean CD34+ population in blood of humans before and after HBO2 treatments
- Data are the fraction of CD34+ stem cells within the gated population using blood obtained from 26 patients before and after their 1st, 10th, and 20th HBO2 treatment



(Thom, et al., 2006)



HBOT: Upregulates Pluripotent Stem Cells in the Blood

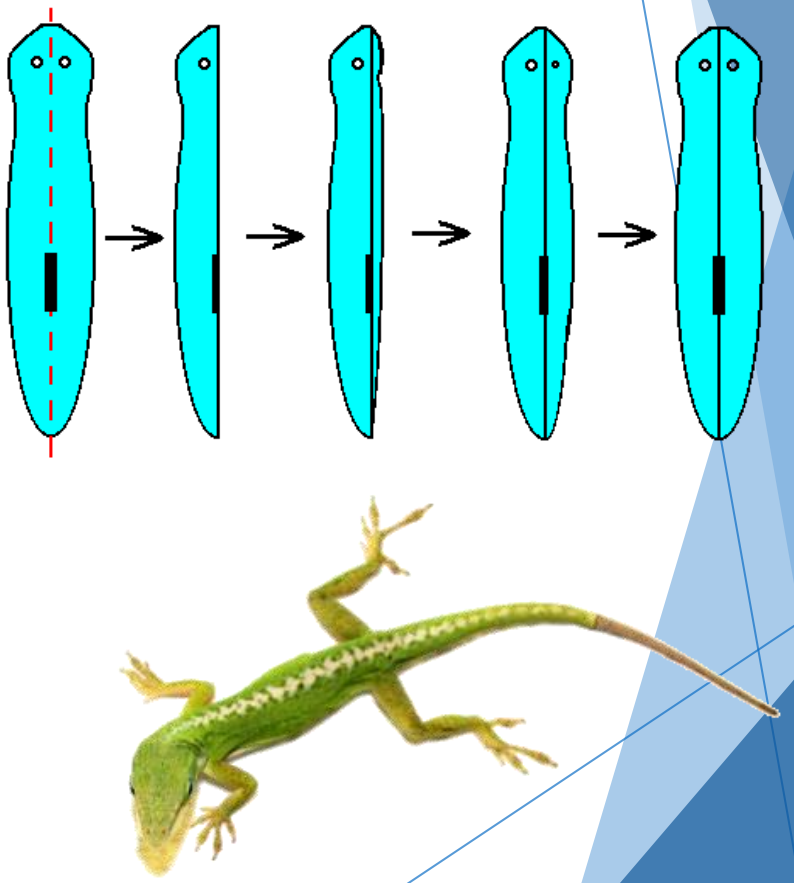
- 2 hours = 3x amount of stem cells circulating stem cells in your blood
- 20 sessions = 800% more stem cells circulating stem cells in your blood
- Released through a nitric oxide process stimulated by HBOT

HBOT: Upregulates Pluripotent Stem Cells (VSELs) in the Blood

- Repairing tissue damage with endogenous VSELs and growth factors is the body's primary way to stop the cause pain
- VSELs can be also harvested by blood draw, isolated, and activated

Pluripotent (VSELs) vs. Multipotent (Mesenchymal-MSCs)

- Many stem cell clinics are focused on the use of mesenchymal stem cells (MSCs)
- MSCs are derived from bone marrow, umbilical, or fat
- MSCs have merit for homologous use (bone marrow to bone marrow or fat to fat transplantation)
- MSCs do not actually transform, in vivo, to new tissues



Pluripotent (VSELs)	Multipotent (Mesenchymal)
Recently discovered in peripheral blood	From bone marrow, fat, and cord blood
Also known as very small embryonic-like stem cells (VSELs)	Mesenchymal stem cells (MSCs)
Does not have a specialized trajectory of development	On a development trajectory
Give rise to all the cell types	Specialization potential limited to one or more cell lines
Lineage uncommitted	Lineage committed
Long lifespan	Short-lived
Not restricted by FDA	Increased FDA restriction for non-homologous tissue use
Best for regeneration	Best for homologous use

Clinical Indications

Multipotent (Mesenchymal)

- **Tissue Replacement (Homologous Only):**
  - Bone marrow transplant
  - Breast, lips, cheeks, eyes, buttocks
- **Systemic inflammatory conditions:**
  - Autoimmune disorders
  - Acute renal failure
  - Myocardial infarction
  - Type I diabetes
  - Graft-vs-host disease
  - Systemic lupus
  - Pulmonary fibrosis

Pluripotent (VSELs)

- **Degenerative diseases:**
  - Diabetes
  - Osteoarthritis / osteoporosis
  - Alzheimer's disease
- **Regenerative applications:**
  - Traumatic brain injury
  - Joint / ligament repair
  - Anti-aging
  - Post cancer treatment
  - Fertility

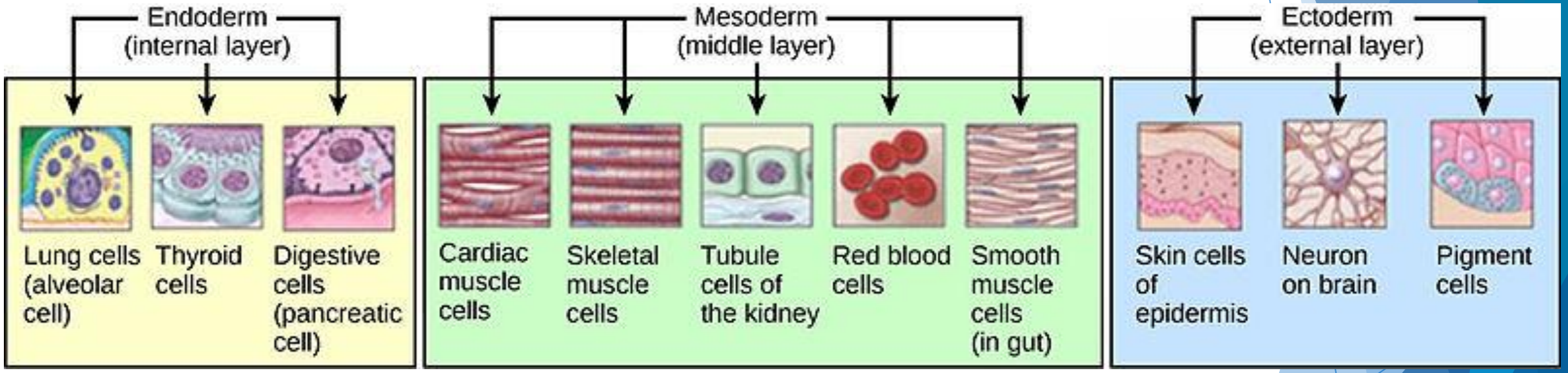
Mesenchymal Stem Cells (Multipotent): **Clinical Indications**

- These cells **do not** develop into new cartilage cells – they only provide growth factors
- Therapeutic effects are short-lived
  - "Recent studies have suggested that less than 1% of systemically administered MSCs persist for longer than a week following injection" (Parekkadan & Milwid, 2010, pg 2).

Mesenchymal Stem Cells (Multipotent): **Dangers**

- Harvesting of bone marrow and fat can be unpleasant
  - Repeat harvesting is limited
- Immunomodulatory effects can predispose the patients to more infections or even cancer
- Reduces inflammation for 6 months – 2 years but have limited regenerative benefits

Pluripotent Stem Cells (VSELs)



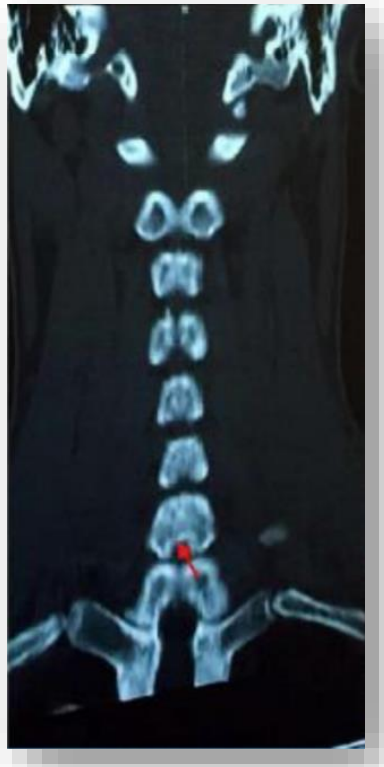
Lineage uncommitted pluripotent stem cells can produce all types of cells in the germ layer

Pluripotent Stem Cells (VSELs)

Pre-Treatment

Displaced (5mm) C-7 proximal spinal fracture failed to heal 9 months post trauma

Post-Treatment



4 months post-treatment of peripheral blood-based stem cells - the fracture is fully healed

Regenerative Treatments with HBOT and Pluripotent Stem Cells for Sports Injuries and Arthritis

HBOT for Sports Injuries

- Reduces swelling
- Blunts the inflammatory process
- Improves range of motion earlier/ PT
- Increases and enhances tissue growth
  - Fibroblast and osteoblast proliferation
- Improves bone regeneration-faster and stronger fracture repair

Case Study

- Injured on January 5th 2009
- Shearing fracture, surgically repaired
- High risk for Non-Union
- Started HBO January 7th 2009
- 30 tx over 6 week period
- Cleared to ski March 3rd 2009

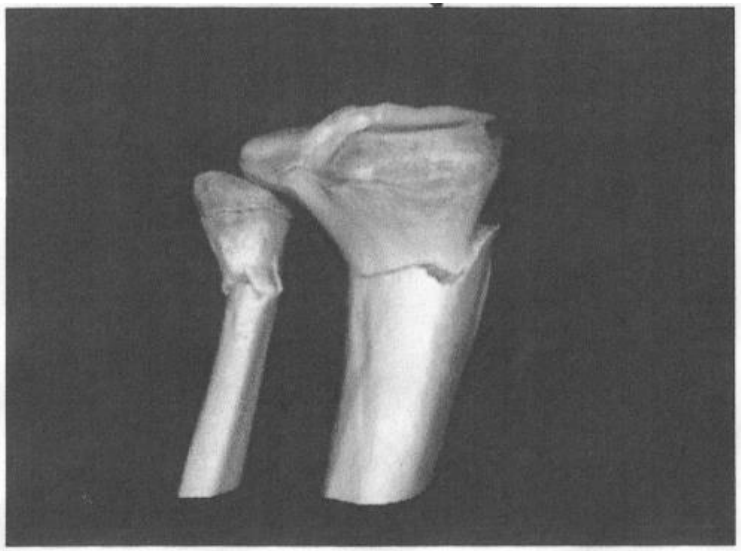


Image 1

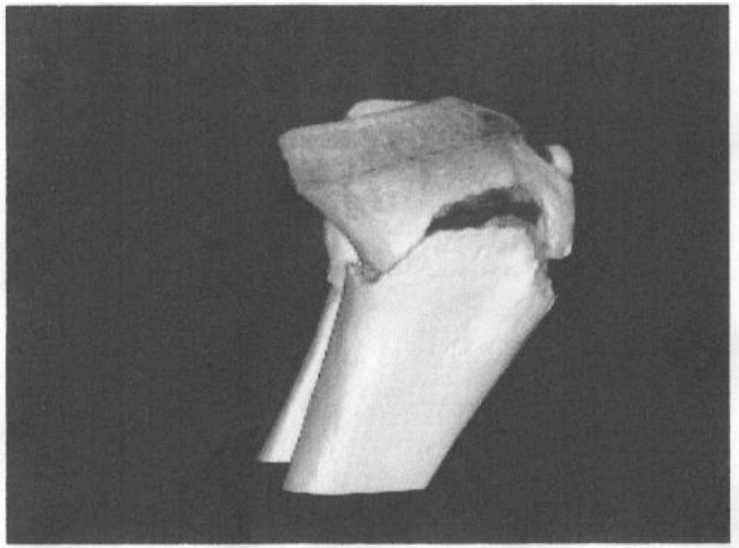
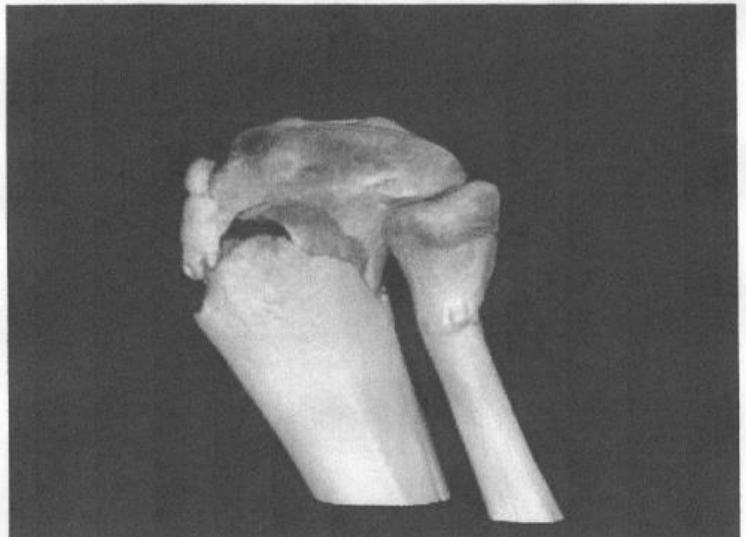
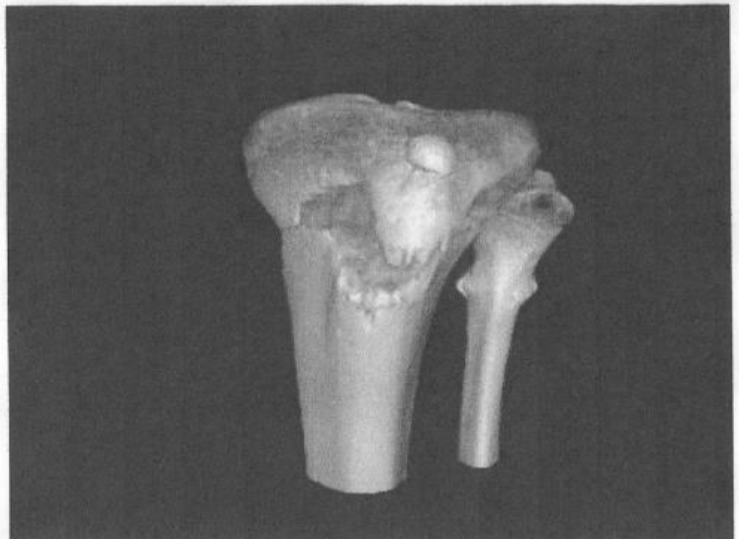


Image 2





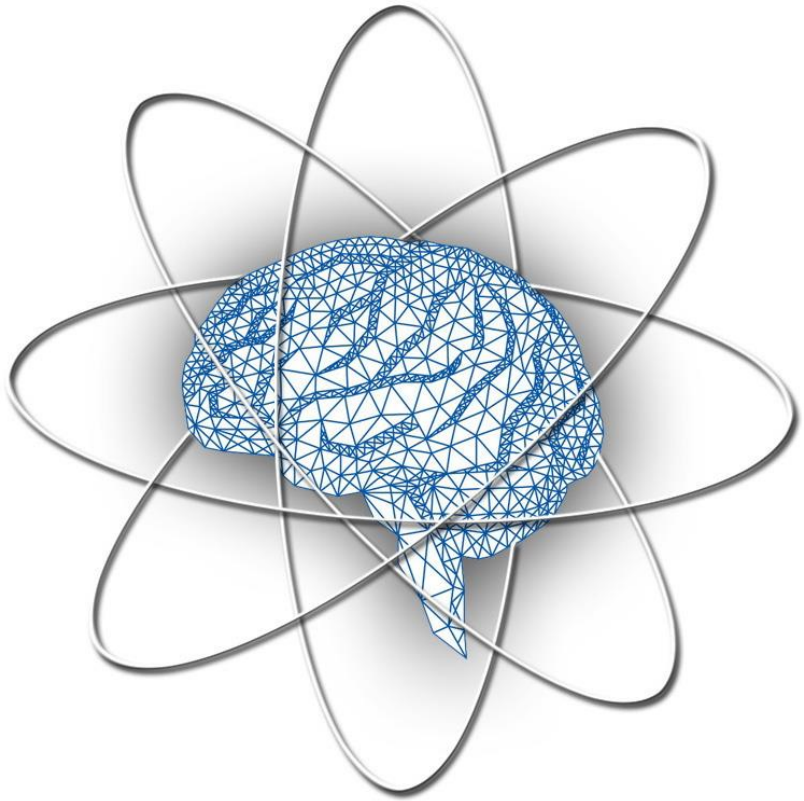
Professional Sports - **Twelve NFL teams own HBOT chambers**

- *“Ward is using hyperbaric chamber to accelerate recovery”* -USA Today
- *“Football superstar Terrell Owens used hyperbaric oxygen therapy to hasten his recovery from an ankle injury so that he could play in the Super Bowl.”* -Fox Sports
- *Cincinnati Bengals defensive tackle Bryan Robinson says “hyperbaric oxygen therapy was the catalyst in getting a nagging ankle injury to heal.”* -Cincinnati Inquirer
- *“Linebacker Kevin Burnett credits hyperbaric oxygen therapy for helping him get back onto the playing field quickly after surgery to repair cartilage damage in his knee.” -D*allas Cowboys Official Weekly

HBOT and Brain Injuries

- Induces neuroplasticity
- Increases tissue oxygenation
- Generates new capillary networks
- Restores blood supply
- Increases stem cells in the blood

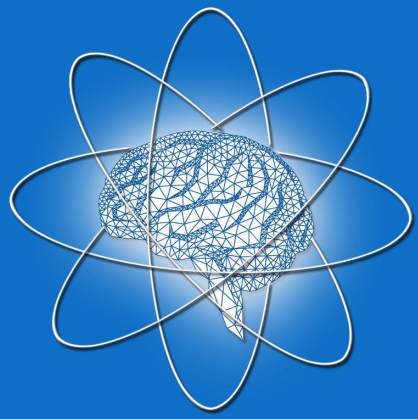
Traumatic Brain Injury: Pre Treatment



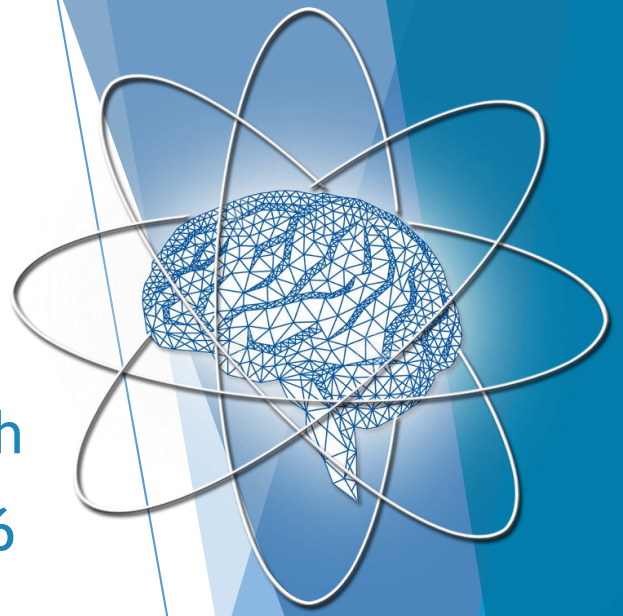
- 10 treatments in a HBOT medical grade facility
  - 1.5 to 1.75 ATA
  - Or at least 3-4 weeks in a home HBOT chamber
- Stem cell enhancing supplements are taken 2 weeks before stem cell harvesting

Protocol for Traumatic Brain Injury: PRP and VSEL Treatment

Day 1:	Consultation HBOT Cranial therapy IV therapy Intranasal (IN) PRP and insulin
Day 2:	IV and IN NAD+ IV and IN pluripotent stem cells (VESLs) from the blood HBOT



Protocol For Traumatic Brain Injury: Post Treatment



- Medical grade HBOT: 10-30x (at 1.5 to 1.75 ATA) over next month
  - Repeat 20 treatments at 3 months; repeat 20 treatments at 6 months
- *Alternative:* Home low pressure O2 chambers (at 1.3 ATA) 5-7 days/week for 1.25 hours for 3 months
  - Then at least 4 days/week for 9 months
- Home administration of intranasal insulin 10 days or more
- PT, cranial osteopathy, functional medicine (including hormone management), and other therapeutic modalities (vision therapy, neurofeedback, LLLT, ketogenic diet)

“In June 2017, I went in for my second intranasal stem cell procedure and by August I felt well enough that I started saying yes again to facilitating events and speaking gigs. I also experienced relief from anxiety. With the stem cell procedures, the results were never immediate but 8-12 weeks post procedure I experienced a noticeable jump in my healing. Even though, I’m still not 100% back to what I was, TBI Therapy has turned me into a TBI THRIVER, not just a survivor. I’m happy. I enjoy life again, can travel and am doing work in the world that’s more aligned with myself than ever.”

“I am now officially 5 weeks post intranasal/IV stem cell and PRP treatment and the results for me have been are nothing short of MIRACULOUS! Trust me when I say that losing who you are from a traumatic brain injury is absolutely devastating! Over the years I learned how to coexist with my brain injury and the issues that came along with it but only a select few close to me could tell I was still struggling at times. Until now... Popeye may have his spinach but I have stem cells and PRP! Yes, my brain is strong!”

Arthritis Case Report

- 80 year old with tricompartmental arthritis x 10 years, confirmed by xray, worse in R knee
- Treated with VSELs in Bilat Knee joints, menisci, and associated ligaments on 2/9/2018
- Reports on 4/13/2018 that her left knee does not hurt
- Reports improvements in walking with less R knee pain on 6/7/2018. Patient provided booster PRP injection into R knee joint and IT band at 6/7/2018
- *"The only consistent symptom I have is that it is always uncomfortable when I stand up from a sitting position and when I first get up in the morning. Usually just a few steps and the discomfort is gone."*

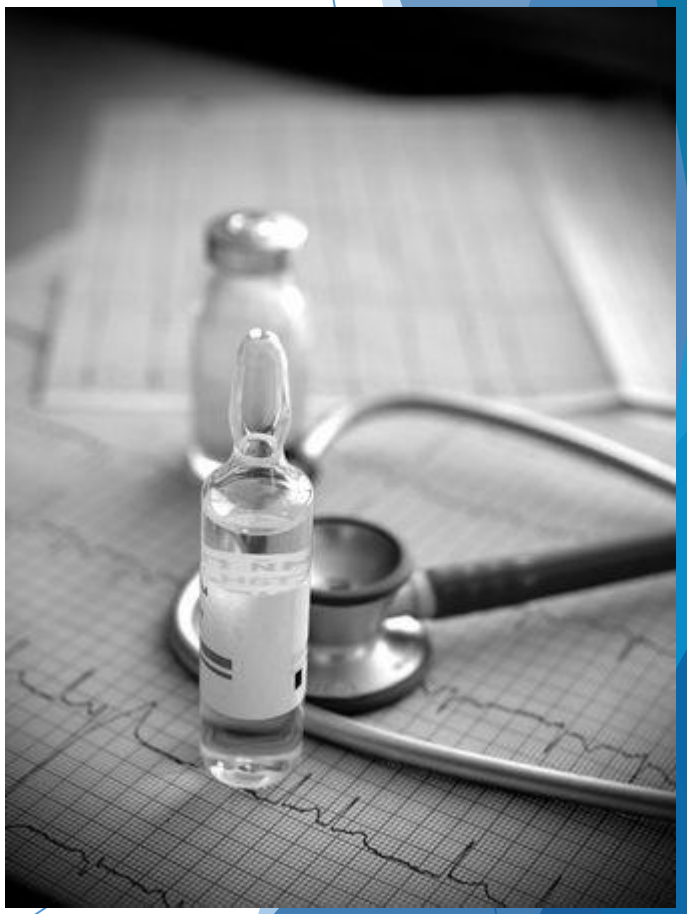
HBOT: Adjunctive to IV Therapies for Chronic Infection

HBOT: Adjunctive to IV Therapies for Chronic Infection

- HBOT alone: Helps Osteomyelitis, subcutaneous infections, systemic infections such as herpes, EBV, etc.
- HBOT (2.0+ ATA) + IV ascorbate (in excess of 50g), has an even greater effect on many chronic infectious conditions (including chronic viral (like EBV), immunosuppression, and post-Lyme syndrome)

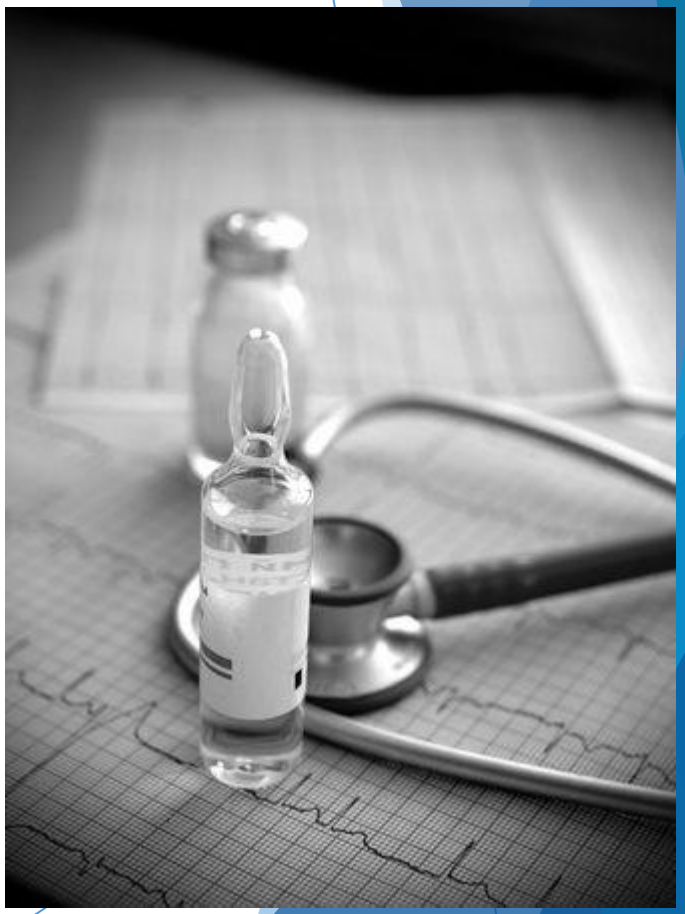
HBOT: Adjunctive to IV Therapies for Chronic Infection: Driving the Fenton Reaction with Ascorbate

- With catalytic metal ions, ascorbate has pro-oxidant effects
- Ascorbate reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron. **Increase Ascorbate = Increase Fe^{2+}**
  - $AscH^- + Fe^{3+} \rightarrow Asc^{\bullet -} + Fe^{2+}$
- Fe^{2+} can readily react with O_2, reducing it to superoxide radical. **Increase O_2 = Increase $O^{\cdot -}_2$**
  - $Fe^{2+} + O_2 \rightarrow Fe^{3+} + O^{\cdot -}_2$
- The superoxide radical dismutes to H_2O_2 and O_2
  - $O^{\cdot -}_2 + O^{\cdot -}_2 + 2H^+ \rightarrow H_2O_2 + O_2$ **Increased H_2O_2**



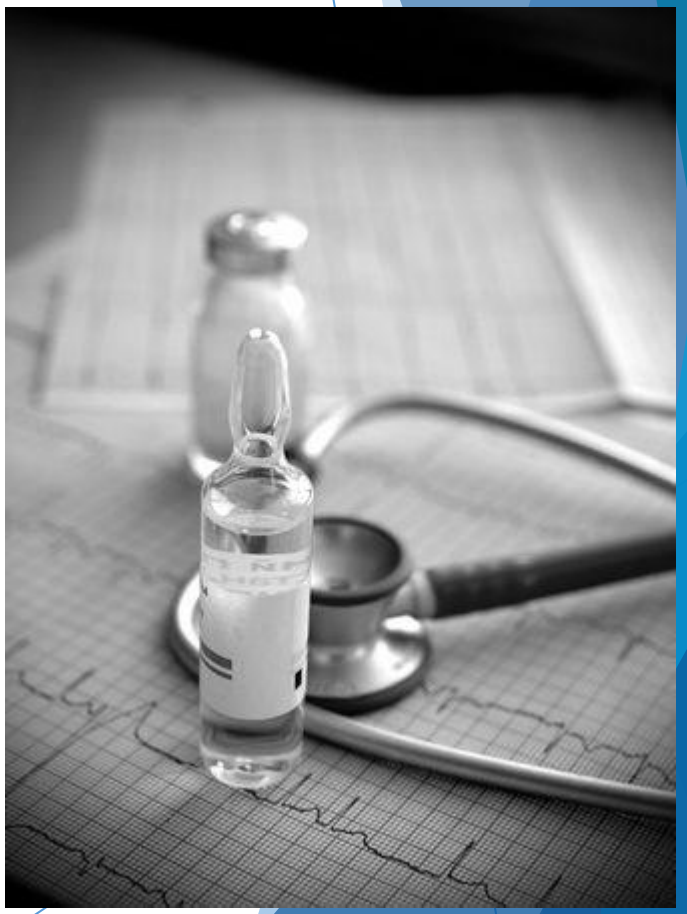
HBOT: Adjunctive to IV Therapies for Chronic Infection: Driving the Fenton Reaction with Ascorbate

- In a classic Fenton reaction, Fe^{2+} reacts with H_2O_2 to generate Fe^{3+} and the very oxidizing hydroxyl radical.
  - $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$
- This OH radical is incredibly deadly to viruses, bacteria, spirochetes, other pathogens, and, reportedly cancer cells
- Healthy cells are protected from peroxide radicals by the enzyme catalyze



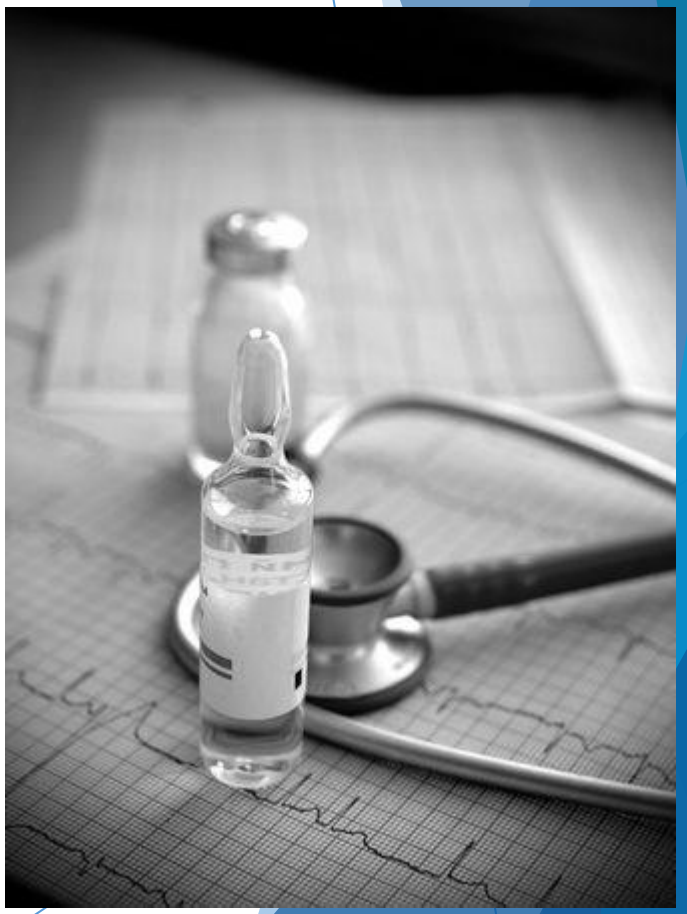
HBOT: Adjunctive to IV Therapies for Chronic Infection: Driving the Fenton Reaction with Ascorbate

- Stimulating this reaction can create interferon like side effects in the patients
- Patients report areas of prior injuries or inflammation can get flared up, achy, or significantly painful
- Most patients report abdominal/diaphragmatic pain that resolves within 2-20 minutes after getting out of the chamber
- *Fun Correlation: This is further evidence that the increased presence of ROS leads to nociceptive pain*



HBOT: Adjunctive to IV Therapies for Chronic Infection: Driving the Fenton Reaction with Ascorbate

- Patients may need more bioavaibie iron: the typical range for the iron dose is 1 part of Fe per 5-25 parts of H2O2
- *pH adjustment to 3-5 :* if the pH is too high the iron precipitate in Fe(OH)3 and will decompose the H2O2 to oxygen.
  - Basically, the optimal pH occurs between 3 and 6
- Do not give the patient a neutralized bag of ascorbate—pH must be at least than 5-6 in the bag



Case Report: Lyme Disease

History

- 60 yo female reported diagnosis of Lyme disease with HHV6, EBV, M.Pn, Babesia, Erlichia
- R ocular pain, R vision loss, extreme fatigue, diagnosed with 9 bands/10 bands for Borellia - Treated with Doxycline and unspecified antibiotic
- Worsened with intractable R eye pain, vision loss, extreme sensitivity to light, tingling in her R UE and LE and wheelchair bound after 6 months
- Received IV Rocephin and other antibiotics including Doxycline and Azithromycin, and nutritional IV therapies including EDTA, turmeric, ascorbate, alpha lipoic acid, glutathione, and amino acids
- Walking again but still suffered extreme R eye pain, vision loss, migraine headache pain, elevated liver function tests, elevated lipase, chronic fatigue, and skin rash
- Reported being unable to work and bed ridden with fatigue

Case Report: Lyme Disease

Treatment

- IV sodium ascorbate
  - Up to 95 g non-corn based ascorbate with minerals (Ca,Mg, K) 3 days/week
- Hyperbaric oxygen therapy
  - Up to 2.4 ATA (1 hour after receiving IV ascorbate) 3 days/week
- After 20 weeks:
  - Improvement in condition of pancreatitis with a resolution of her lipase value and liver function tests
  - Less fatigue and improved energy to think more clearly, improved ability to stay up later and take walks during the afternoon
  - Improvement in her eye pain and ability to use the computer for more than 5 minutes at a time
- Referred to a holistic ophthalmologist for continued care

Case Report: Lyme Disease

- Chronic Lyme disease is often accompanied by toxins and viruses that cannot be eliminated by simply using antibiotic therapy.

- Without HBOT and Vitamin C treatment, this patient would not have gotten better.

Case Report: Mold Toxins

- 34 yo male with L temporal glioma and seizure condition – likely secondary to mold toxins in home
- 11/2017 Diagnosed with glioma - surgically removed
- 12/2017 Tumor just as large as before removal
- 3/2018 Moved out of condo and began IV Ascorbate and HBOT
- 4/2018-6/2018 Chemo therapy and radiation therapy, continued IV ascorbate 1-2x/week at 60 g (stopped HBOT due to seizure)
- 6/26/2018 Complete resolution - no tumor at all seen on MRI, no seizures
- Played intense soccer game with no issues

Anti-cancer effect of ascorbic acid *in vitro*

Janine M. DeBlasi, Nathan P. Ward, Angela M. Poff, Andrew P. Koutnik, Christopher Q. Rogers, Dominic P. D'Agostino

Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL

Background Information

- Cancer is the second leading cause of death in the U.S.
  - Projected to take 595,690 lives in 2016 and cost the nation over $125 billion
- To effectively reduce these detrimental losses, **non-toxic, low-cost therapies** should be further examined to supplement the standard of care
- Anti-carcinogenic and minimally toxic therapy under investigation: **high-dose ascorbic acid (AA)**
- AA can function as a **pro-oxidant** at pharmacological levels (achieved I.V. or I.P.)
  - Delivers hydrogen peroxide (H_2O_2) to tumorous tissue upon oxidation and initiates cell death
- High-dose AA has elicited significant **anticancer effects** in animal models and small-scale human reports at concentrations **nontoxic** to healthy cells
- **We aim to examine the anticancer effect of AA *in vitro* and to mechanistically evaluate AA-induced oxidative stress, as well as investigate AA's synergy with another non-toxic metabolic therapy: Hyperbaric Oxygen Therapy (HBOT)**
  - I. Determine the effect of AA on viability and proliferation *in vitro*
  - II. Evaluate the mechanism of AA-induced cytotoxicity: N-Acetyl cysteine (NAC) is an antioxidant precursor to glutathione, an antioxidant that is highly abundant in the body and scavenges free radicals. If treatment with NAC attenuates the therapeutic effect of AA, this finding would support the hypothesis that **oxidative stress mediates AA-induced cytotoxicity**
  - III. Investigate if synergy exists between HBOT and AA: HBOT is a medical treatment used to heal wounds, radiation injury, decompression sickness, and other health ailments by delivering 100% oxygen at elevated barometric pressure; since HBOT enhances free radical production and **oxidative stress**, we hypothesize that it will synergize with AA and further decrease VM-M3 cell viability
- We anticipate that this approach will yield significant insight into and further investigate the hypothesis that AA is an **effective adjuvant to the standard of care**

Experimental Design

VM-M3 Cells:

- Highly metastatic cells derived from a spontaneous brain tumor in VM/Dk inbred mouse

AA-induced cell death:

- Cytotoxicity/ viability was measured in VM-M3 cells with fluorescence microscopy, using dyes calcein AM and EthD-1 to identify live and dead cells, respectively- cells labeled with both calcein AM and Ethd-1 may indicate early stages of necrosis and were counted as dead (Ethd-1 binds with nucleic acids inside the cell, indicating a loss of membrane integrity)
- Cells were treated with pharmacological concentrations of AA ranging from 0.001 mM to 5 mM

AA's effects on proliferation:

- Standard trypan blue hemocytometry was used to measure proliferation
- Cells were treated with varying concentrations of AA, and were counted after growth periods of 24, 48, 72, and 96 hours

Treatment with antioxidant NAC and AA:

- Cells were treated with a cytotoxic concentration of AA (0.5 mM), in the presence or absence of 5 mM NAC

AA and HBOT Combination:

- VM-M3 cells were treated with one session of HBOT (100% O_2, 60 mins, 2.5 ATA)
- AA concentrations below 0.5 mM were used since ≥ 0.5 mM AA already induces high % cell death

Ascorbic Acid Inhibits VM-M3 Cells *In Vitro*

VM-M3 Cell Death

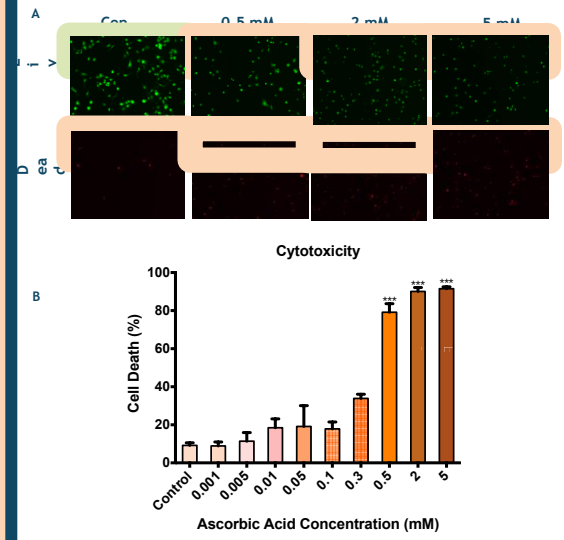


Figure 1. AA mediates VM-M3 cell death in a concentration-dependent manner. (A,B) 24 hour treatment with 0.5, 2, and 5 mM AA significantly induced cytotoxicity compared to control and all other tested concentrations (One-way ANOVA, p<0.001).

VM-M3 Cell Proliferation

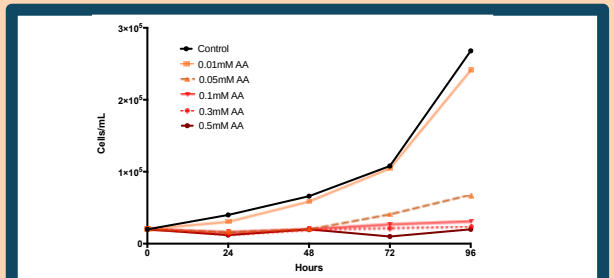


Figure 2. AA may decrease VM-M3 cell proliferation *in vitro*. 0.05, 0.1, 0.3, and 0.5 mM AA exhibited a trend of decreased proliferation compared to the control and 0.01 mM AA. This experiment will be repeated to test for statistical significance.

Additional Preliminary Findings

Effect of NAC on AA-mediated Cytotoxicity

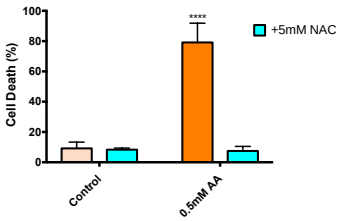


Figure 3. Antioxidant NAC attenuates the effect of AA *in vitro*. 24 hour treatment with 5mM NAC mitigated AA-induced cytotoxicity (One-way ANOVA, p<0.0001). 0.5mM AA was also considered significant when compared to control and control + 5mM NAC (p<0.0001).

Potential Synergy of AA and HBOT

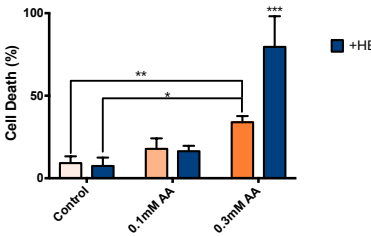


Figure 4. HBOT and AA synergize *in vitro*. 24 hour treatment with HBOT and 0.3 mM AA significantly increased cytotoxicity compared to all other treatments (One-way ANOVA, p<0.001). 0.3mM AA was also considered significant when compared to control (p=0.002) and control + HBOT (p=0.015). The addition of HBOT did not affect control and 0.1 mM AA.

Conclusions/ Future Directions

- High-dose AA shows an **anticancer effect** *in vitro* and exhibits cytotoxicity through an **oxidative stress** mechanism
  - HBOT may enhance this therapeutic effect
- These findings indicate that high-dose AA should be further investigated as an **adjuvant to the current standard of care**
- Further studies include:
  - Evaluating the effect of HBOT on the proliferation of AA-treated VM-M3 cells
  - Evaluating role of hydrogen peroxide (H_2O_2) in AA-induced cytotoxicity with treatment of catalase- an enzyme that breaks down H_2O_2 to water and oxygen

Acknowledgements

Facilities use at Laboratory of Metabolic Medicine (Director, Dr. Dominic D'Agostino) and Hyperbaric Biomedical Research Laboratory (Director, Dr. Jay B. Dean). Work supported by: USF Foundation (501c3); Metabolic Therapy and Cancer Research Account (#250244): Scivation: Florida High Tech Corridor Funding (#MED052-0061361)

Turning Science Into Health®

Take Home

1) Physician who treat patients with chronic orthopedic or neuropathic pain or inflammation should consider the primary use of HBOT to alleviate that pain or as an adjunctive therapy in combination with other modalities to effectively address the source of the pain.

2) Stem cell mobilization by HBOT is perhaps one of the most effective uses of the HBOT in regenerative medicine. These stem cells can be extracted easily from the blood and injected locally to address a variety of pain conditions.

3) Used in combination with high dose ascorbate, HBOT can be one of the most effective ways to eliminate pathogens in patients suffering from acute, chronic, localized, or systemic infections.

References

Boussi-Gross, R., Golan, H., Fishlev, G., Bechor, Y., Volkov, O., et al. (2013) Hyperbaric Oxygen Therapy Can Improve Post Concussion Syndrome Years after Mild Traumatic Brain Injury – Randomized Prospective Trial. *PLoS ONE 8*(11): e79995. doi: 10.1371/journal.pone.0079995.

Brabazon, F. P., Khayrullina, G. I., Frey, W. H., & Byrnes, K. R. (2014, June). INTRANASAL INSULIN TREATMENT OF TRAUMATIC BRAIN INJURY. *In JOURNAL OF NEUROTRAUMA (Vol. 31*, No. 12, pp. A106-A106). 140 HUGUENOT STREET, 3RD FL, NEW ROCHELLE, NY 10801 USA: MARY ANN LIEBERT, INC.

Cell Applications. https://www.cellapplications.com/stem-0

Cellular Differentiation. http://oerpub.github.io/epubjs-demo-book/content/m46036.xhtml

Chen, Q., Espey, M. G., Krishna, M. C., Mitchell, J. B., Corpe, C. P., Buettner, G. R., ... & Levine, M. (2005). Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proceedings of the National Academy of Sciences*, *102*(38), 13604-13609.

Danielyan, L., Beer-Hammer, S., Stolzing, A., Schäfer, R., Siegel, G., Fabian, C., ... & Novakovic, A. (2014). Intranasal delivery of bone marrow-derived mesenchymal stem cells, macrophages, and microglia to the brain in mouse models of Alzheimer's and Parkinson's disease. *Cell transplantation*,*23*(1), S123-S139.

European Society of Endocrinology. (2010). Vitamin D deficiency associated with chronic fatigue in brain injured patients. ScienceDaily. Retrieved August 15, 2016 from www.sciencedaily.com/releases/2010/04/100427182609.htm

Gladstone Institutes. (2008). Collagen May Help Protect Brain Against Alzheimer's Disease. *ScienceDaily*. Retrieved August 15, 2016 from www.sciencedaily.com/releases/2008/12/081210150713.htm

Gunther, N. & Queen, E. (2013). What Physical and Cognitive Rest Really Mean After a Concussion. *Brainline*. Retrieved from http://www.brainline.org/content/multimedia.php?id=9022

Haller, H., Cramer, H., Werner, M., & Dobos, G. (2015). Treating the sequelae of postoperative meningioma and traumatic brain injury: a case of implementation of craniosacral therapy in integrative inpatient care. *The Journal of Alternative and Complementary Medicine*, *21*(2), 110-112.

Han, G., Liu, K., Li, L., Li, X., & Zhao, P. (2017). Effects of hyperbaric oxygen therapy on neuropathic pain via mitophagy in microglia. *Molecular Pain*, *13*, 1744806917710862. http://doi.org/10.1177/1744806917710862

Huskisson, E., Maggini, S., & Ruf, M. (2007). The role of vitamins and minerals in energy metabolism and well-being. *Journal of international medical research*, *35*(3), 277-289.

Kunter, U., Rong, S., Boor, P., Eitner, F., Müller-Newen, G., Djuric, Z., ... & Milovanceva-Popovska, M. (2007). Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *Journal of the American Society of Nephrology*, *18*(6), 1754-1764.

Kurtz, S. (2008). *U.S. Patent Application No. 12/077,296*. Retrieved August 15, 2016 from https://www.google.com/patents/US20090012039

MacCord, K. (2012). Mesenchyme. *The Embryo Project Encyclopedia*. Retrieved from https://embryo.asu.edu/pages/mesenchyme

McNally, M. A., & Hartman, A. L. (2012). Ketone bodies in epilepsy. *Journal of neurochemistry*, *121*(1), 28-35.
Mesenchymal Stem Cell Reagents. Retrieved from http://www.sigmaaldrich.com/life-science/cell-biology/cell-biology-products.html?TablePage=22692887

Mischley, L. K., Conley, K. E., Shankland, E. G., Kavanagh, T. J., Rosenfeld, M. E., Duda, J. E., ... & Padowski, J. M. (2016). Central nervous system uptake of intranasal glutathione in Parkinson's disease. *npj Parkinson's Disease*, *2*, 16002.

Moskalenko, Y., Frymann, V., Kravchenko, T., & Weinstein, G. (2003). Physiological background of the Cranial Rhythmic Impulse and the Primary respiratory Mechanism. *Am Acad Osteopath J*, *13*(2), 21-33.

Murnaghan, I. (2016). Multipotent stem cells. *Explore Stem Cells*. Retrieved from http://www.explorestemcells.co.uk/multipotentstemcells.html

Rho, J. M., & Stafstrom, C. E. (2012). The ketogenic diet as a treatment paradigm for diverse neurological disorders. Frontiers in pharmacology, 3, 59.

Rose, D. (2012). Hyperbaric oxygen therapy for chronic refractory osteomyelitis. *American family physician*, *86*(10), 888-author.

Parekkadan B, Milwid JM. Mesenchymal Stem Cells as Therapeutics. *Annual review of biomedical engineering*. 2010;12:87-117. doi:10.1146/annurev-bioeng-070909-105309.

Stout, C. L., Ashley, D. W., Morgan, J. H., Long, G. F., Collins, J. A., Limnios, J. I., ... & Young, H. E. (2007). Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma. *The American Surgeon*, *73*(11), 1106-1110.

Sundin, M., Örvell, C., Rasmusson, I., Sundberg, B., Ringden, O., & Le Blanc, K. (2006). Mesenchymal stem cells are susceptible to human herpesviruses, but viral DNA cannot be detected in the healthy seropositive individual. *Bone marrow transplantation*, *37*(11), 1051-1059.

Sutherland, A. M., Clarke, H. A., Katz, J., & Katznelson, R. (2016). Hyperbaric oxygen therapy: a new treatment for chronic pain?. *Pain Practice*, *16*(5), 620-628.

Thom, S. R., Bhopale, V. M., Velazquez, O. C., Goldstein, L. J., Thom, L. H., & Buerk, D. G. (2006). Stem cell mobilization by hyperbaric oxygen. *American Journal of Physiology-Heart and Circulatory Physiology*, *290*(4), H1378-H1386.

Tithon Biotech (n.d.). Minutevideo retrieved from http://mv.pac.io/post/55b2e2b41e1818650cc7b872

Tithon Human Sciences (2015). Peripheral blood derived pluripotent stem cell technology. Rhttp://aspenintegrativemedicine.com/wp-content/uploads/Tithon-Human-Sciences-Ortho-Case-Study.pdf

UHN Staff. (2015). Vitamins for Memory Loss and Stroke Prevention – These 3 Are Critical. University Health News Daily. Retrieved August 15, 2016 from http://universityhealthnews.com/daily/memory/vitamins-for-memory-loss-and-stroke-prevention-these-3-are-critical/

Van Velthoven, C. T., Kavelaars, A., van Bel, F., & Heijnen, C. J. (2010). Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. *Pediatric research*, *68*, 419-422.

Young, H. E., Duplaa, C., Romero-Ramos, M., Chesselet, M. F., Vourc'h, P., Yost, M. J., ... & Tamura-Ninomiya, S. (2004). Adult reserve stem cells and their potential for tissue engineering. *Cell biochemistry and biophysics*, *40*(1), 1-80.

Young, H. E., & Black, A. C. (2004). Adult stem cells. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*, *276*(1), 75-102.

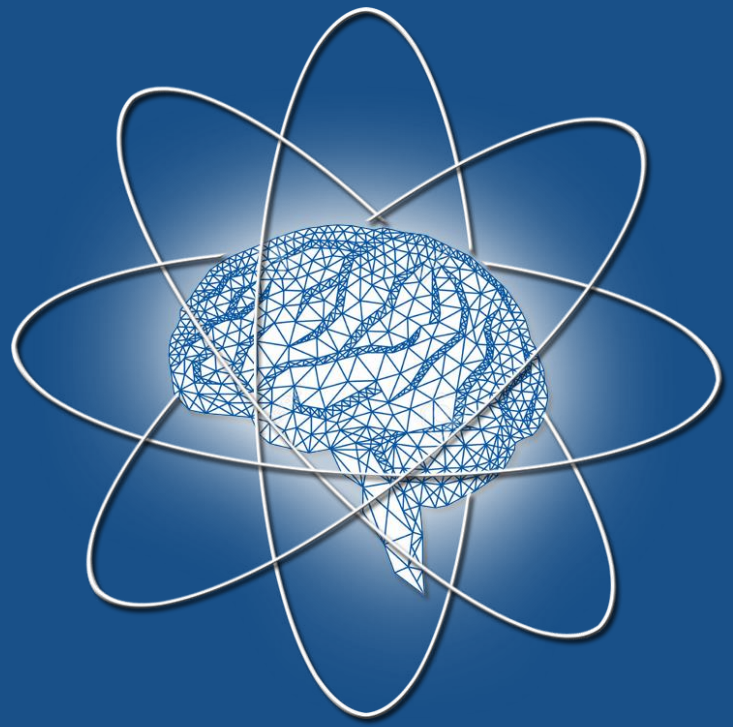
Zhao, B., Pan, Y., Xu, H., & Song, X. (2017). Hyperbaric oxygen attenuates neuropathic pain and reverses inflammatory signaling likely via the Kindlin-1/Wnt-10a signaling pathway in the chronic pain injury model in rats. *The Journal of Headache and Pain*, *18*(1), 1. http://doi.org/10.1186/s10194-016-0713-y

"Facts, like telescopes and wigs for gentlemen, were a seventeenth-century invention."

MacIntyre, *Whose Justice? Which Rationality?* p. 357

Colorado Center for Hyperbaric Medicine

www.cohyperbarics.com



TBI Therapy

Treats TBI patients by combining regenerative therapies: HBOT, stem cells, PRP, and nutritional therapies.

tbitherapy.com

ASPEN INTEGRATIVE MEDICINE

Treats chronic pain and major medical problems using natural and alternative medicine whenever possible.

aspenintegrativemedicine.com