

# THE NORMOBARIC OXYGEN PARADOX : FROM BREATH-HOLD DIVING TO THE PATIENT'S BED



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Belgium

# Disclaimer

- I declare **no real or potential conflict of interest.**
- Any views or opinions presented in this presentation are solely those of the author and do not necessarily represent those of any organization that author belongs to.



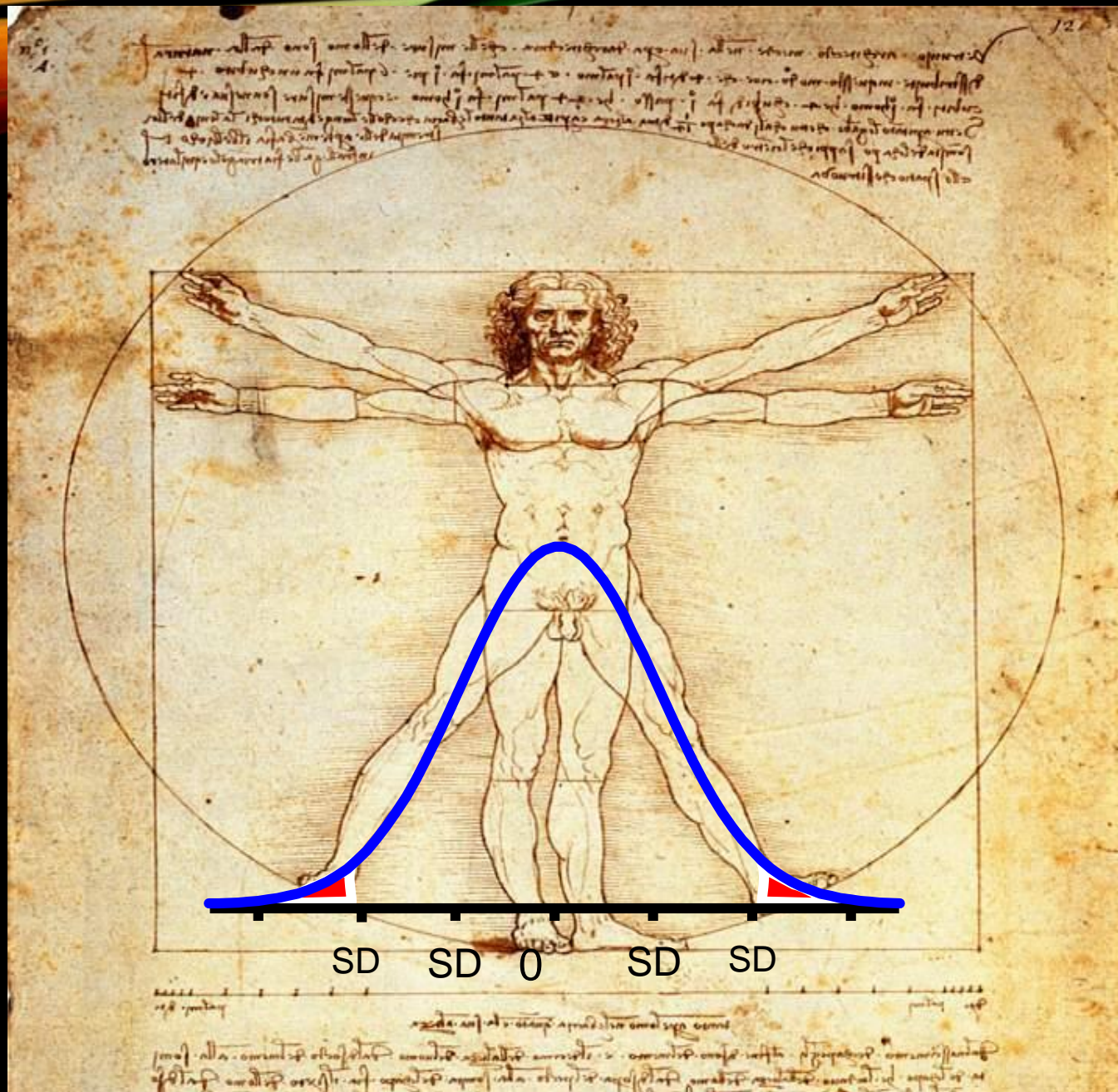
# OXYGEN SENSING FOOLS JANUS



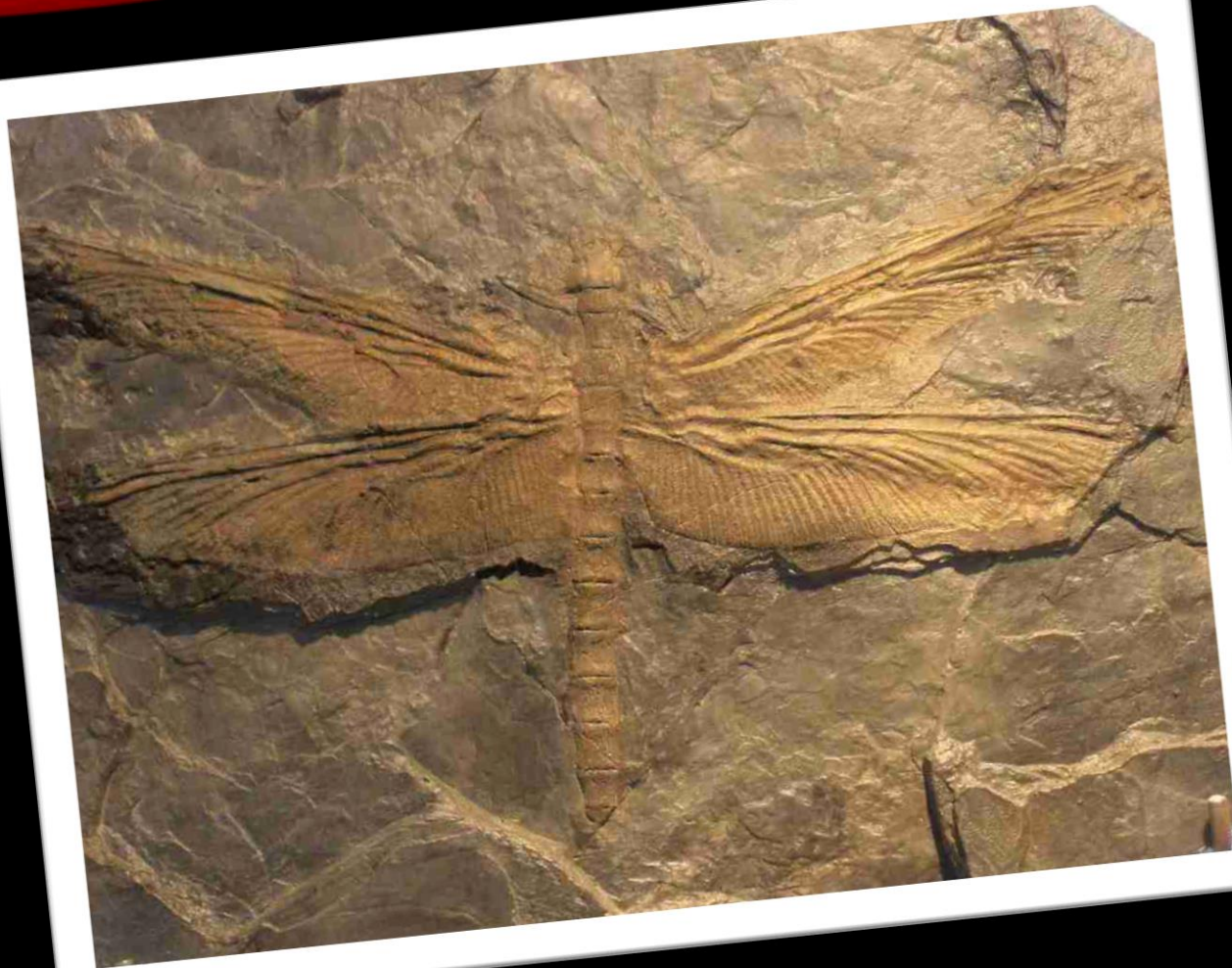








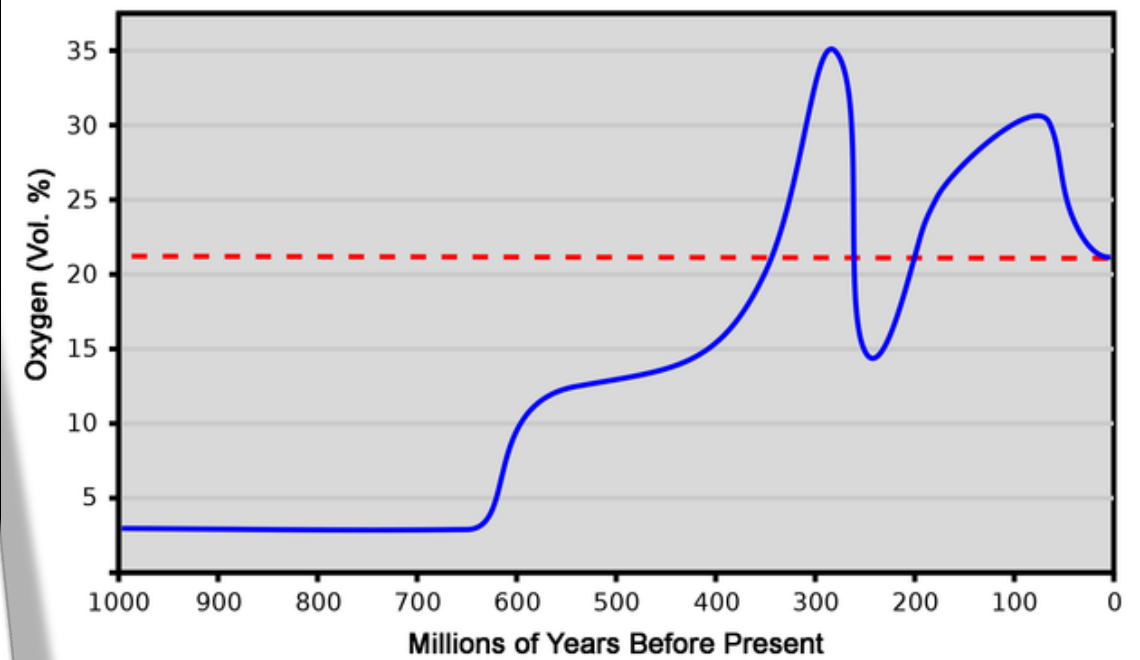




Meganeuropsis Dragonfly

## Oxygen Content of Earth's Atmosphere

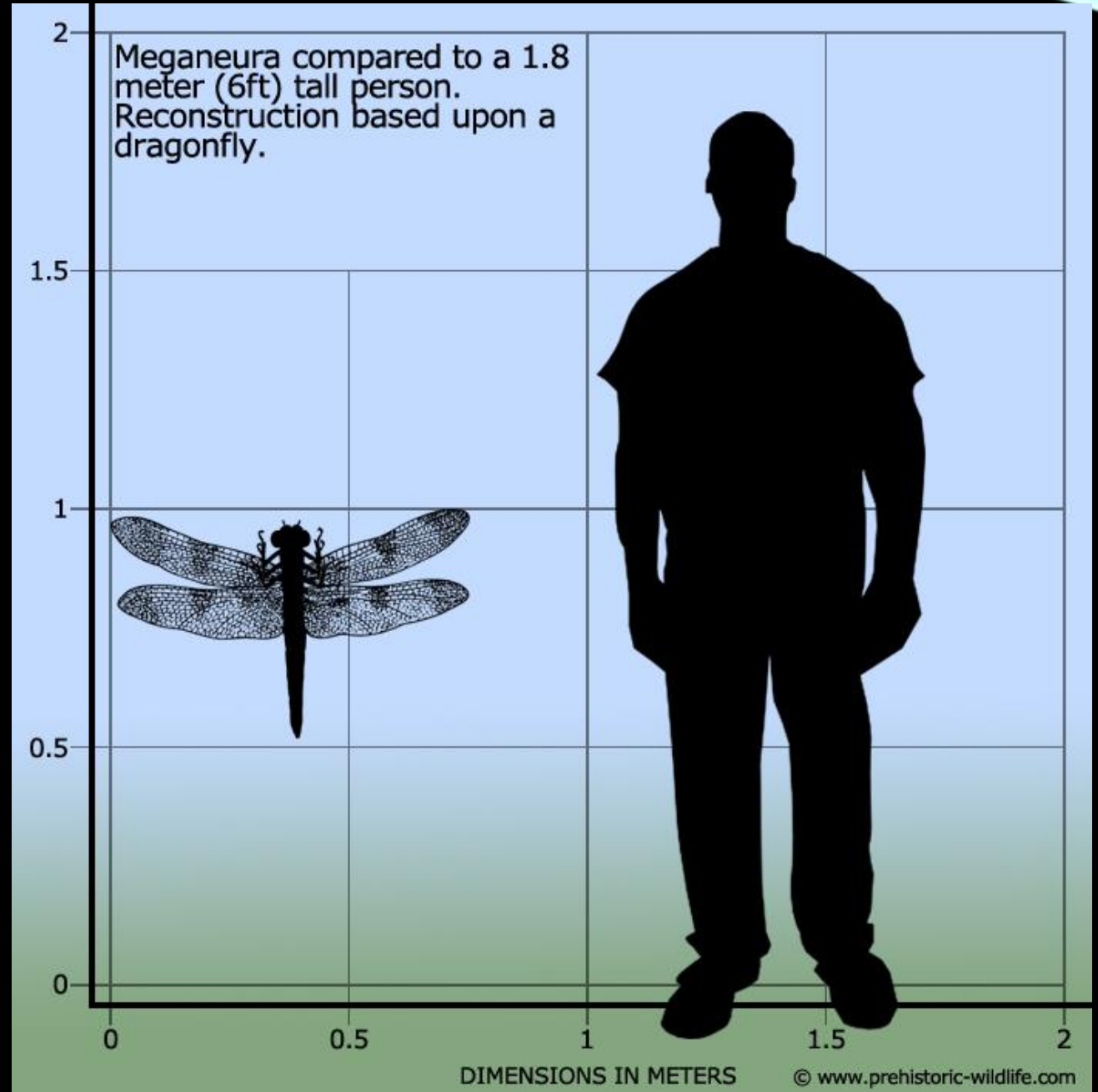
During the Course of the Last Billion Years





# GIGANTISM=PROTECTION

During Carboniferus  
Oxygen content in  
the atmosphere  
was Around 28%



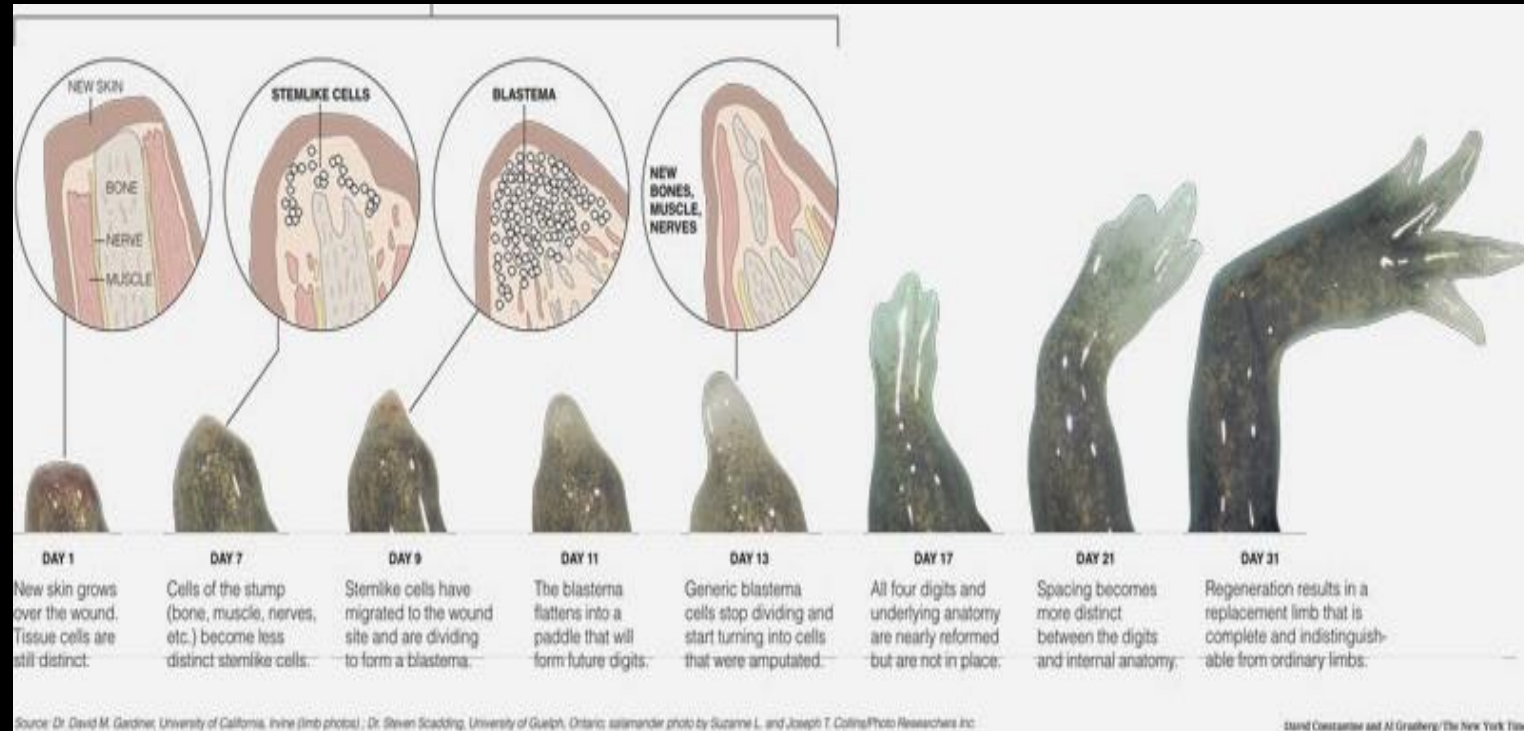
# REJUVINATION RECONSTRUCTION

WE NEED BASICALLY 3 INGREDIENTS

Trigger (deltas- Hif)

Target (Stem Cells)

Supporting environment  
(Oxygenated tissue)





## CALL FOR PAPERS | *Regulation and Function of Stem Cells in the Cardiovascular System*

### Stem cell mobilization by hyperbaric oxygen

Stephen R. Thom,<sup>1,2</sup> Veena M. Bhopale,<sup>1</sup> Omalda C. Velazquez,<sup>3</sup>  
Lee J. Goldstein,<sup>3</sup> Lynne H. Thom,<sup>1</sup> and Donald G. Buerk<sup>4</sup>

<sup>1</sup>Institute for Environmental Medicine and Departments of <sup>2</sup>Emergency Medicine, <sup>3</sup>Surgery,  
and <sup>4</sup>Physiology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania

Submitted 19 August 2005; accepted in final form 7 November 2005

Thom, Stephen R., Veena M. Bhopale, Omalda C. Velazquez, Lee J. Goldstein, Lynne H. Thom, and Donald G. Buerk. Stem cell mobilization by hyperbaric oxygen. *Am J Physiol Heart Circ Physiol* 290: H1378–H1386, 2006. First published November 18, 2005; doi:10.1152/ajpheart.00888.2005.—We hypothesized that exposure to hyperbaric oxygen (HBO<sub>2</sub>) would mobilize stem/progenitor cells from the bone marrow by a nitric oxide (·NO)-dependent mechanism. The population of CD34<sup>+</sup> cells in the peripheral circulation of humans doubled in response to a single exposure to 2.0 atmospheres absolute (ATA) O<sub>2</sub> for 2 h. Over a course of 20 treatments, circulating CD34<sup>+</sup> cells increased eightfold, although the overall circulating white cell count was not significantly increased. The number of colony-forming cells (CFCs) increased from  $16 \pm 2$  to  $26 \pm 3$  CFCs/100,000 monocytes plated. Elevations in CFCs were entirely due to the CD34<sup>+</sup> subpopulation, but increased cell growth only occurred in samples obtained immediately posttreatment. A high proportion of progeny cells express receptors for vascular endothelial growth factor-2 and for stromal-derived growth factor. In mice, HBO<sub>2</sub> increased circulating stem cell factor by 50%, increased the number of circulating cells expressing stem cell antigen-1 and CD34 by 3.4-fold, and doubled the number of CFCs. Bone marrow ·NO concentration increased by  $1,008 \pm 255$  nM in association with HBO<sub>2</sub>. Stem cell mobilization did not occur in knockout mice lacking genes for endothelial ·NO synthase. Moreover, pretreatment of wild-type mice with a ·NO synthase inhibitor prevented the HBO<sub>2</sub>-induced elevation in stem cell factor and circulating stem cells. We conclude that HBO<sub>2</sub> mobilizes stem/progenitor cells by stimulating ·NO synthesis.

nitric oxide; CD34; CD133; CXCR4; cKit; colony-forming cells; progenitor cells

28, 32). Hematopoietic SPCs are typically obtained for the purpose of bone marrow transplantation by administration of chemotherapeutic agents and growth factors (36). Utilizing these agents to obtain autologous SPCs for treating disorders such as organ and limb ischemia, and refractory wounds, has been considered, but application is thwarted because of risks such as acute arterial thrombosis, angina, sepsis, and death (7, 20, 21, 27, 29, 30, 36).

Nitric oxide (·NO) plays a key role in triggering SPC mobilization from the bone marrow via release of the stem cell active cytokine, cKit ligand (stem cell factor, SCF) (1, 8). Because HBO<sub>2</sub> can activate ·NO synthase in different tissues, we hypothesized that exposure to HBO<sub>2</sub> may stimulate SPC mobilization to the peripheral circulation (33, 34). In a murine model, we found HBO<sub>2</sub> augments SPC mobilization and recruitment to ischemic wounds and hastens ischemic wound healing (Goldstein LJ, Gallagher K, Baireddy V, Bauer SM, Bauer RJ, Buerk DG, Thom SR, Velazquez OC, unpublished observations). SPCs have been shown to home to ischemic wounds, where they are required for angiogenesis (3).

HBO<sub>2</sub> therapy is administered for a variety of maladies in a hyperbaric chamber where patients breathe pure O<sub>2</sub> at partial pressures up to 3.0 atmospheres absolute (ATA). HBO<sub>2</sub> is used in a standard fashion as prophylactic treatment to reduce the incidence of osteoradionecrosis (ORN) in patients who must undergo surgery involving tissues previously exposed to radiotherapy (6, 15). We obtained peripheral blood samples from normal human volunteers and from a group of patients under-

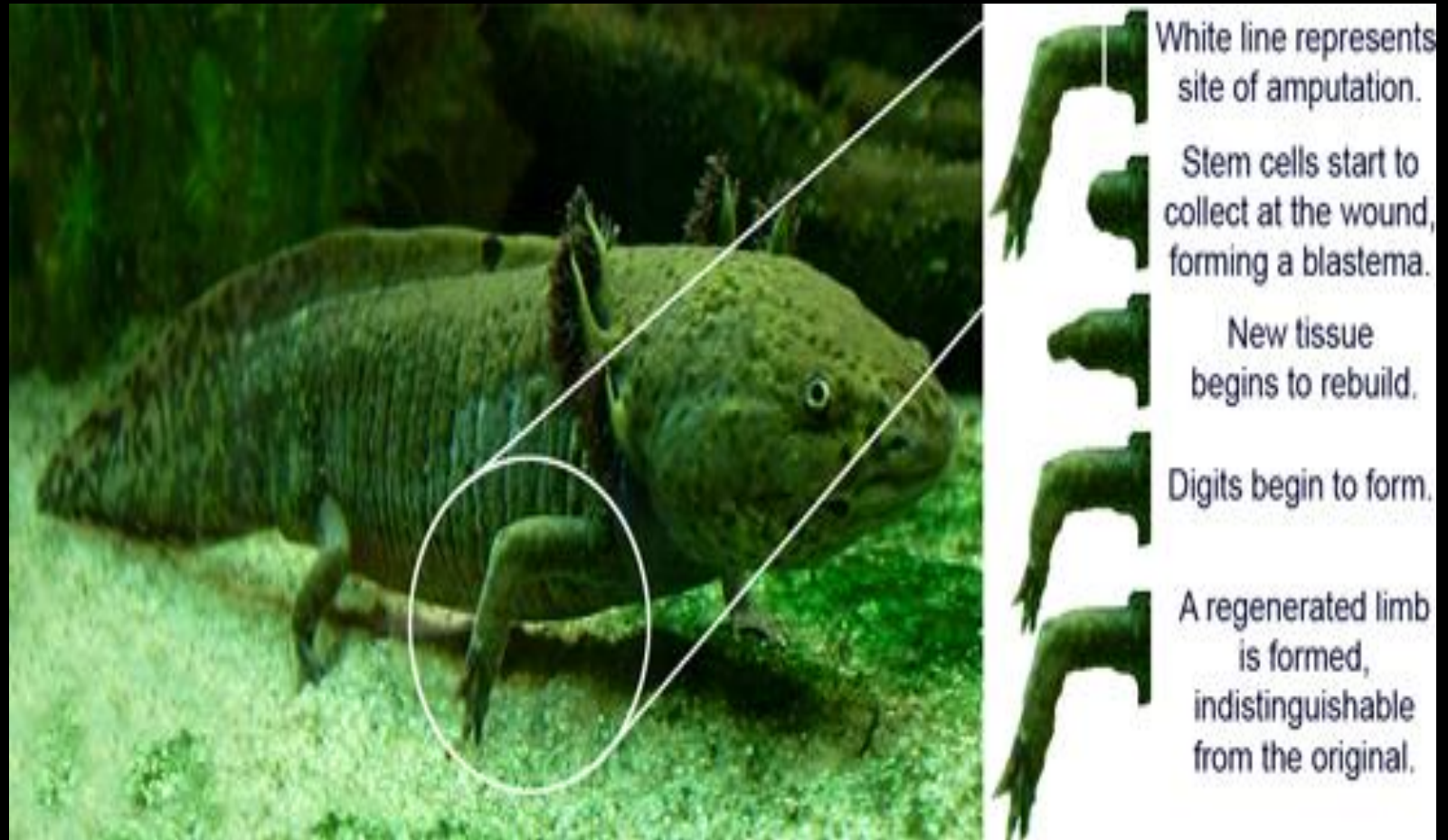
# LESS STEM CELLS NEEDED THAN FORESEEN

Shortly after the limb is amputated, the epithelium layer covers the exposed limb bud, forming the wound epithelium (WE).

A group of stem cells collects below this layer, forming the blastema.

The WE signals the stem cells below it to rebuild the limb, recreating the limb from the point of injury out towards the hand.

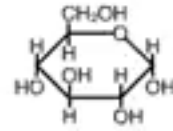
The final regenerated limb is indistinguishable from the original.





## Glycolysis

(Embden-Meyerhof glycolytic pathway)



Glucose

NAD <sup>+</sup>	ATP
P <sub>i</sub>	ADP
	ADP

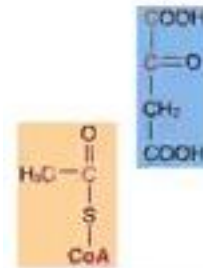
Products

ADP	10
ATP	11
ATP	11

By-products

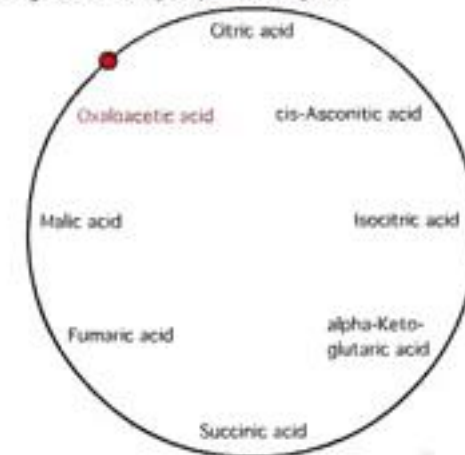


Pyruvic acid (from glycolysis) is decarboxylated to form a two-carbon acetate residue that reacts with coenzyme A to form **acetyl CoA**, which then reacts with **oxaloacetic acid** to begin the Krebs cycle (citric acid cycle).

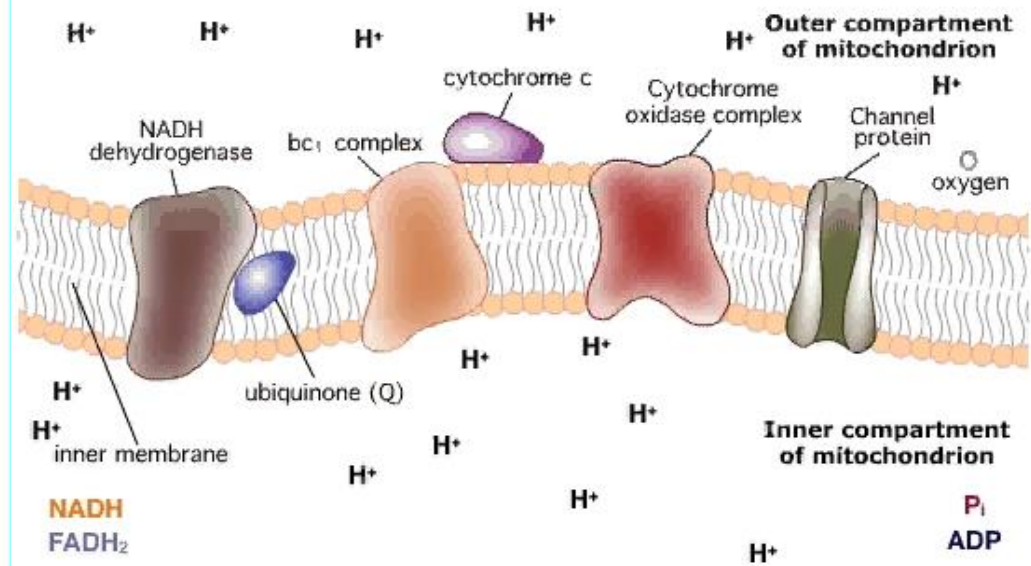
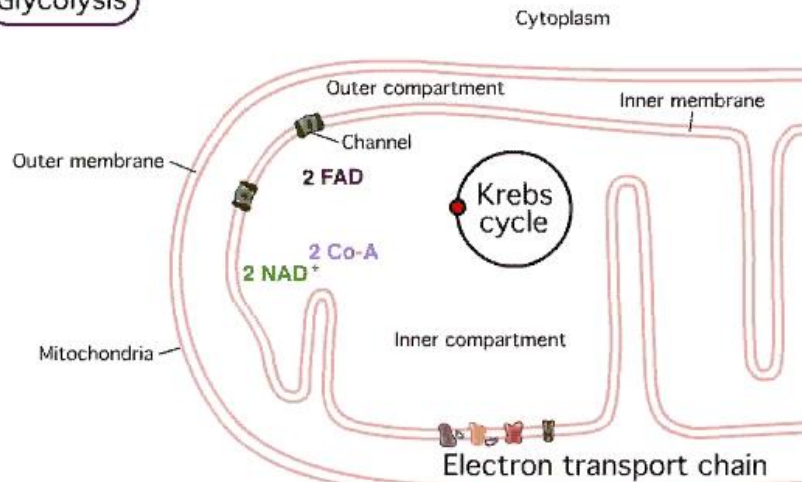


NADH	0
FADH <sub>2</sub>	0
GTP	0
CO <sub>2</sub>	0

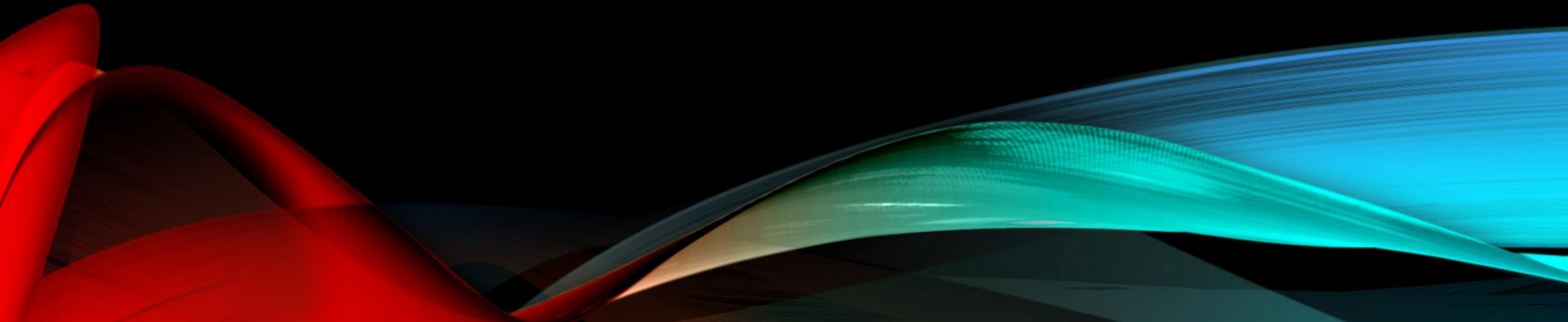
By-products



## Glycolysis



WHAT DOSE DO WE NEED.....ARE WE USING TOO  
HIGH DOSES?





# FISH AND MOUSE



# THERAPEUTIC USE OF ENVIRONMENTAL CHANGES

- Apart from some « sanatoria » and other plane flights to cure pertussis.
- The real therapeutic use of environmental changes combined **Oxygen** and **Pressure**





# ORIGINAL PAPERS

## Life without blood

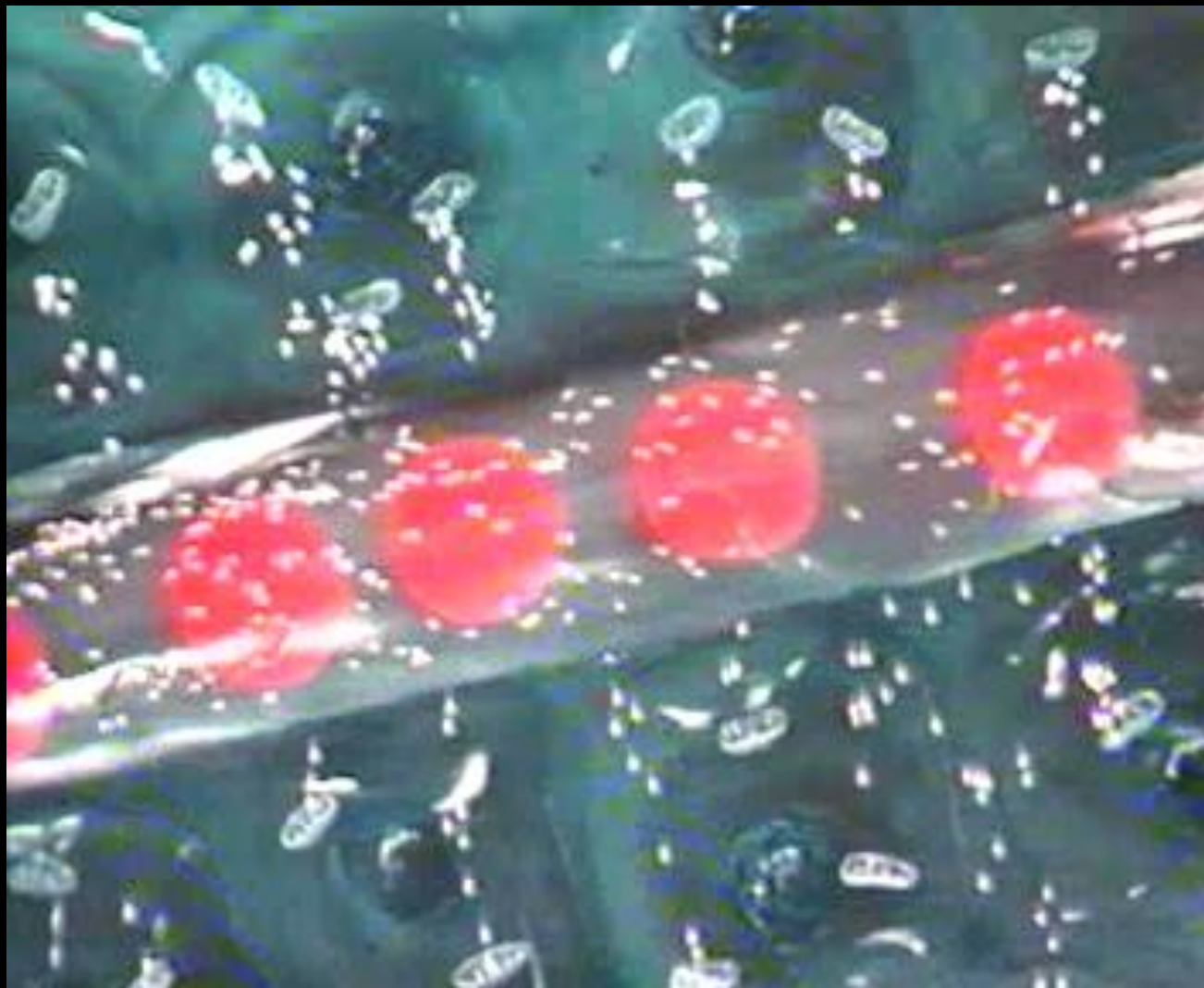
Study of the influence of high atmospheric pressure and hypothermia  
on dilution of the blood)

*by*

I. BOEREMA (\*), N. G. MEYNE, W. K. BRUMMELKAMP  
BOUMA, M. H. MENSCH, F. KAMERMANS, M. STERN HANF  
and W. VAN AALDEREN

from the Surgical Department of the University of Amsterdam)

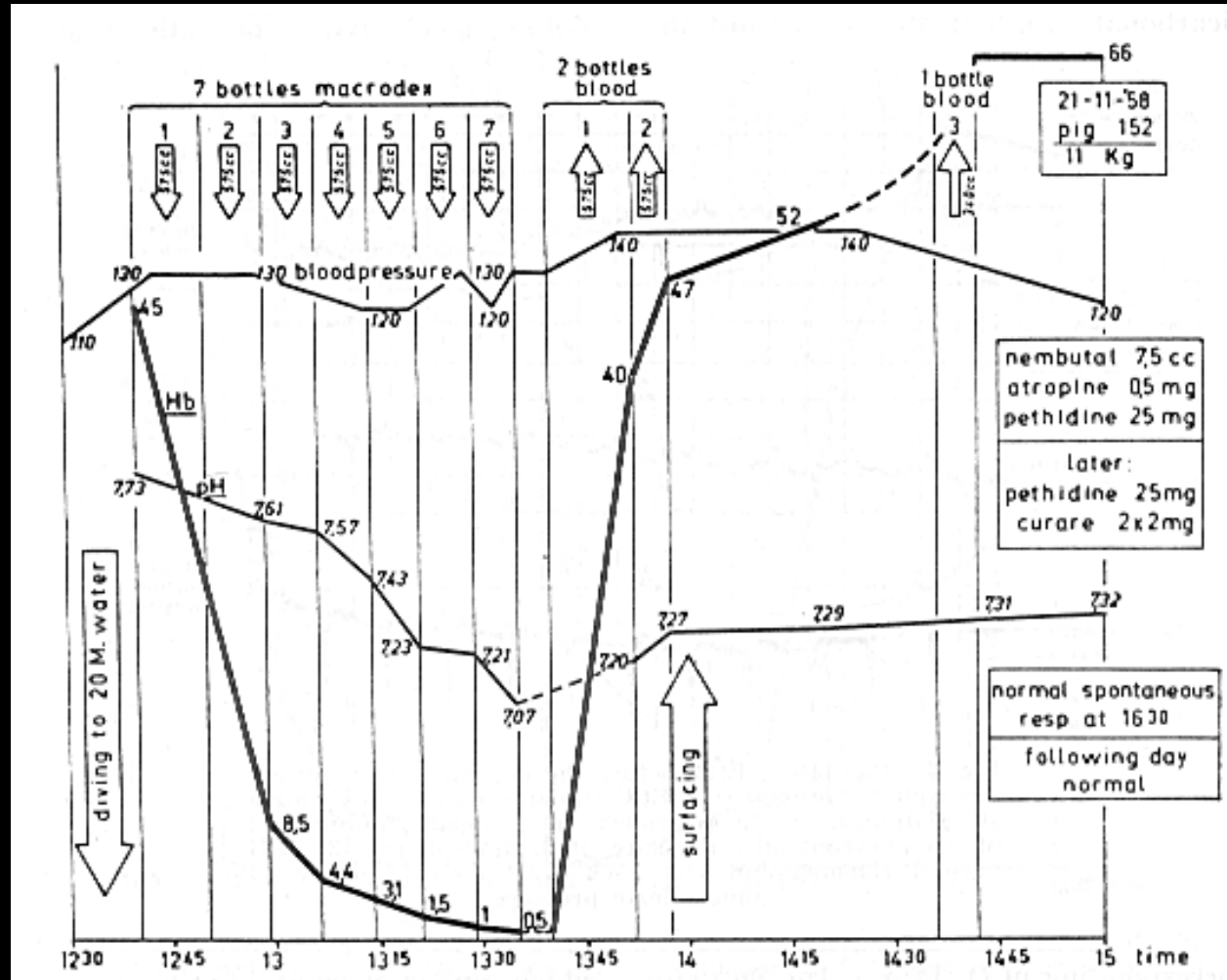
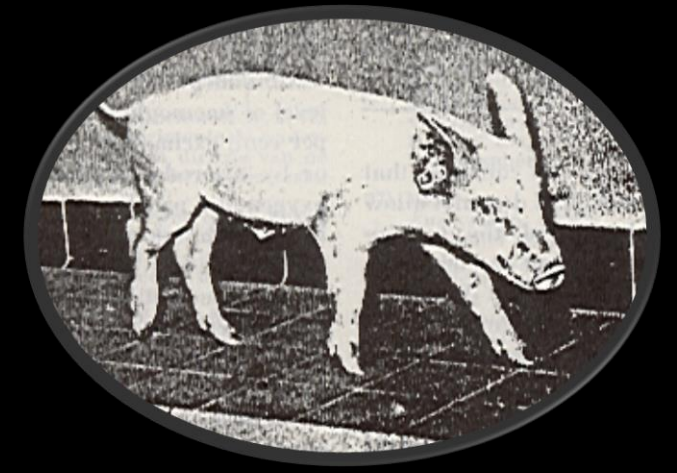
1948 we (first al research) minutes; the reason for this



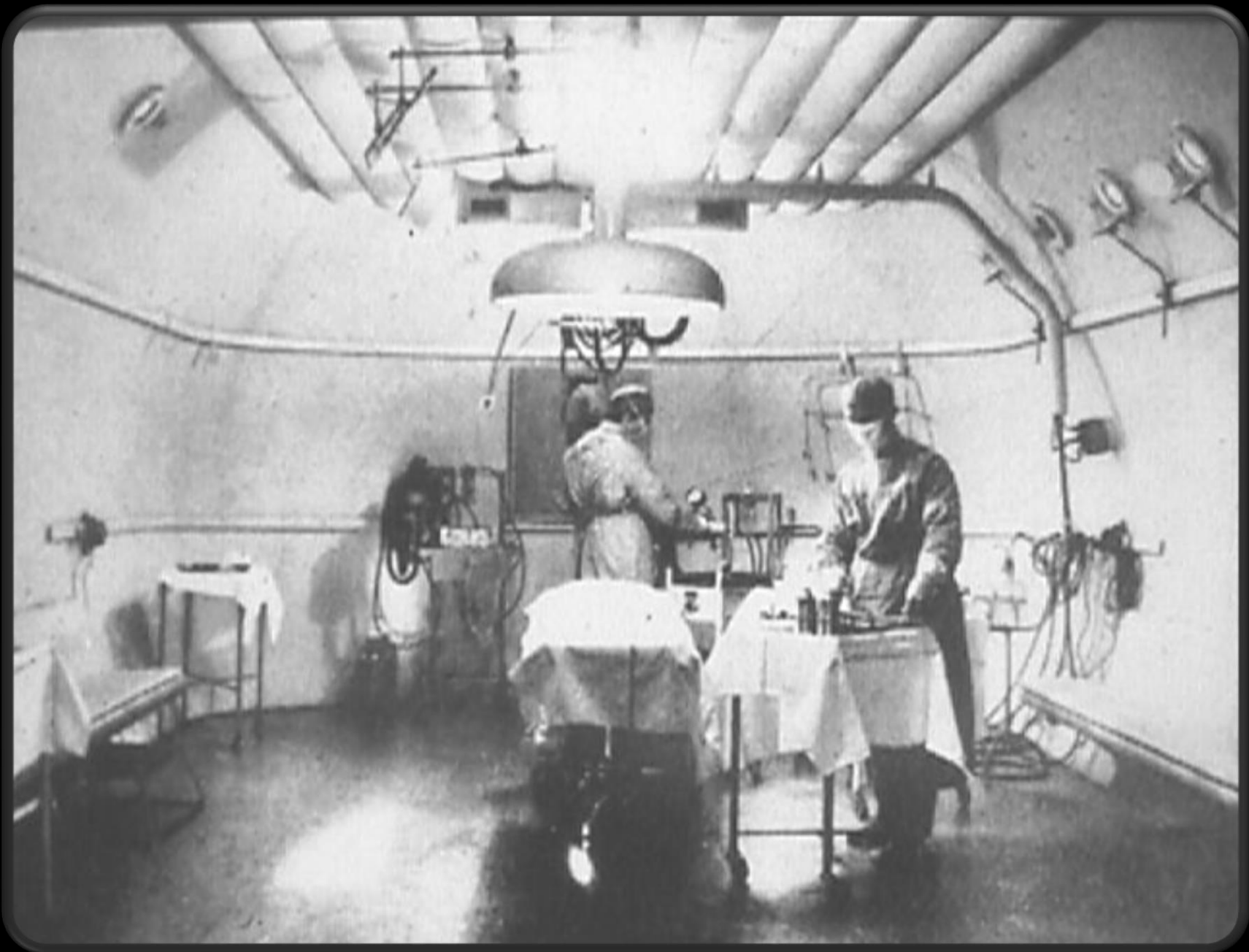
# LIFE WITHOUT BLOOD BOEREMA 1960



# « LIFE WITHOUT BLOOD » BOEREMA 1960



Hyperbaric  
oxygen.  
20 m depth.  
0,5 %  
hématocrit



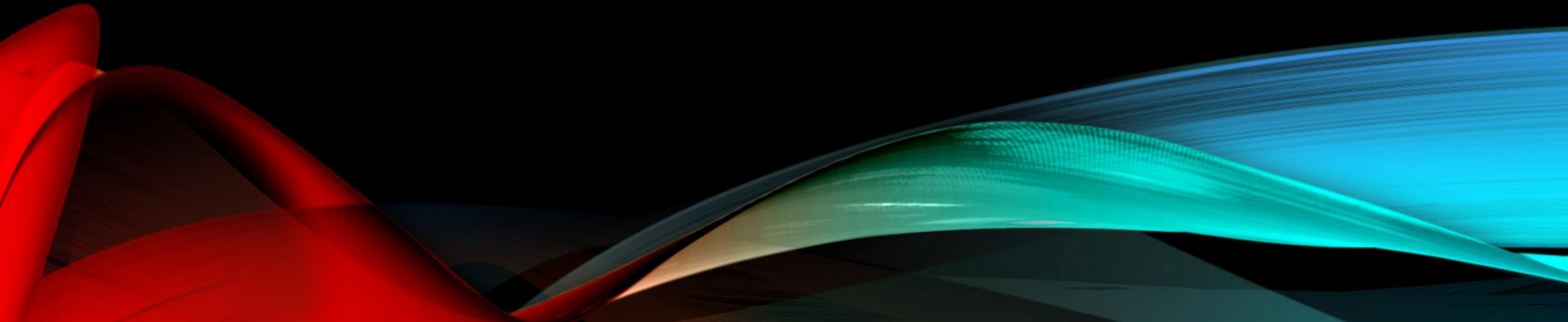


# EMPIRICAL USE, ADAPTATION OF WHAT WAS ALREADY USED FOR DIVERS

Is empirism wrong?



WOULD A TOO LOW DOSE BE A PLACEBO?





# THE PERFECT PROTOCOL

- One homogenous sample measured before and after intervention
- The same sample being measured before and after the time needed for the intervention but without it

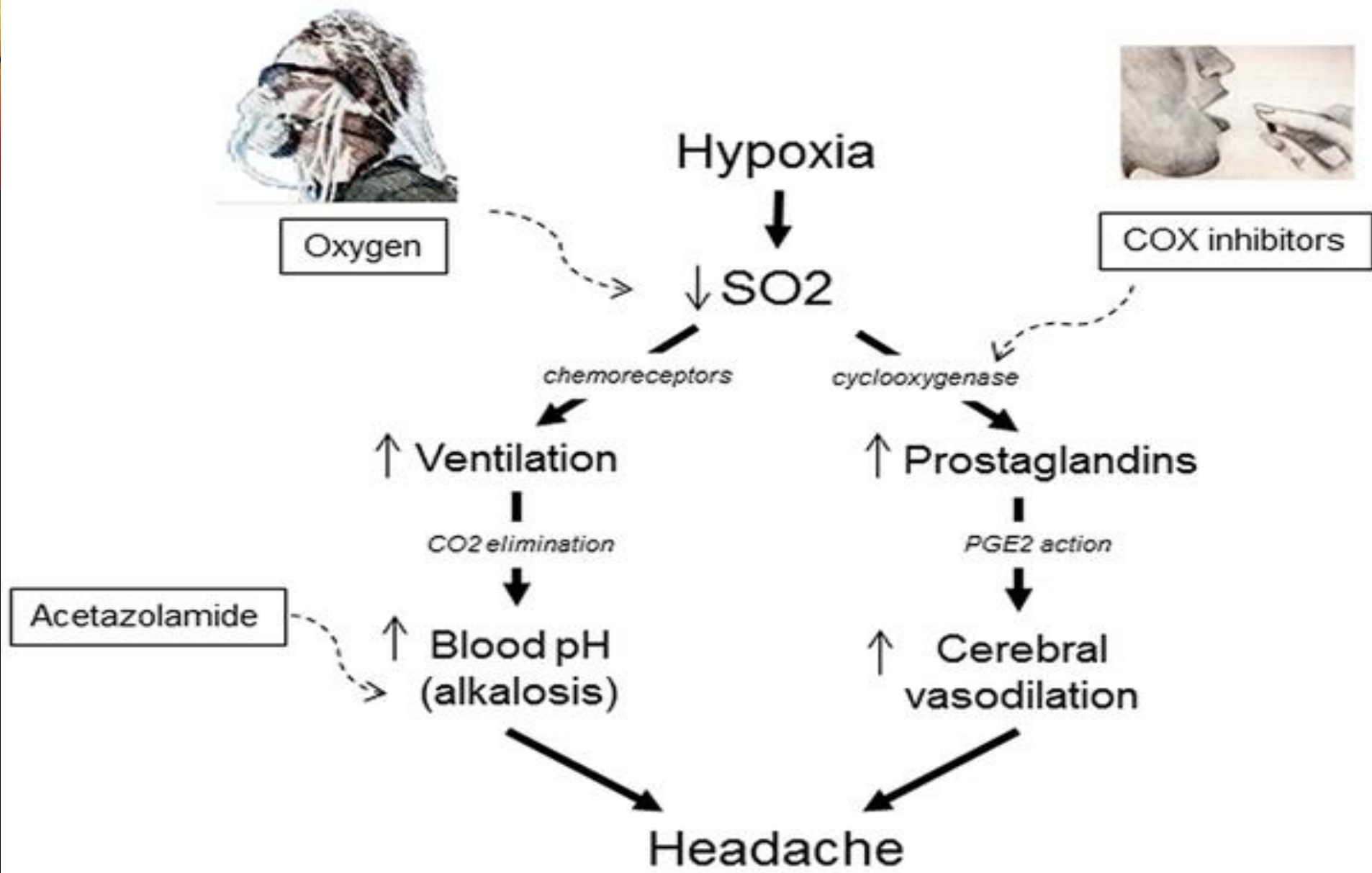
• Time machine needed !!



## PLACEBO

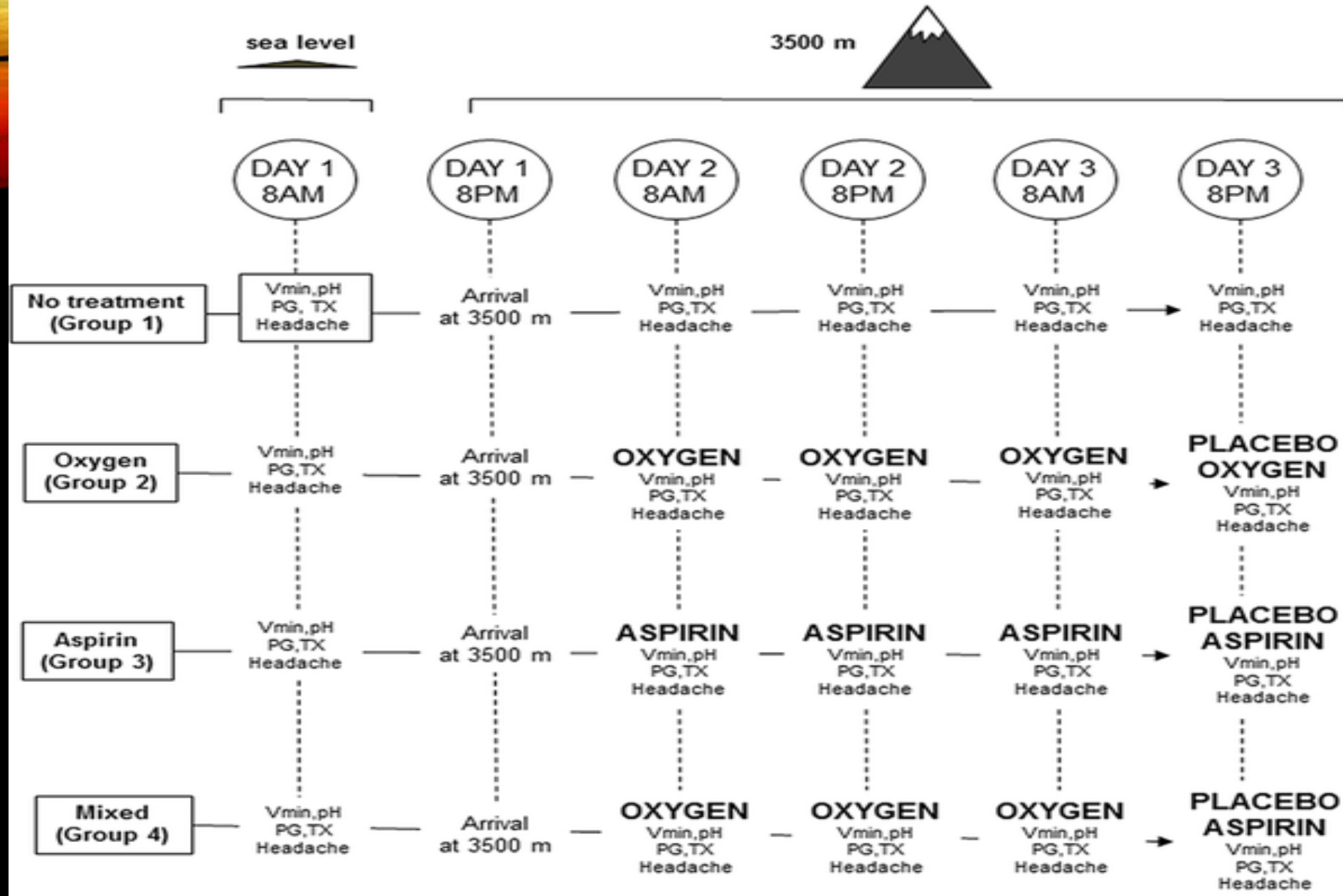
The term “placebo” comes from the St. Jerome’s Latin translation of the Bible in Psalm 114:9 *Placebo Domino in regione vivorum*: “I will please”. This reference was used in the offices for the dead and is related to **liturgy** that funnels by definition a number of **feelings, sensibility, understanding and analysis**.





Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

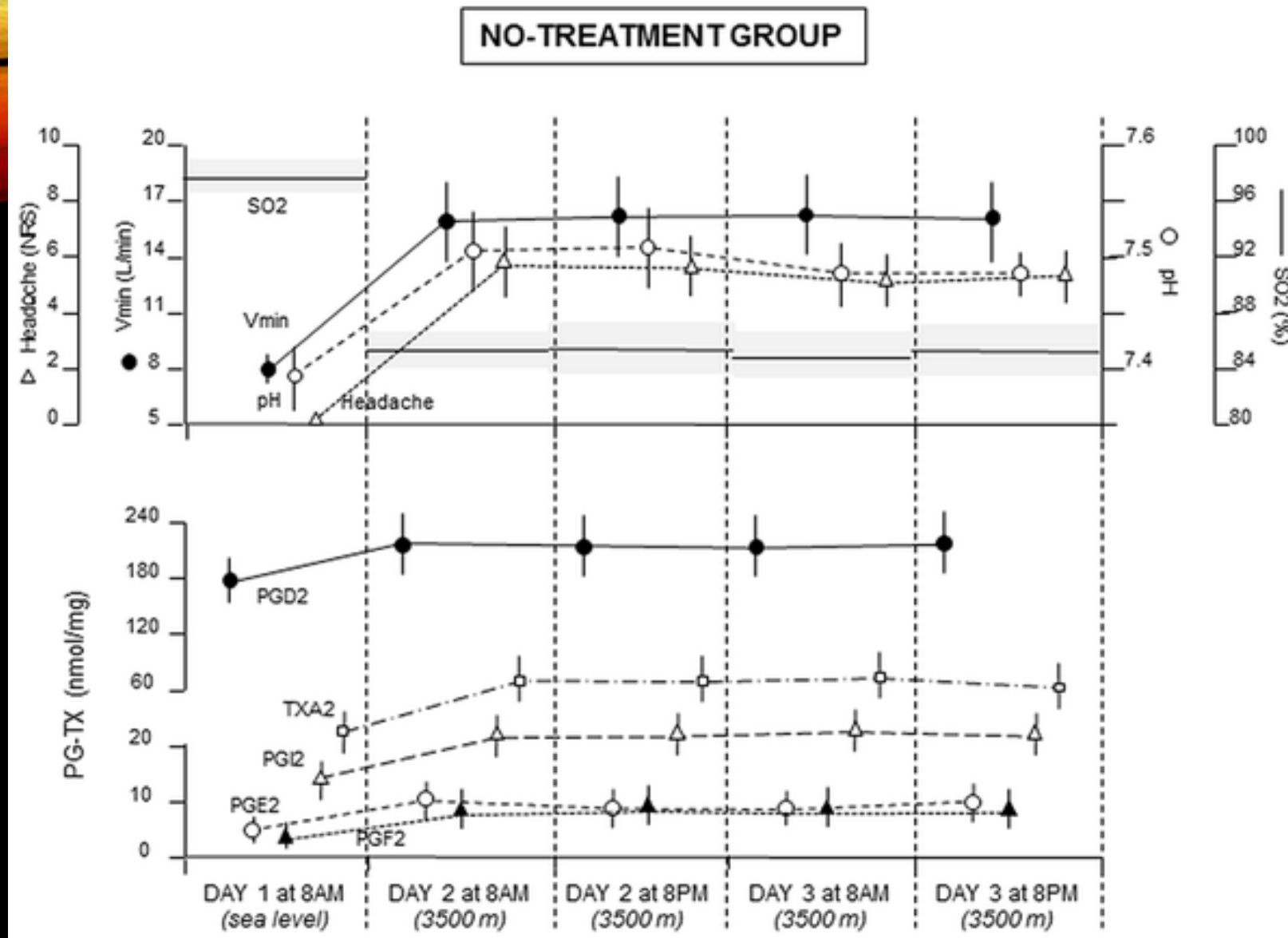
**Fig 1. Rationale of the study.**



Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967

<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

**Fig 2. Experimental design.**

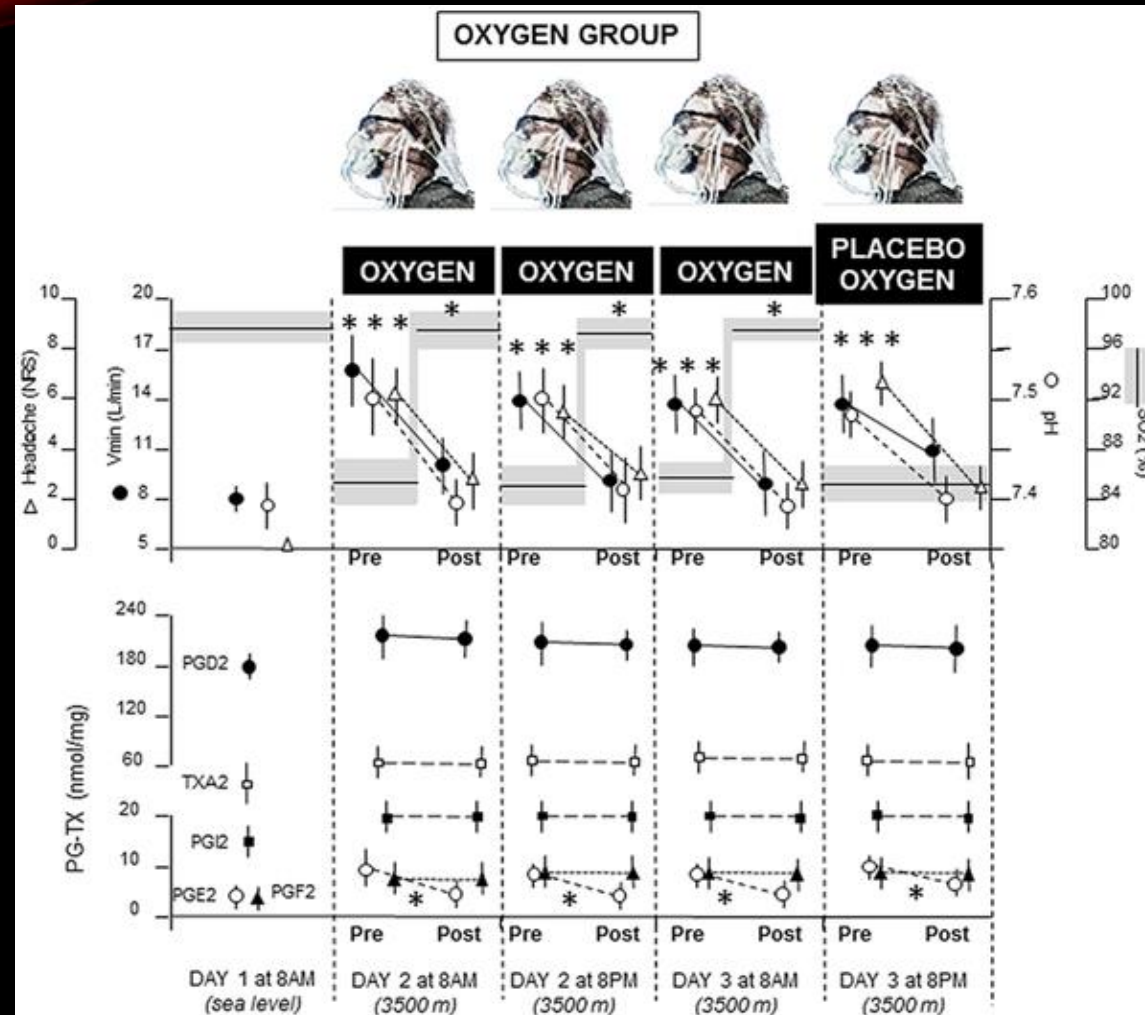


Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

**Fig 3. No-treatment group.**

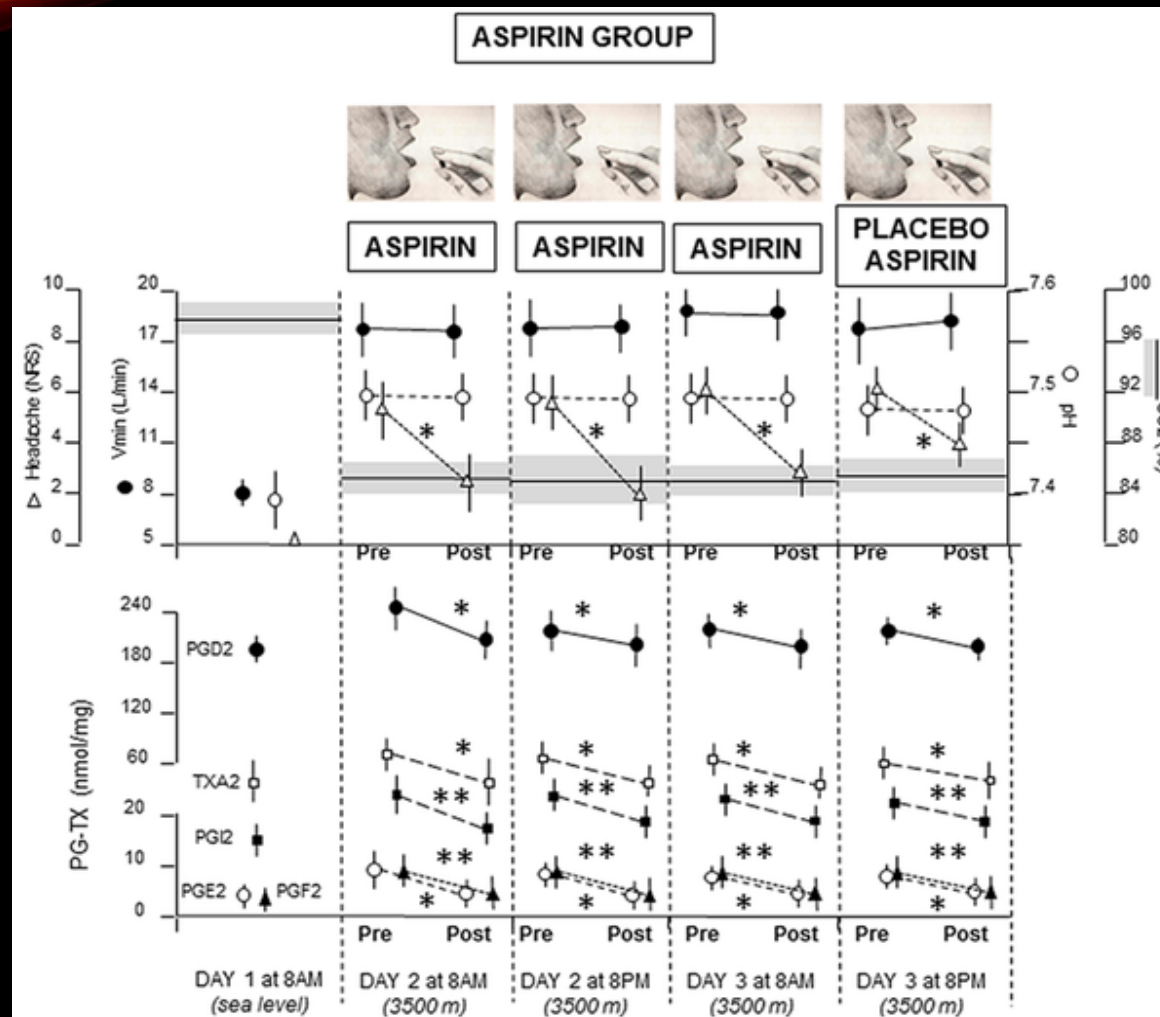


Fig 4. Means (+SD) for all the measurements in the oxygen group.



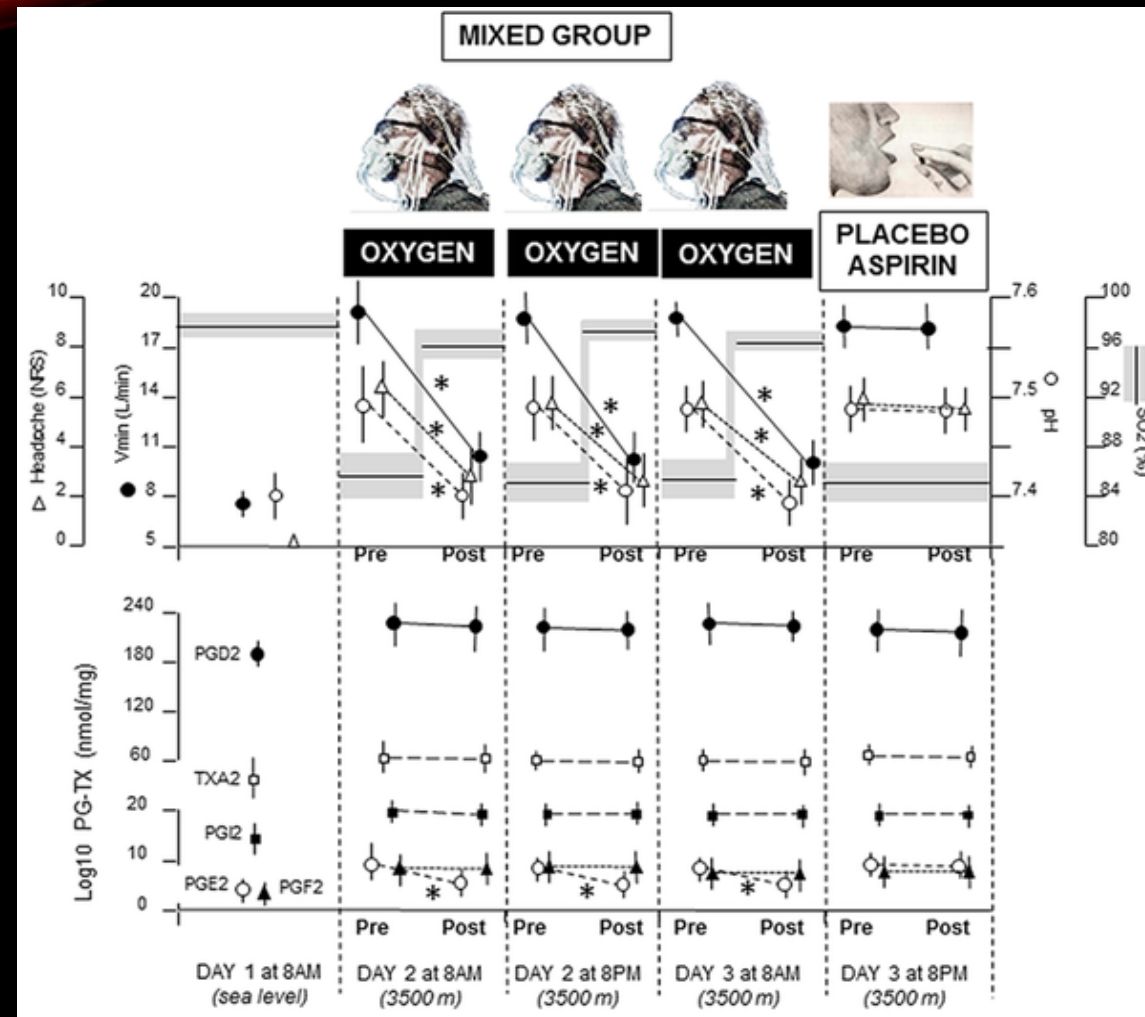
Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

Fig 5. Means ( $\pm$ SD) for all the measurements in the aspirin group.



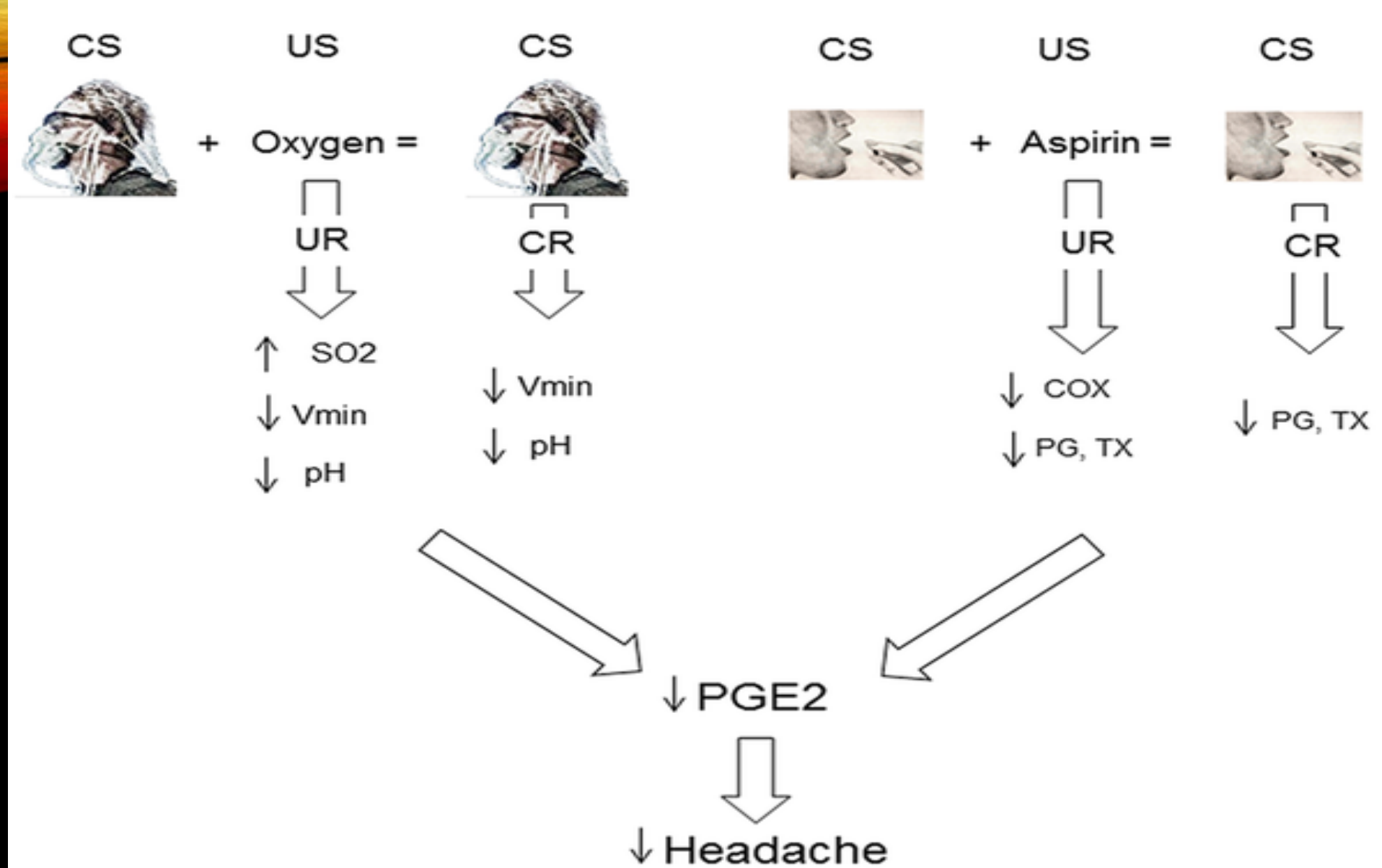
Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

Fig 6. Means (+SD) for all the measurements in the mixed group.



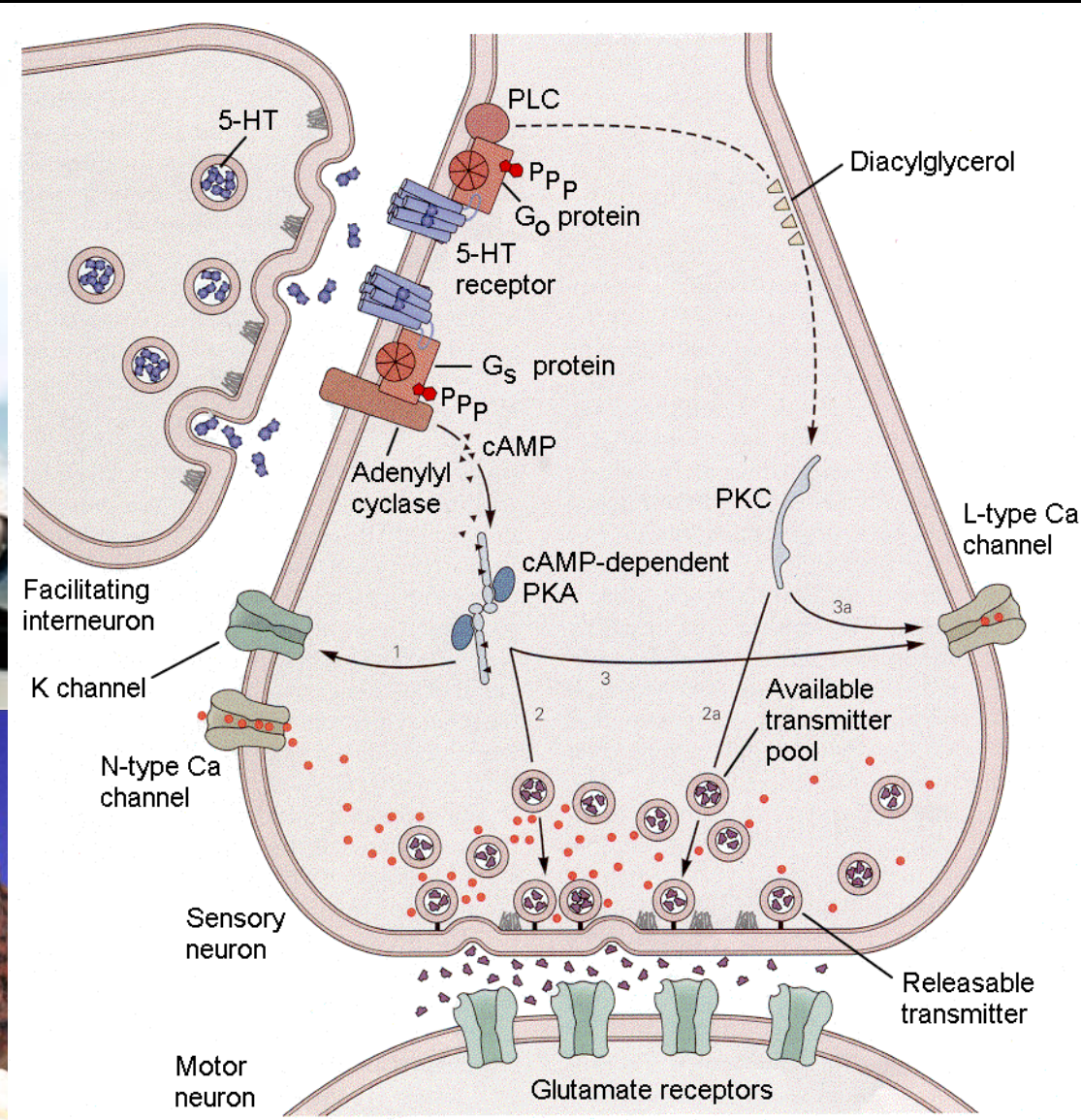
Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>





Benedetti F, Dogue S (2015) Different Placebos, Different Mechanisms, Different Outcomes: Lessons for Clinical Trials. PLoS ONE 10(11): e0140967. doi:10.1371/journal.pone.0140967  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0140967>

**Fig 8. Model that explains the findings of the present study.**



Eric Kandel – Aplysia Californica

## IN A NUTSHELL

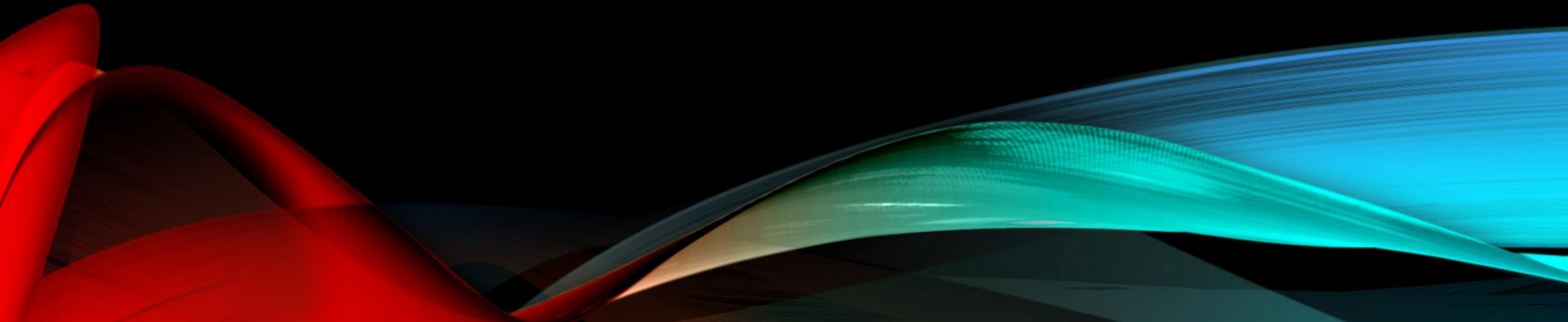
- Ritual is the clue, mixing placebos doesn't work (goes back to the origin of placebo)
- Physiotherapy sessions or Hyperbaric Oxygen sessions are a «ritual» by essence.
- It appears that totally exclude placebo effect is not feasible.



MISTAKE.....



IF VASCULARISATION IS STILL PRESENT AND  
EFFECTIVE  
LOWER DOSES ARE OF INTEREST





# NORMOBARIC OXYGEN & LYMPH DRAINAGE

Original Research

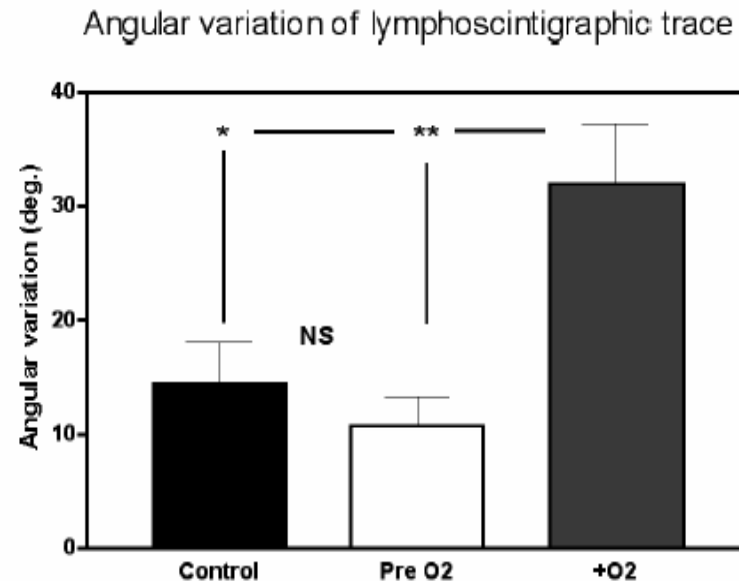


## Normobaric oxygen can enhance protein captation by the lymphatic system in healthy humans.

C. BALESTRA<sup>1,2,3\*</sup>, P. GERMONPRÉ<sup>1,4\*</sup>, T. SNOECK<sup>1,2,3</sup>, M. EZQUER<sup>1,2,3</sup>, O. LEDUC<sup>2</sup>, A. LEDUC<sup>2</sup>, F. WILLEPUT<sup>2</sup>, A. MARRONI<sup>1</sup>, R. CALI CORLEO<sup>1</sup>, R. VANN<sup>1</sup>

<sup>1</sup>DAN Europe, IDAN Research Division. <sup>2</sup>Université Libre de Bruxelles Institut Supérieur d'Education Physique et Kinésithérapie, Brussels, Belgium. <sup>3</sup>Haute Ecole Paul Henri Spaak, General Human Biology Dept., Brussels, Belgium. <sup>4</sup>Center for Hyperbaric Oxygen Therapy, Military Hospital Queen Astrid, Brussels, Belgium.

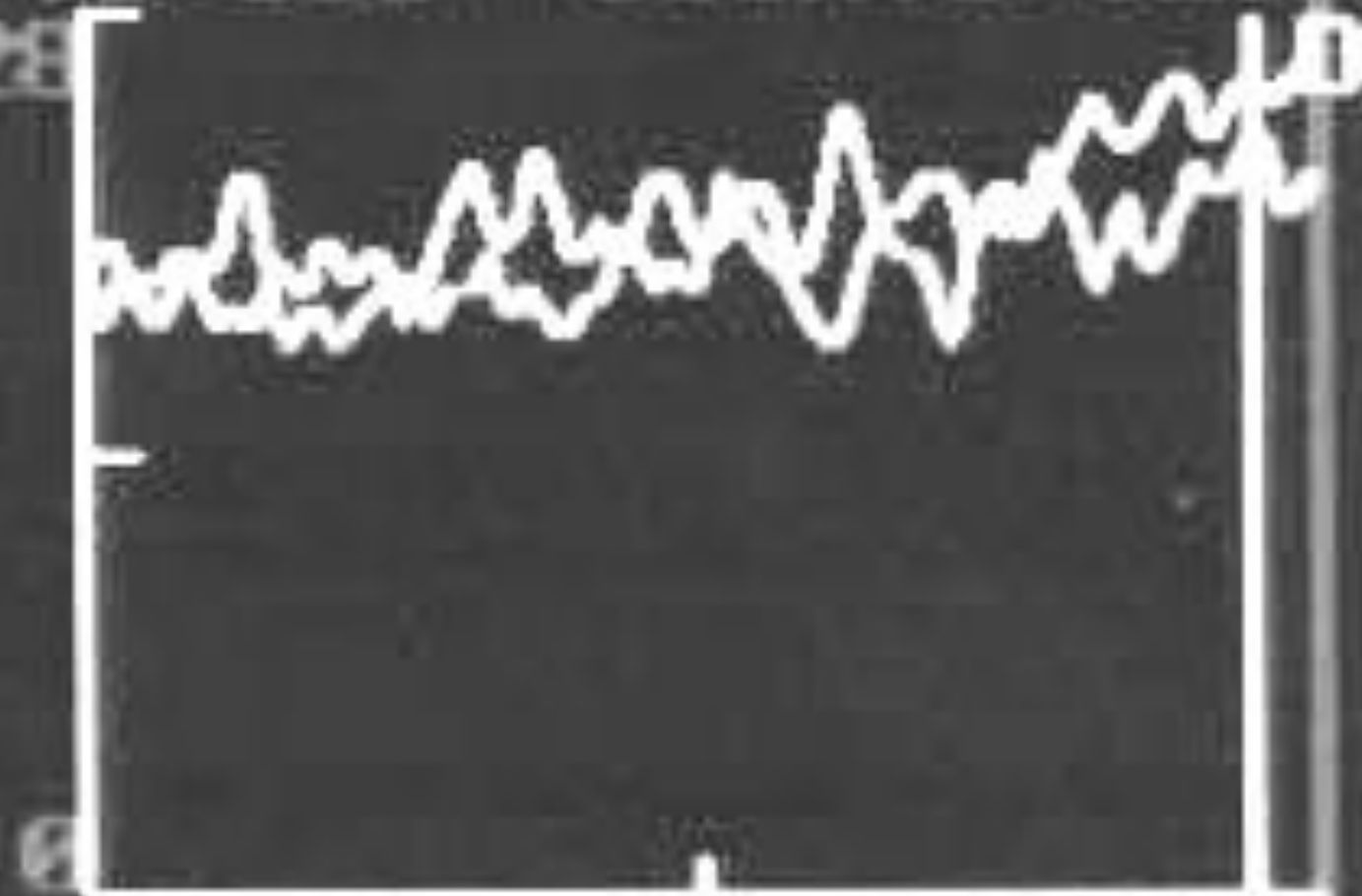
\* These authors contributed equally to the work.



**Figure 2.** Statistical comparison between the control absorption speed (angular variation) in the axillary region; the pre-oxygen situation and after 30 minutes of normobaric oxygen breathing. \*= $p>0,05$ ; \*\*= $p>0,01$

\*1/100 COUNTS/SEC

228



0

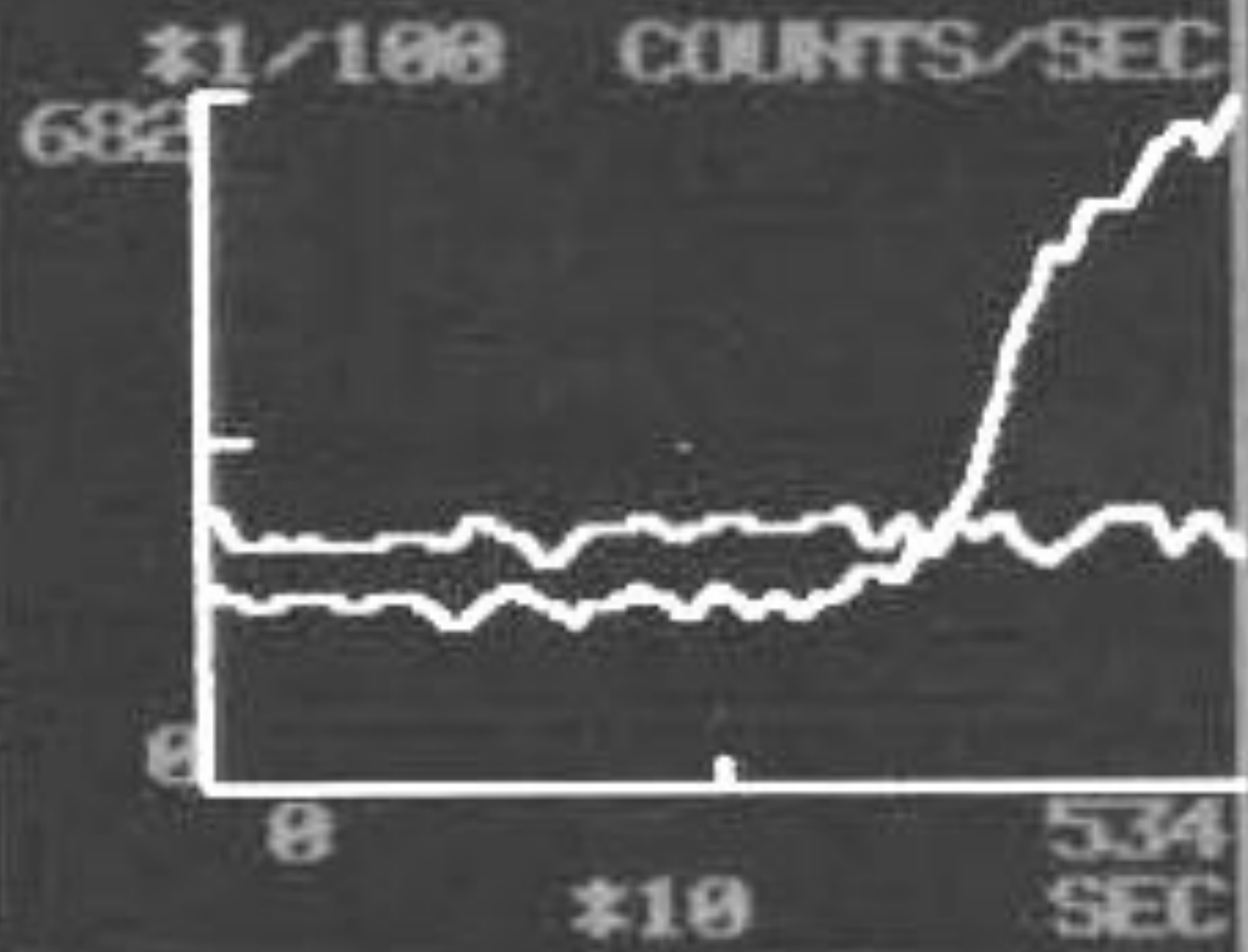
\*10

534

SEC

06

1



58

1



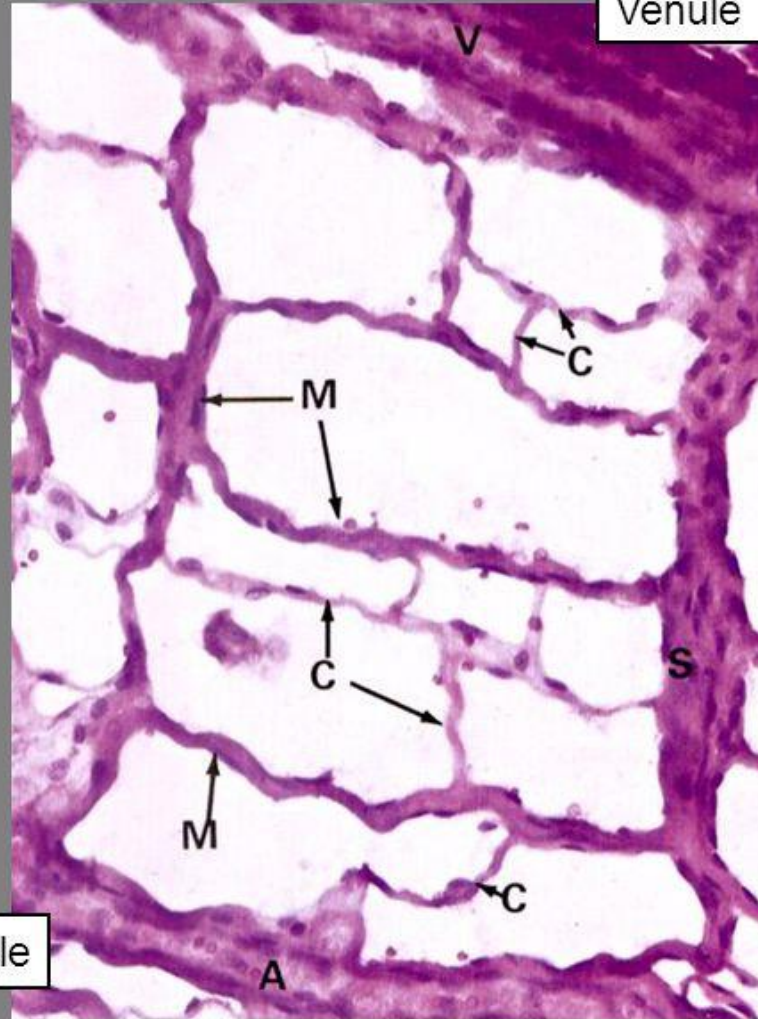
Microvascular unit

Venule

Metarteriole

Capillaries

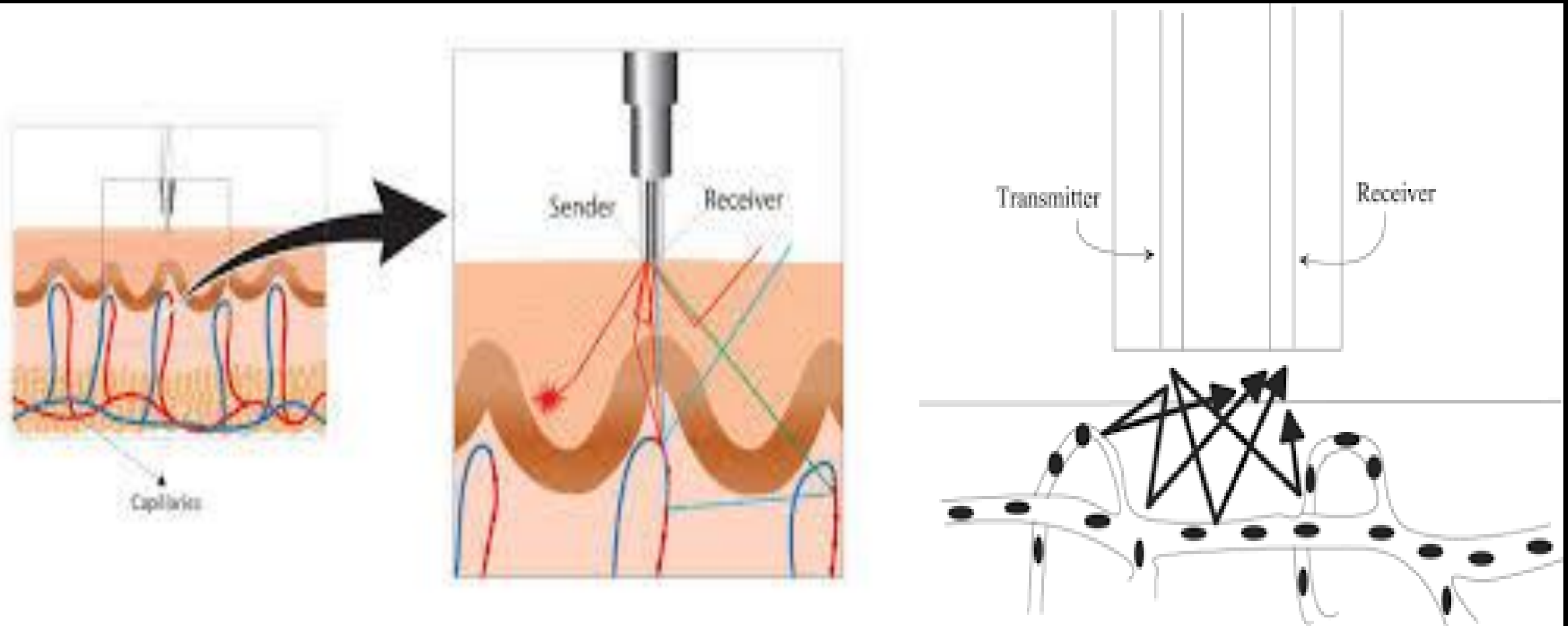
Arteriole

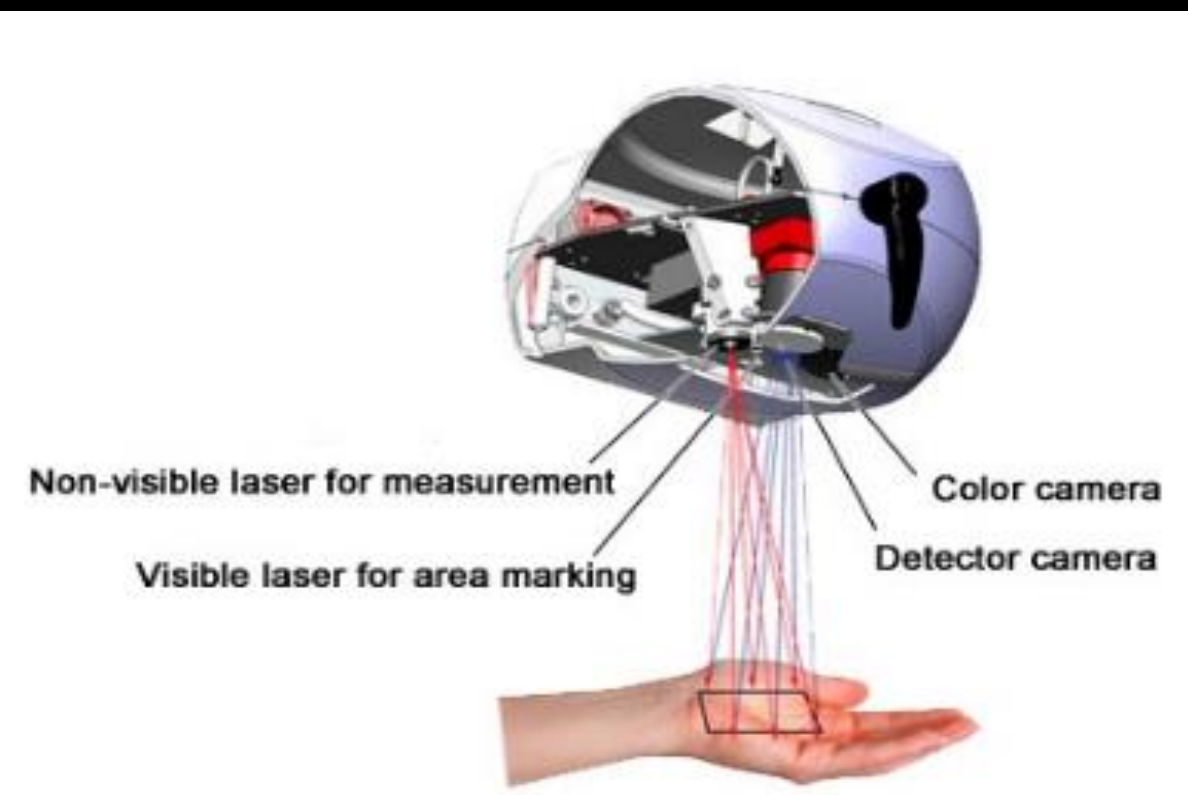


Arteriovenous  
shunt (S)

Pre-capillary  
sphincters exist at the  
origin of each  
capillary

# LASERFLOW SPECKLE DOPPLER





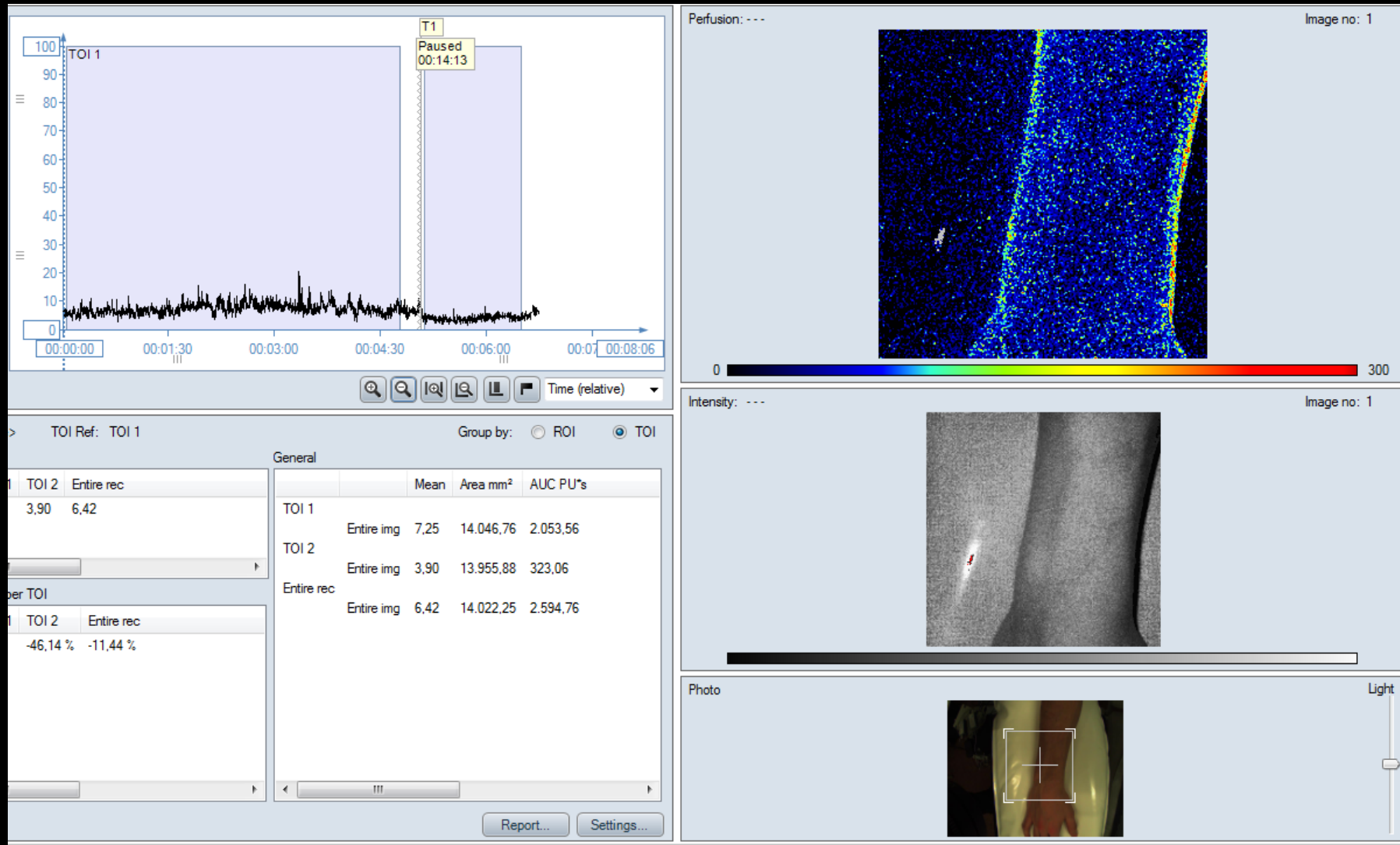




# UPPER LIMB EDEMA TREATMENT

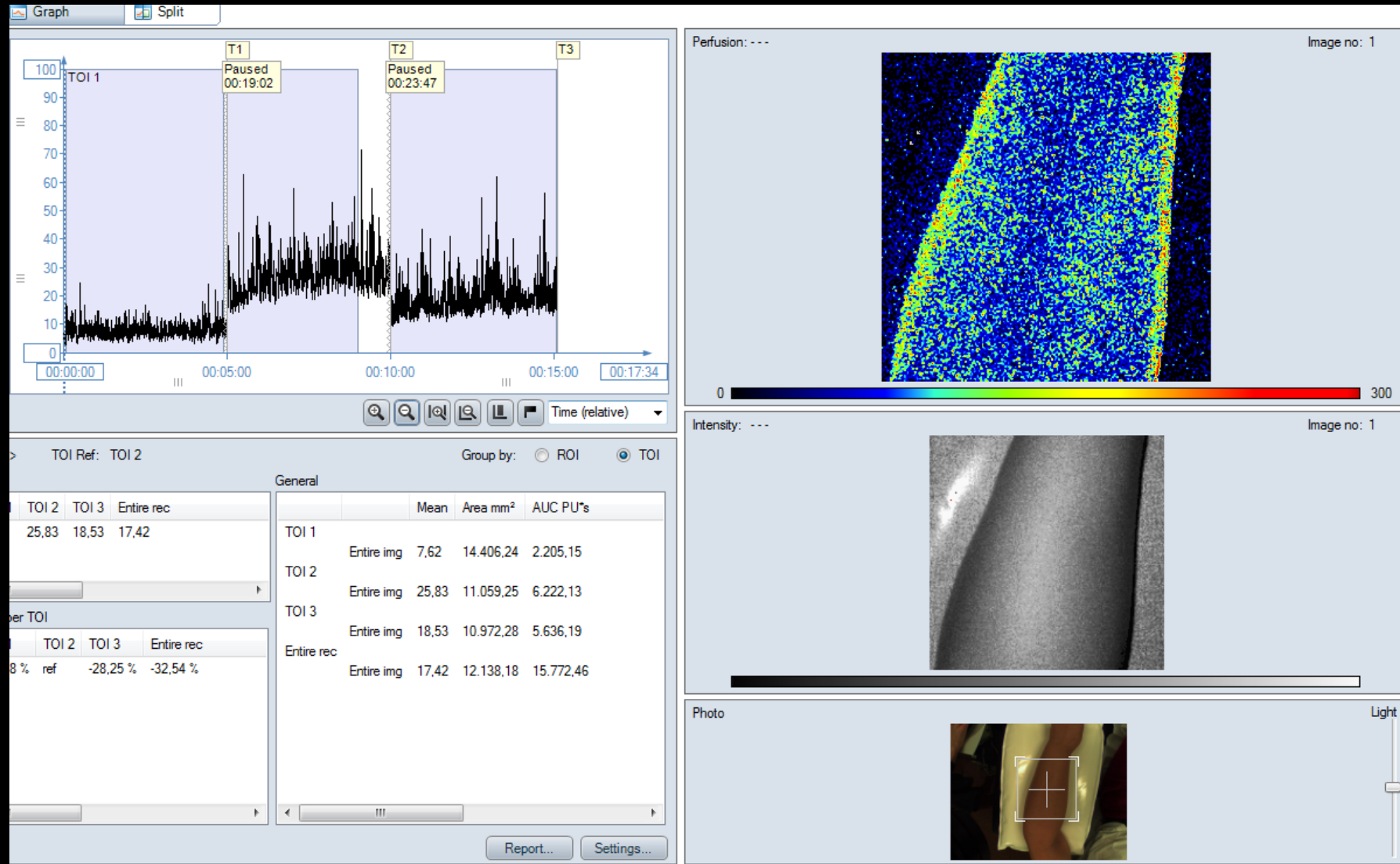


# CUTANEOUS PERFUSION NO EDEMA (MICROVASCULARISATION)

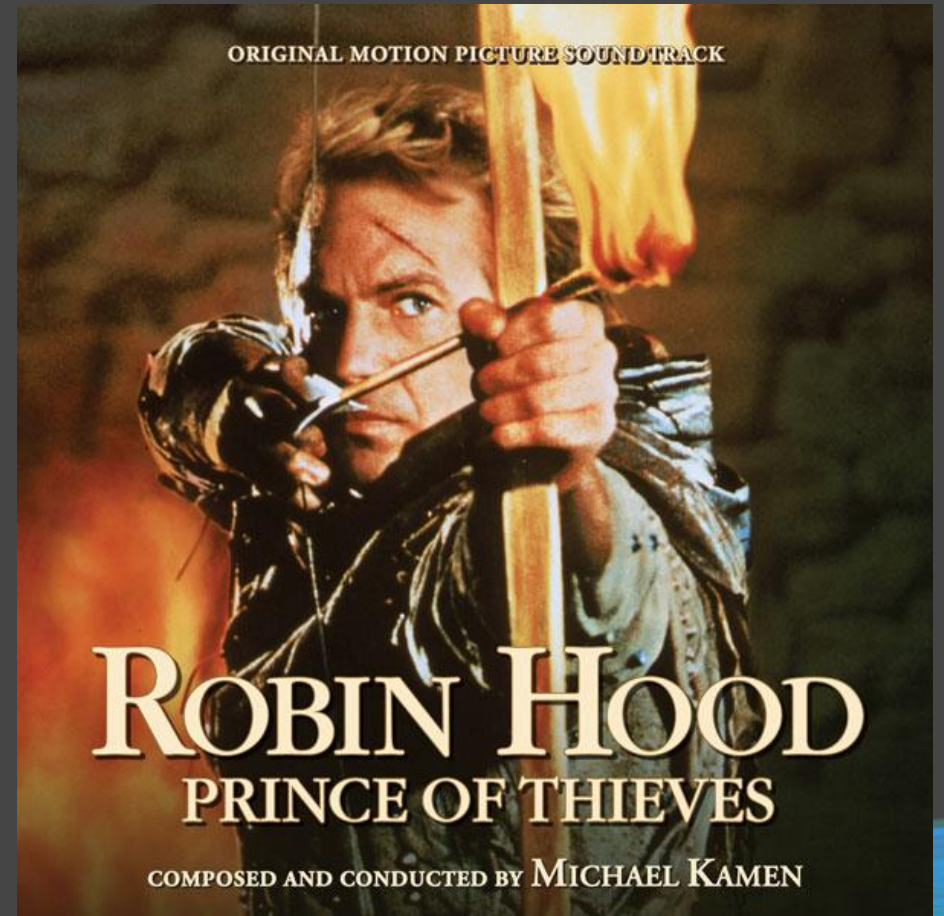
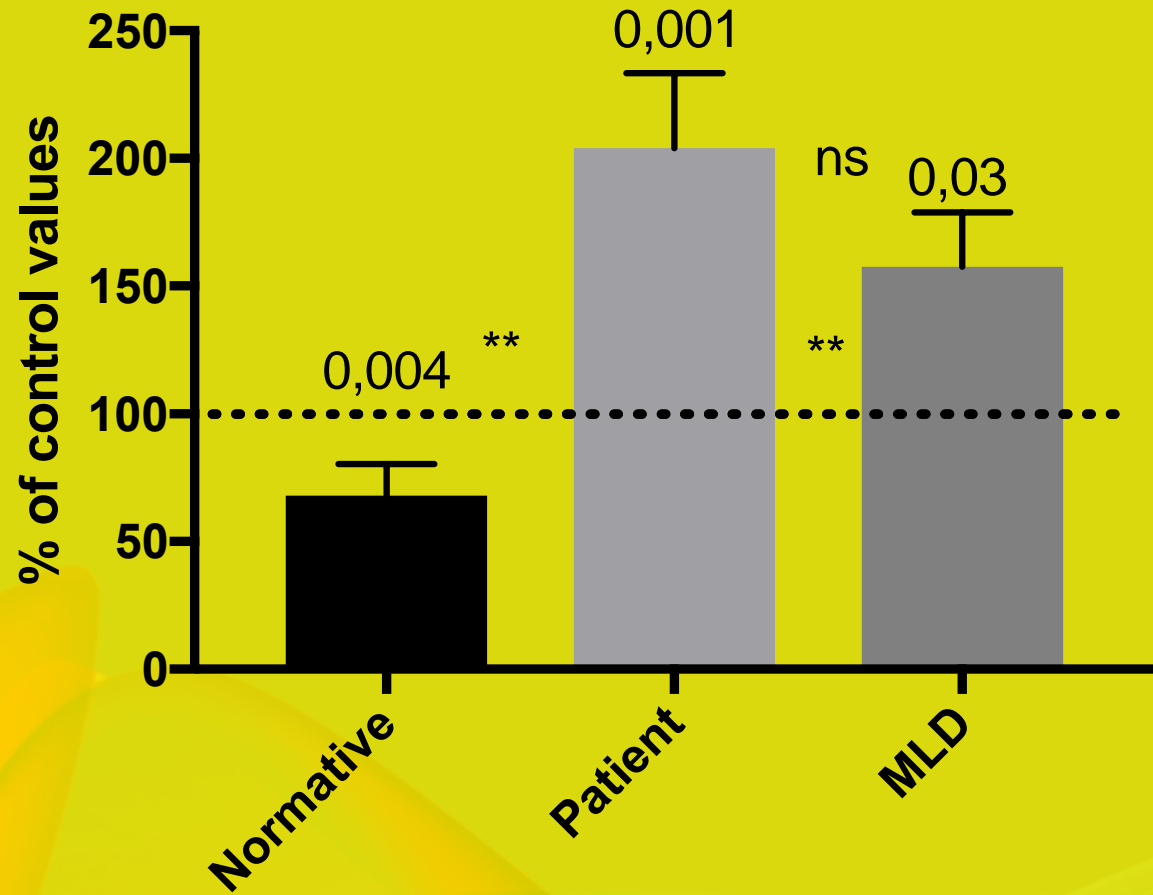


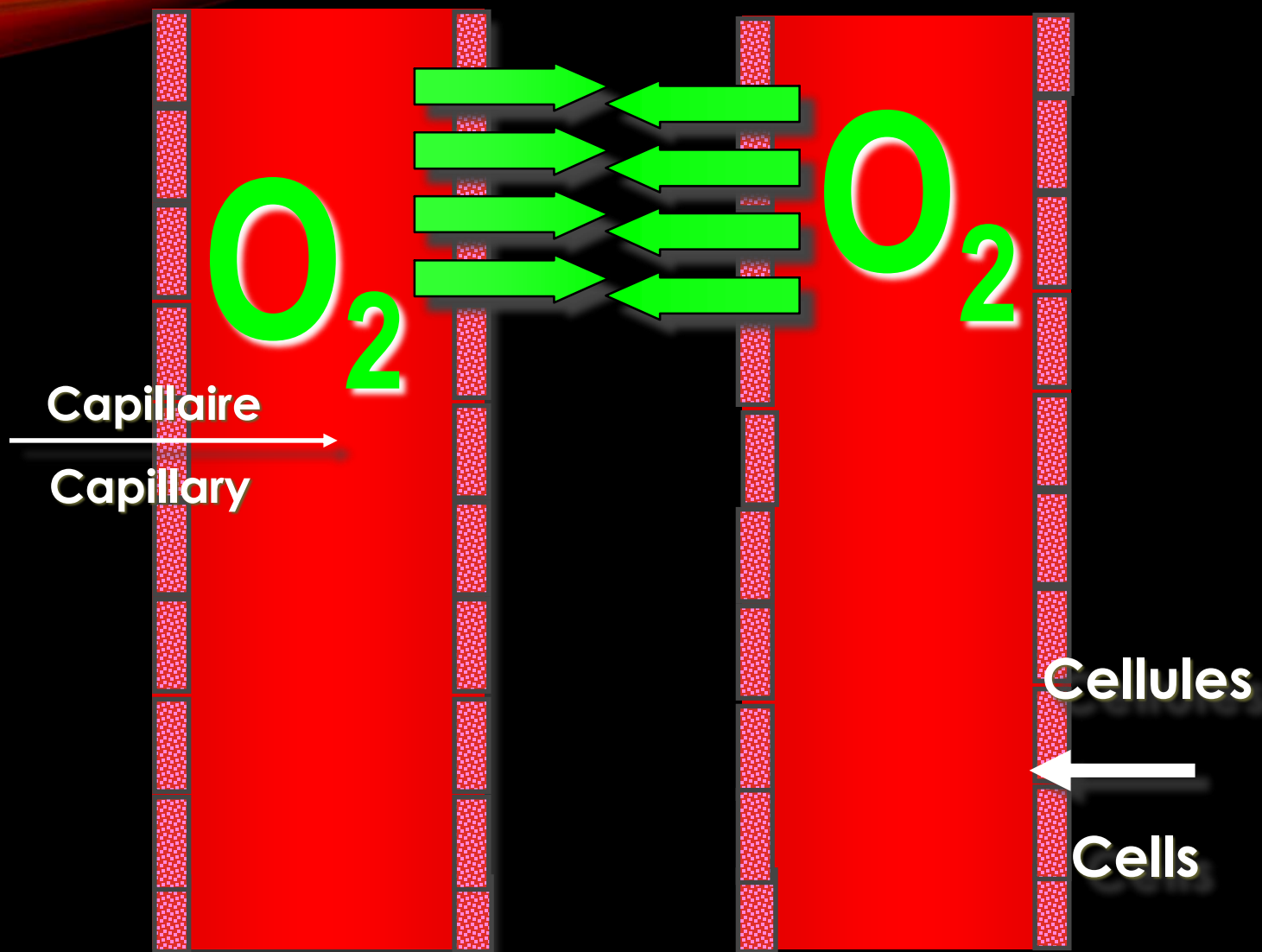


# PERFUSION LYMPHOEDEMA (AXYLLARY LYMPHECTOMY)

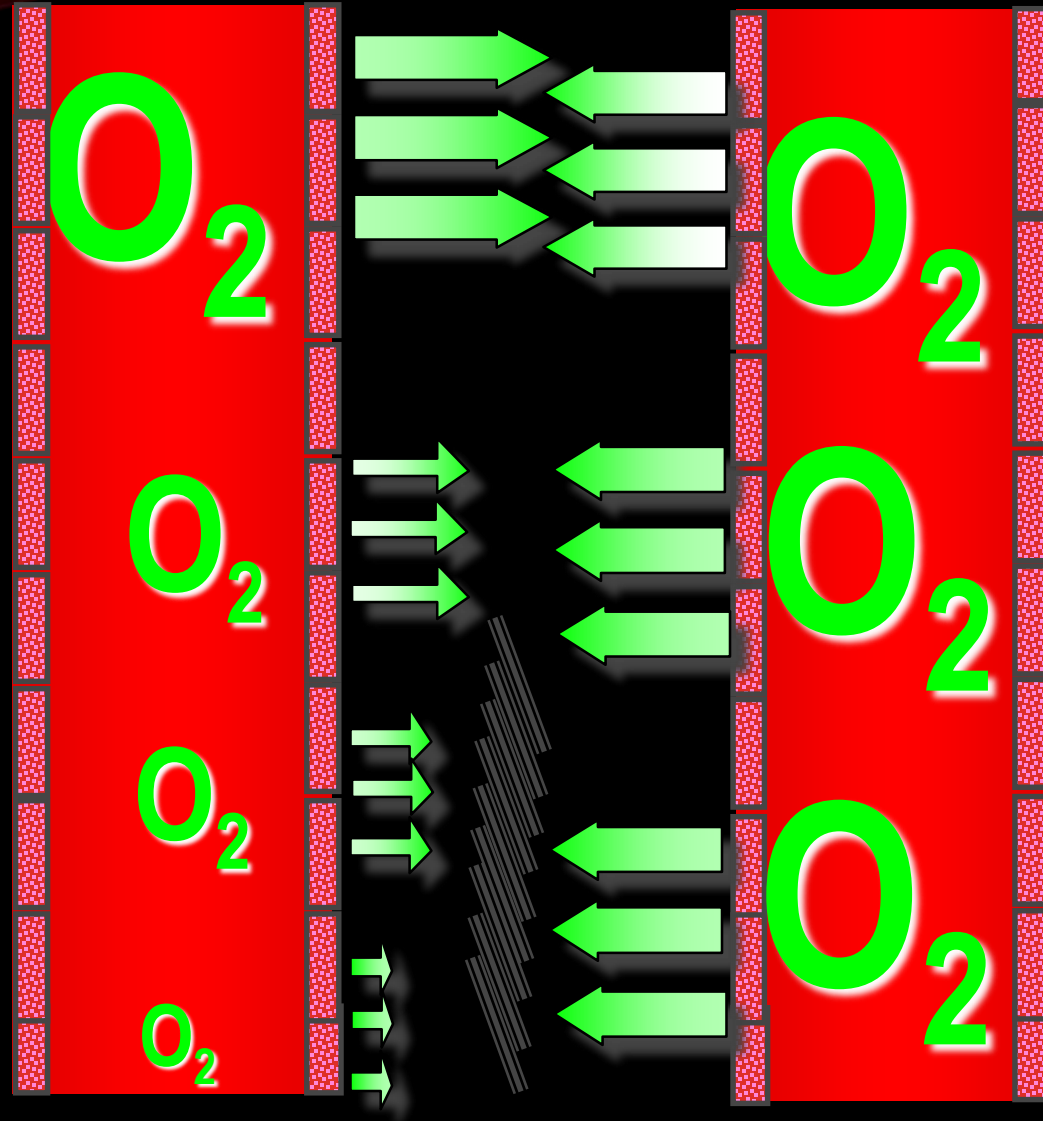


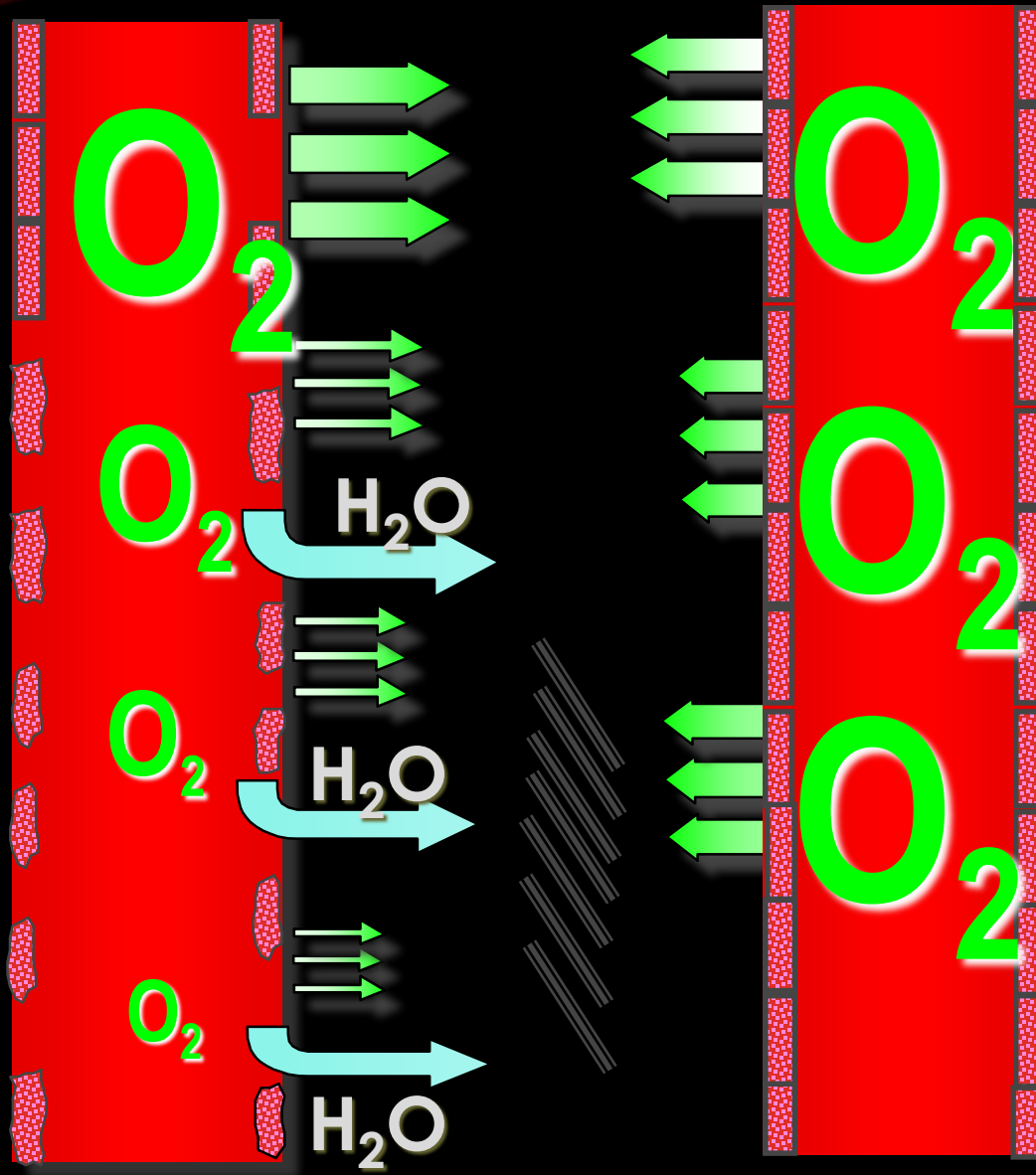
## LaserFlow Doppler Variation n=8 (20 min treatment)

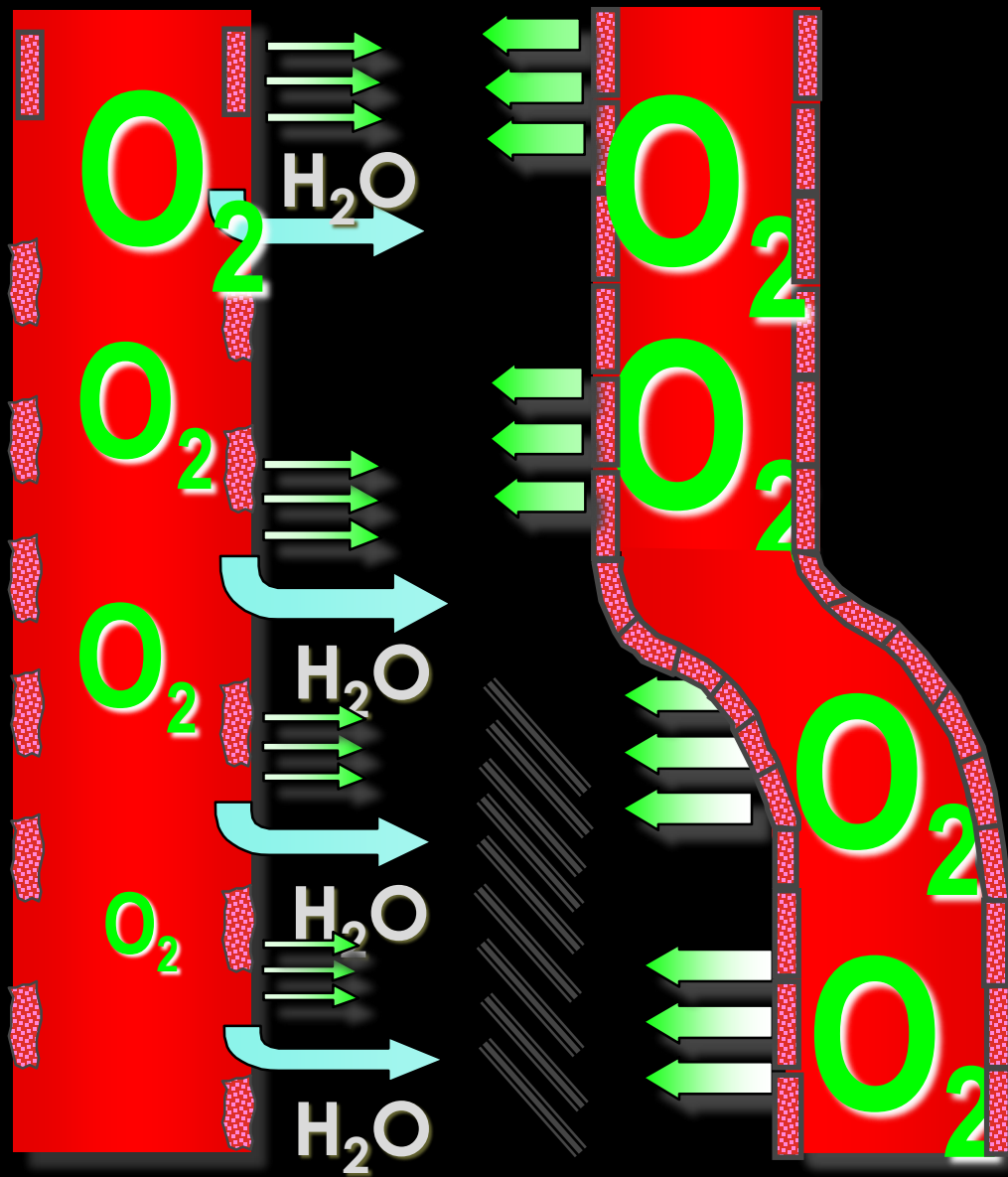




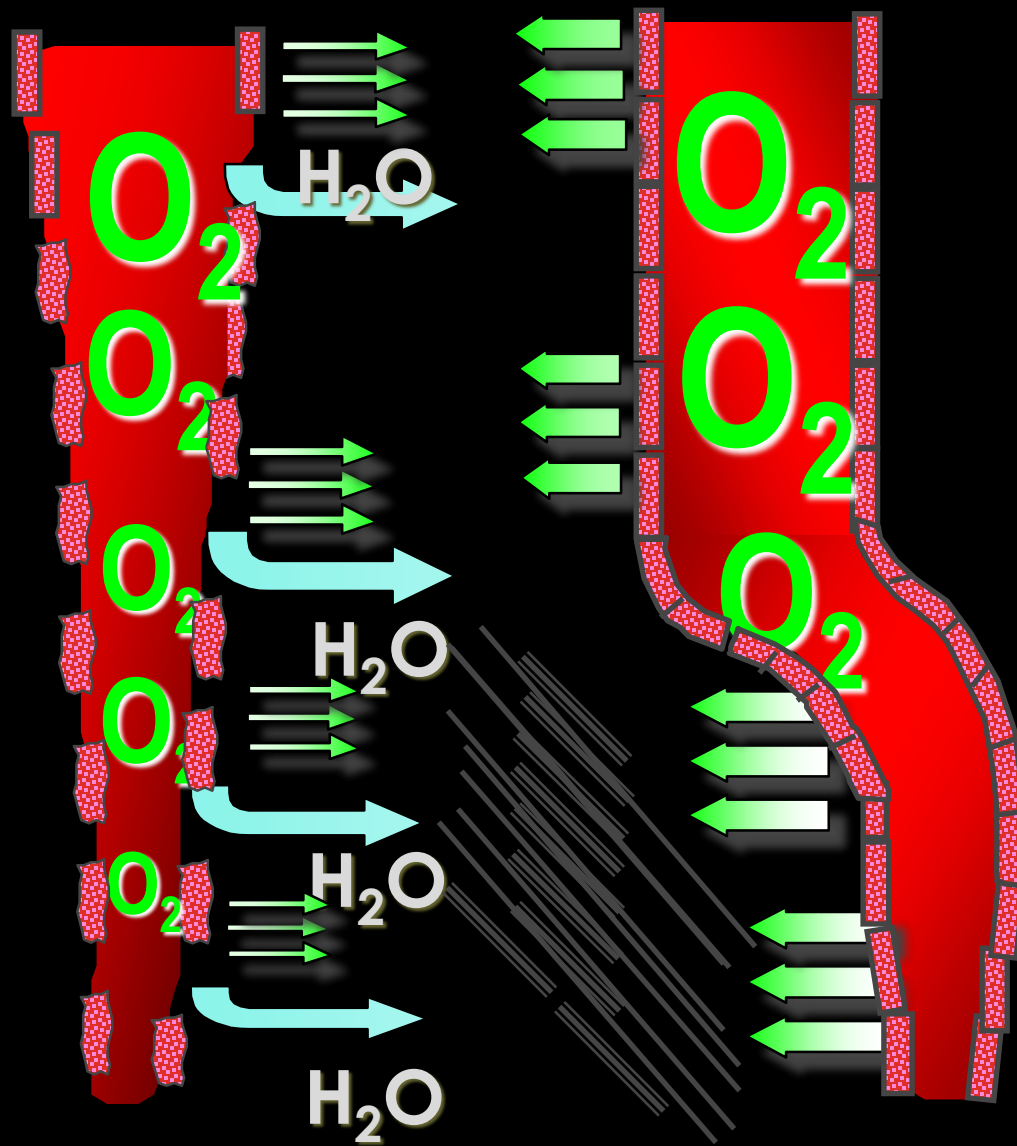




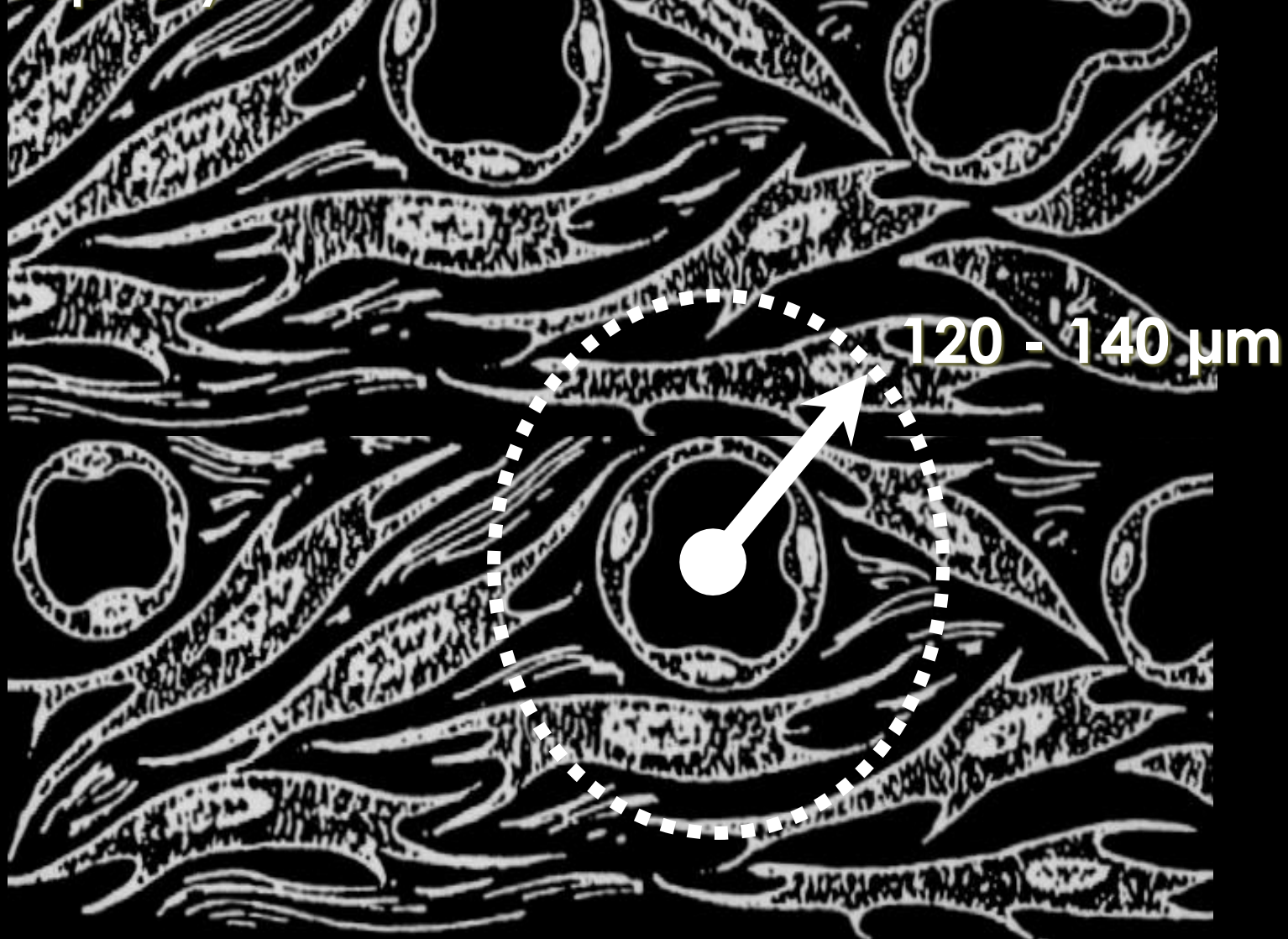








# Maximum O<sub>2</sub> Diffusion Distance From a Functional Capillary



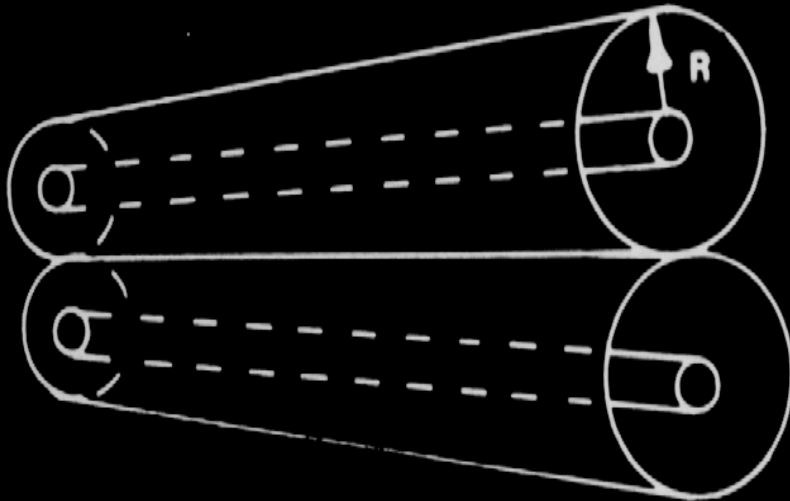
Silver 1976

# Kroch's Mathematical Model (1919)

ARTERIAL END

$PO_2 = 100$  mmHg

$R = 64$  MICROMETERS



VENOUS END

$PO_2 = 34$  mmHg

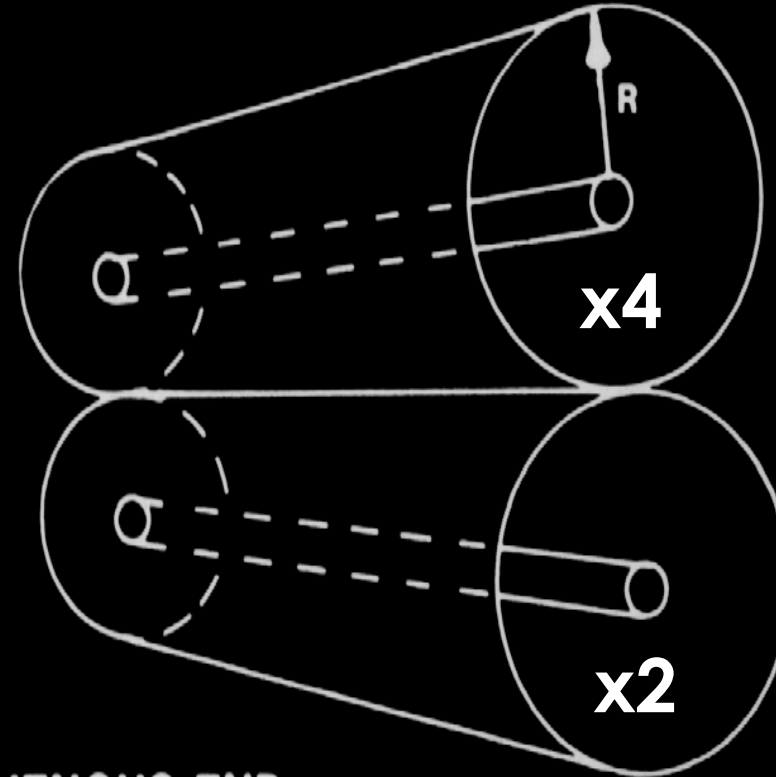
$R = 36$  MICROMETERS

1 ATA - AIR

ARTERIAL END

$PO_2 = 2000$  mmHg

$R = 247$  MICROMETERS



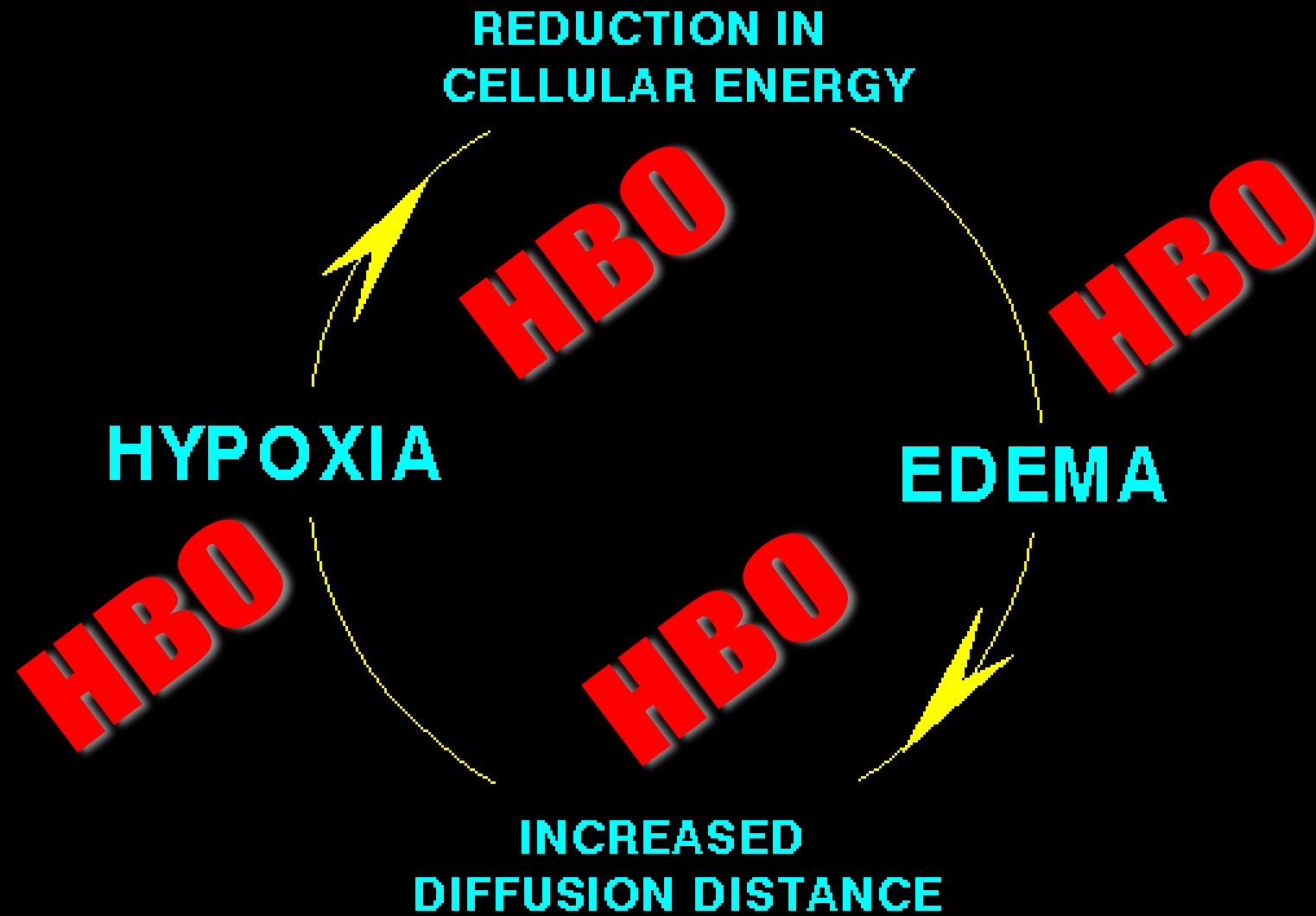
VENOUS END

$PO_2 = 100$  mmHg

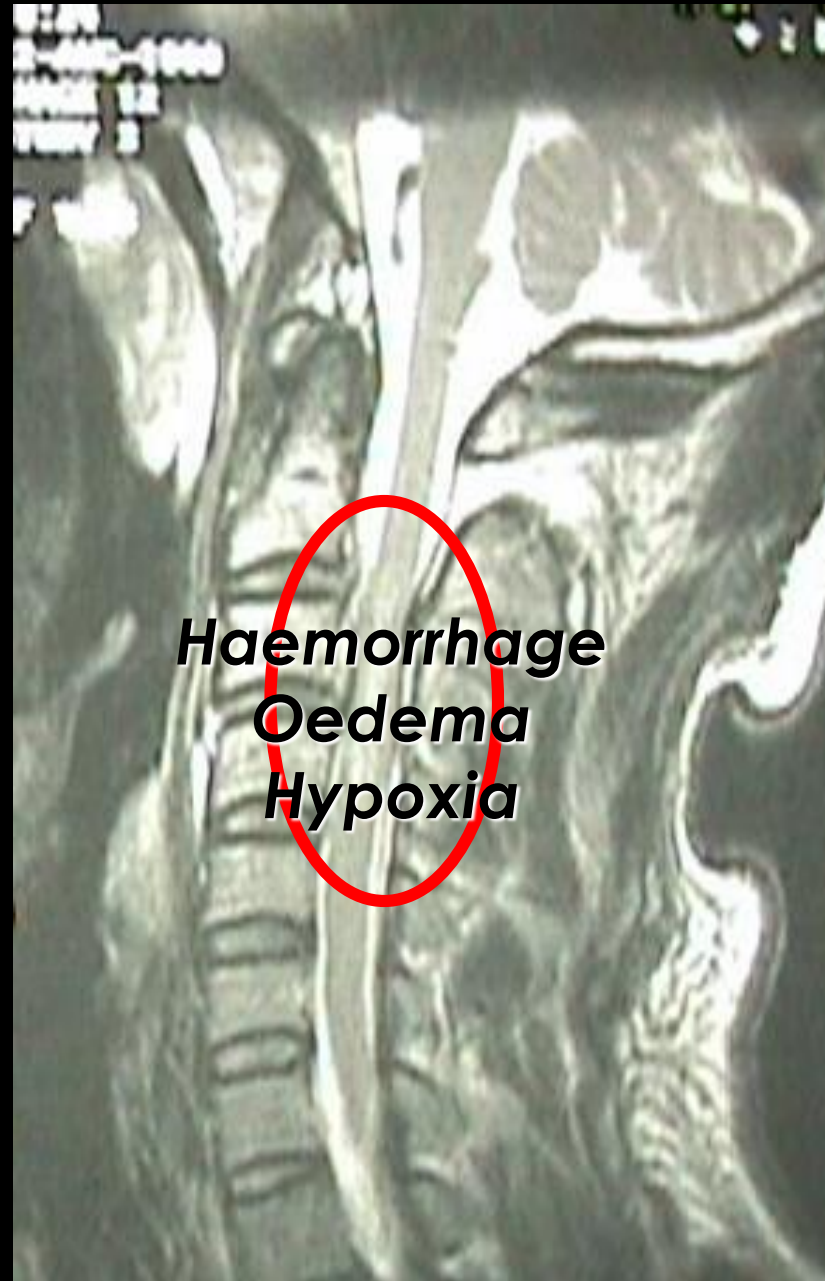
$R = 64$  MICROMETERS

3 ATA - OXYGEN





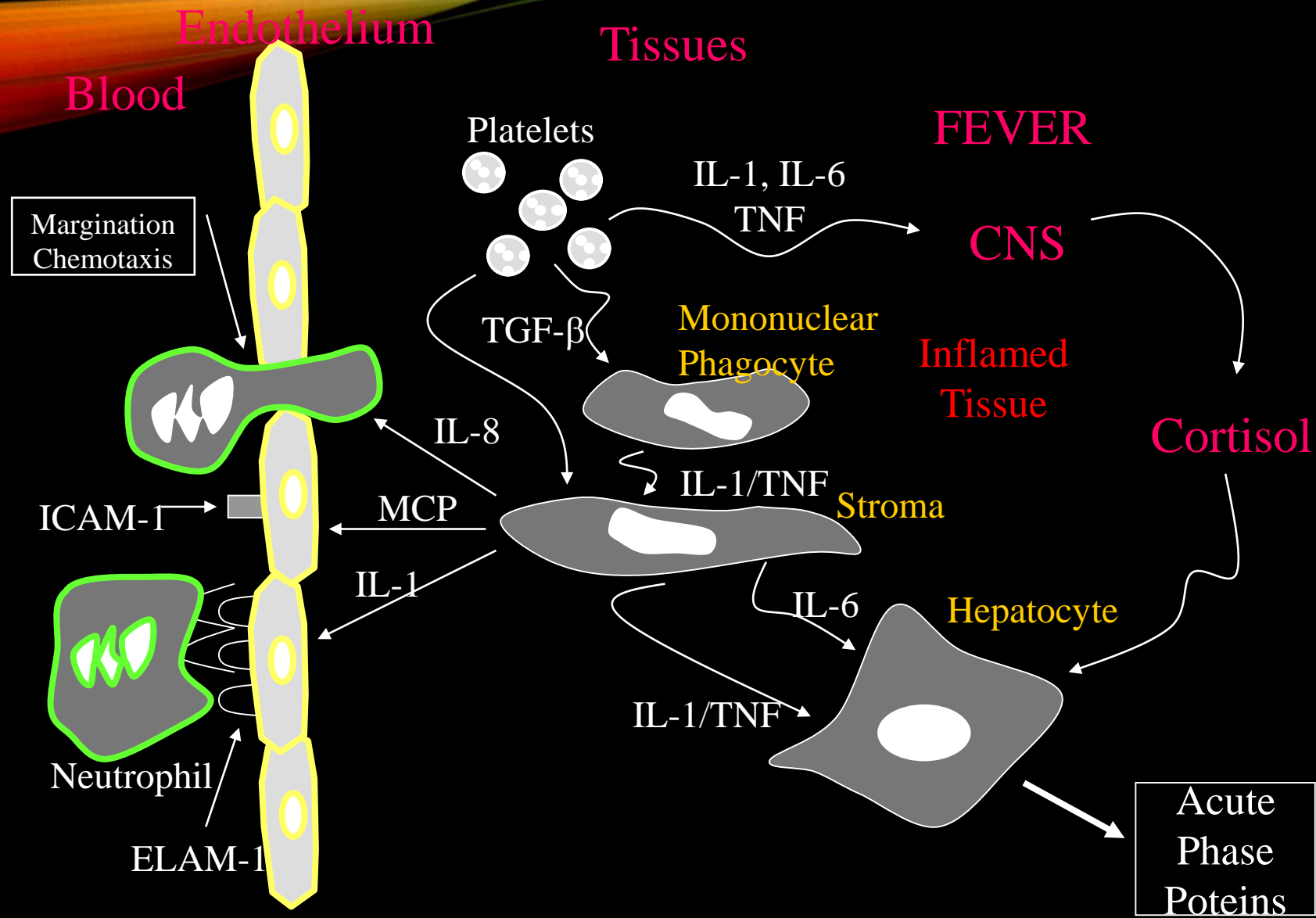
# SPINAL CORD INJURY

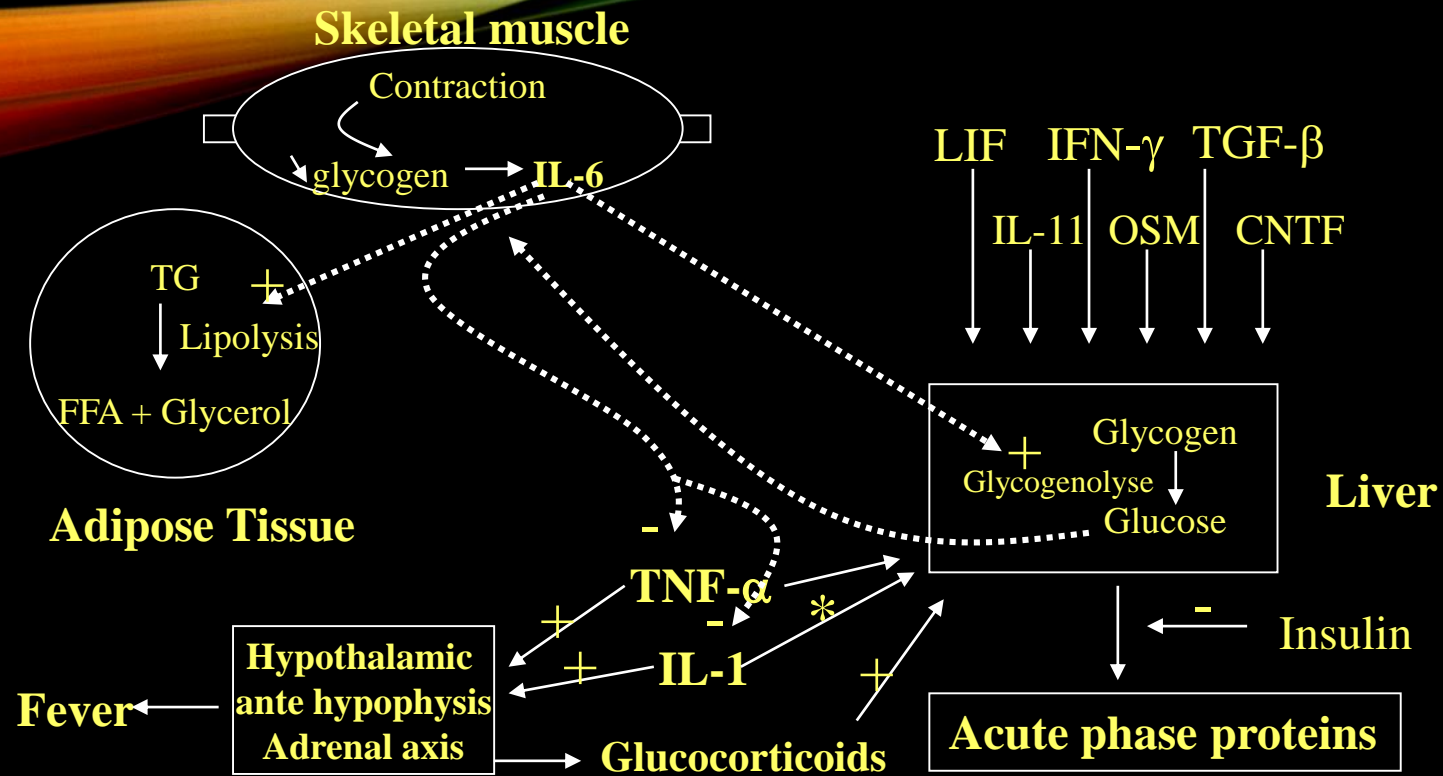




1. 5. 1999





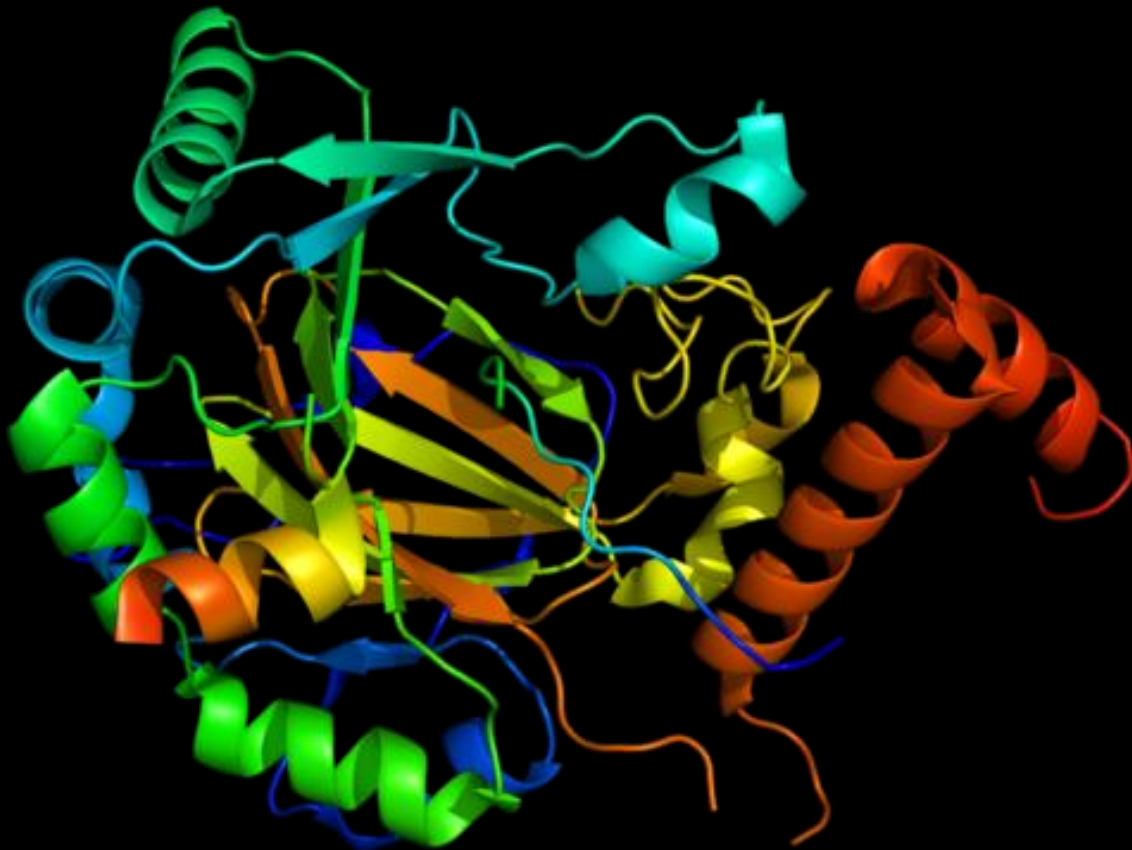


Complement Proteins	Coagulation Proteins	Proteinase Inhibitors	Metal binding Proteins	Acute Phase Reactants	Negative AP Reactants	Other Proteins
C2,C3,C4, C5,C9	Fibrinogen Von Willebrand	α1-antitrypsin α1-antichymotrypsin α2-antiplasmin Heparin cofactor II Plasm. Activator	Haptoglobin Hemopexin Ceruloplasmin Mn SOD	Serum Amyloid A C-reactive prot. Serum amyloid P component	Albumin Pre-albumin Transferrin ApoAI ApoAII α2-Hsglycoprot. Inter-α-trypsin inhib. Histidine rich glycoprot.	α1-ac. Glycoprot. Heme oxygenase Mannose-binding prot. Leukocyte prot.1 Lipoprotein Lipopolysaccharide binding prot.
Factor B C1 Inhibitor C4 binding prot. * Increasing activity		Inhib.1				

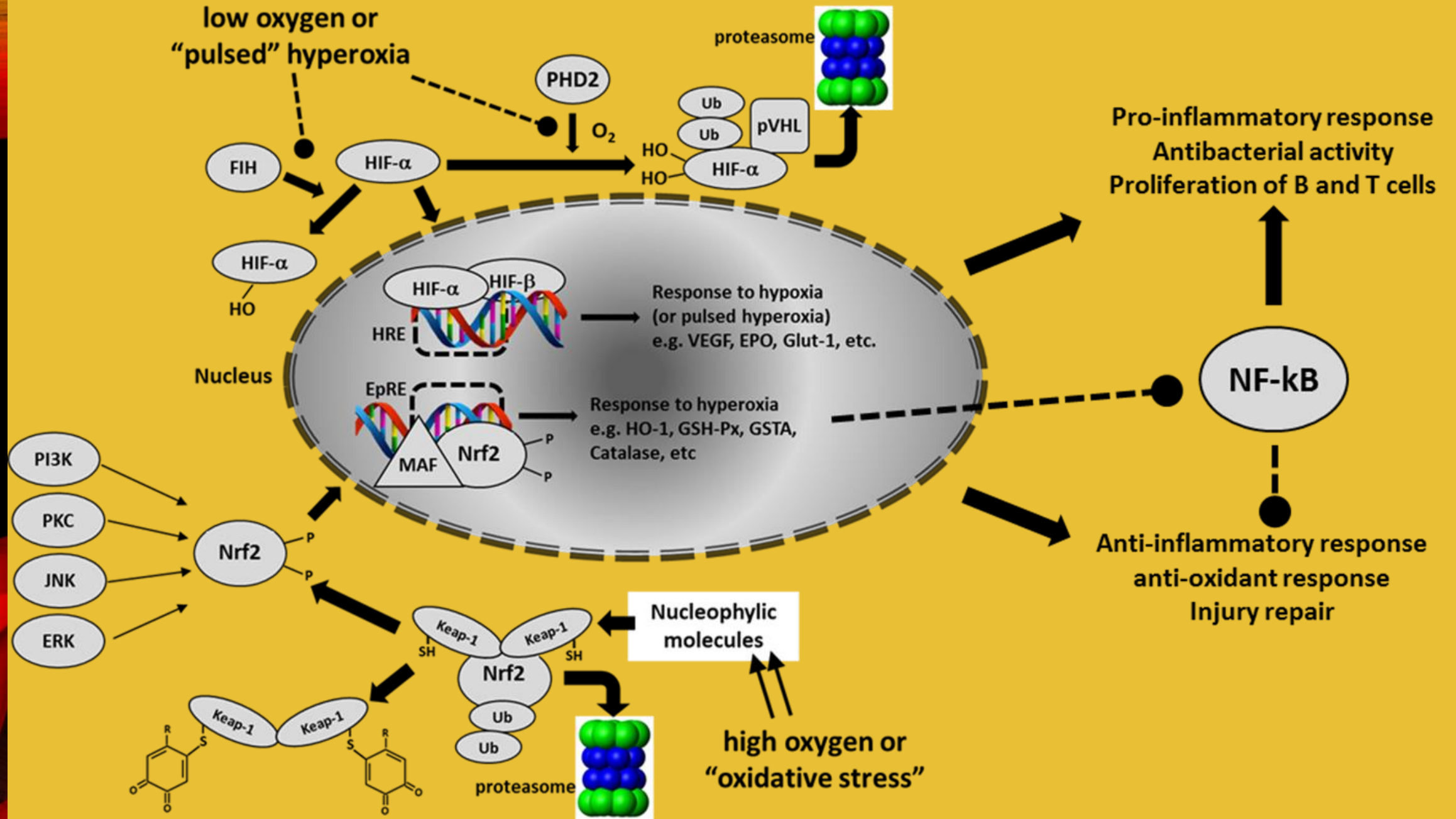
FOCUSING.....



1991 GREG SEMENZA  
HIF-1 ALPHA

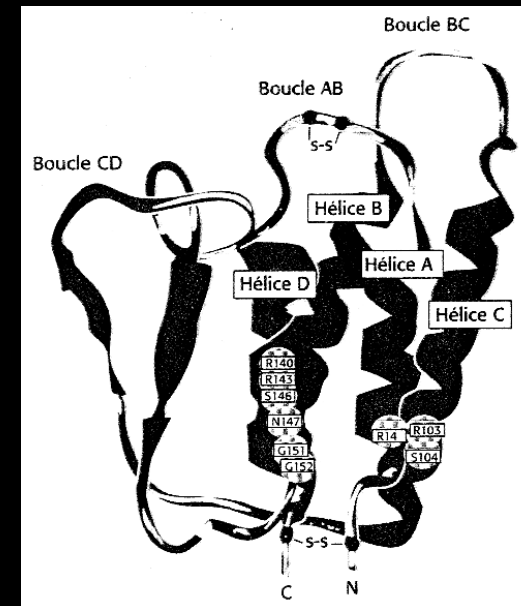
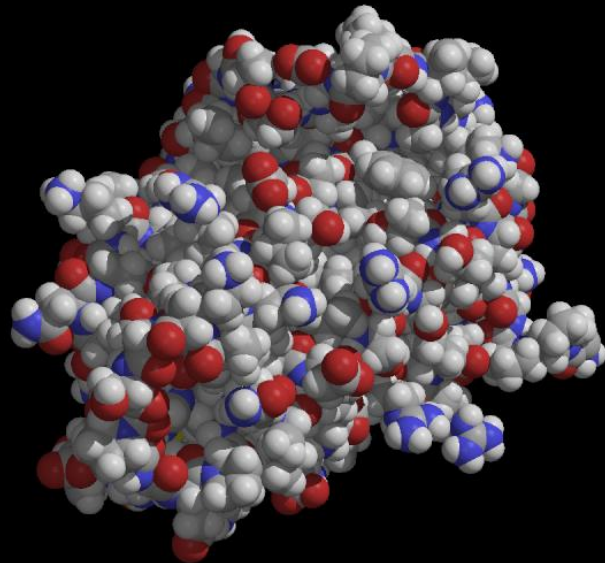






# INTRODUCTION

- Erythropoietin (EPO) regulates RBC homeostasis but also known as antiapoptotic neuroprotective agent
- Polypeptid (cytokine) hormone (Bazan, 1990)



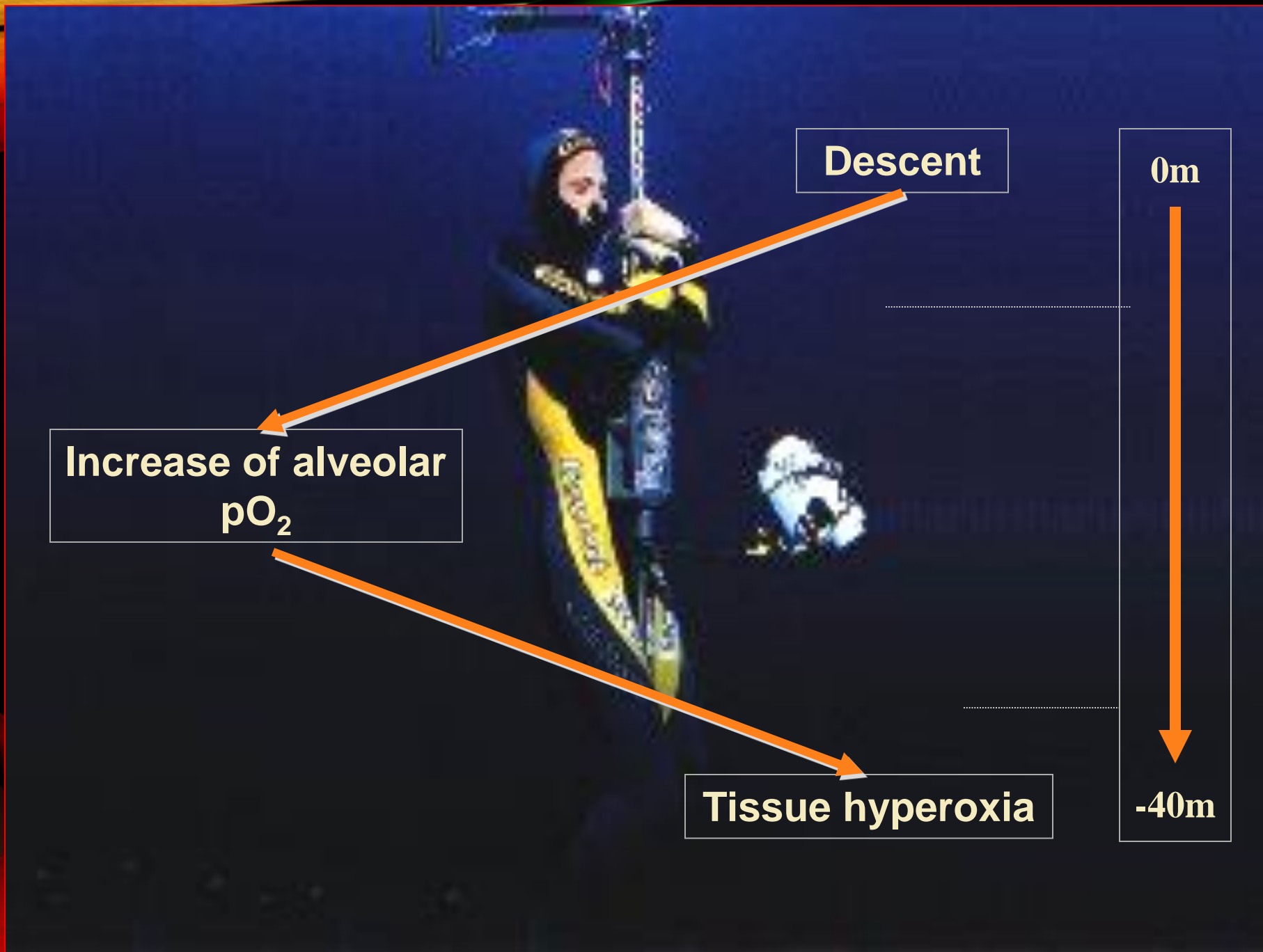


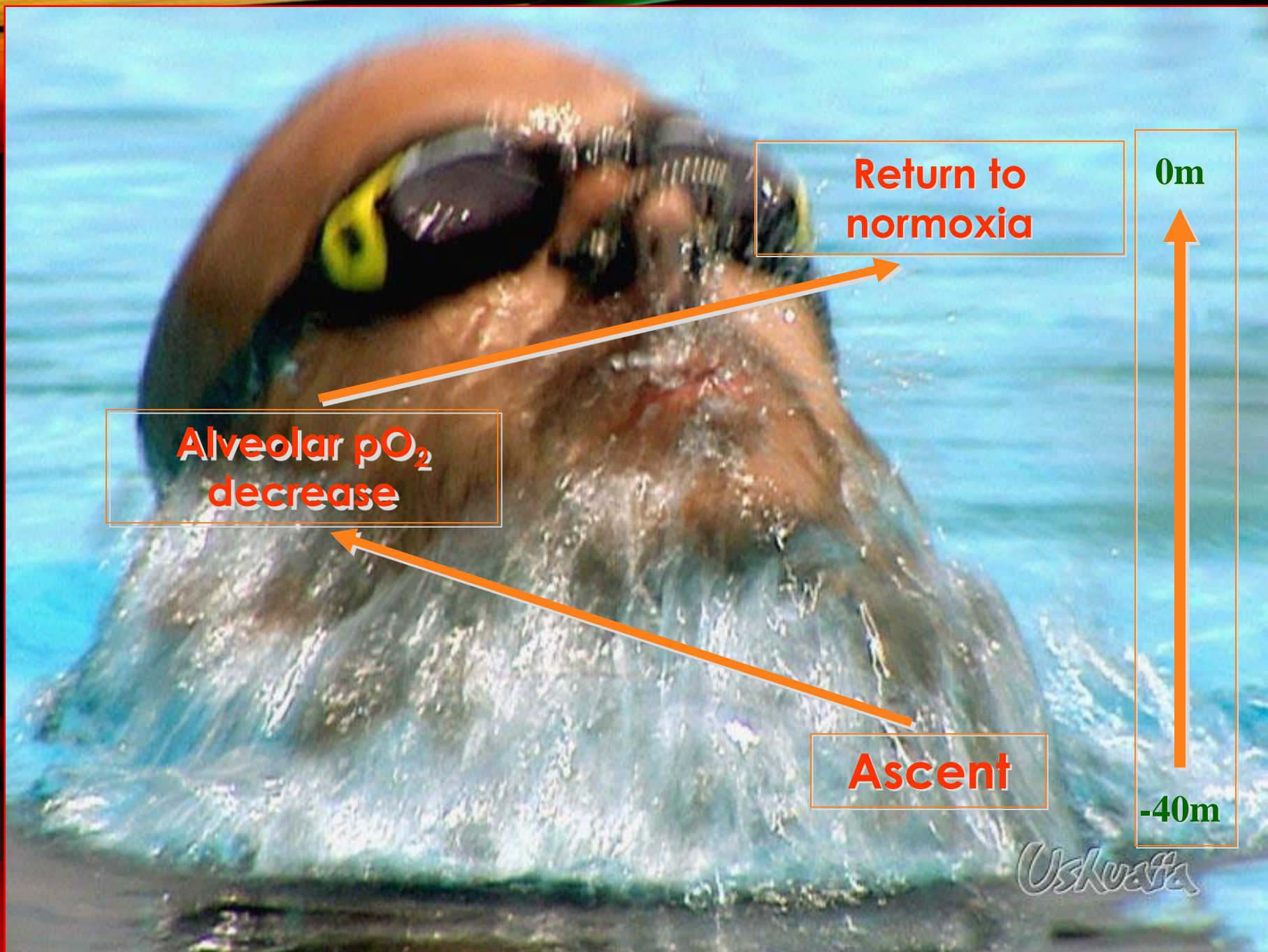
- ✦ Production in renal proximal tubular cell, in response to tissue hypoxia (Goldwasser, 1957)
- ✦ Normal plasma values : 5 - 25 mU/ml
- ✦ Circadian rhythm: literature discordances (Wide 1989, Cahan 1992, Miller 1981, Cotes & Brozovic 1982, Klausen 1993)

## POSSIBLE OTHER STIMULUS ?

- Serum EPO measurements in apnea divers after 3 dives to - 40 m (1999 Malta EUBS Meeting)
- [EPO] markedly elevated !
- Hypothesis: tissular  $pO_2$  variation as a stimulus for EPO production

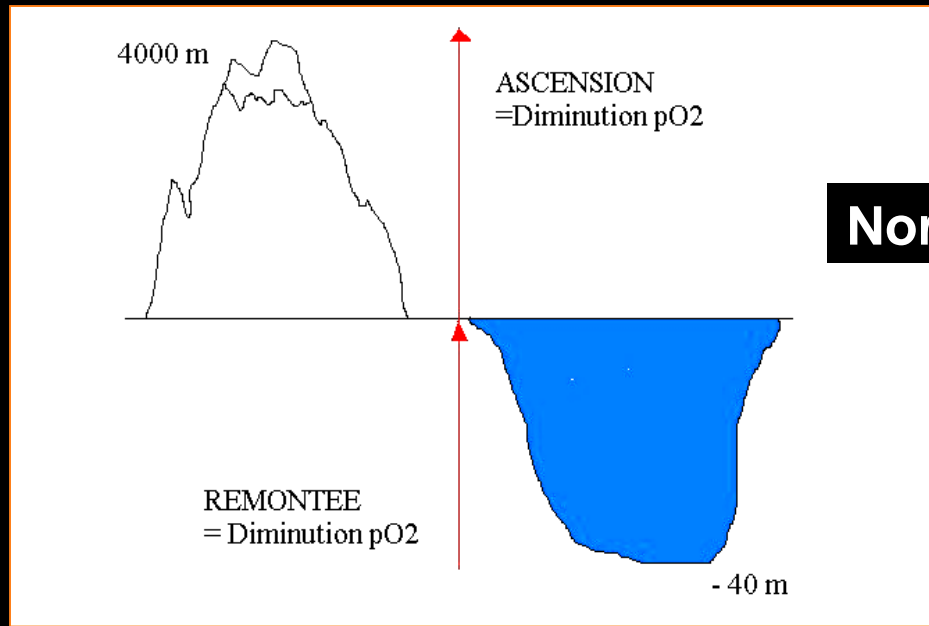








# CONCEPT OF "RELATIVE HYPOXIA"



Normoxia

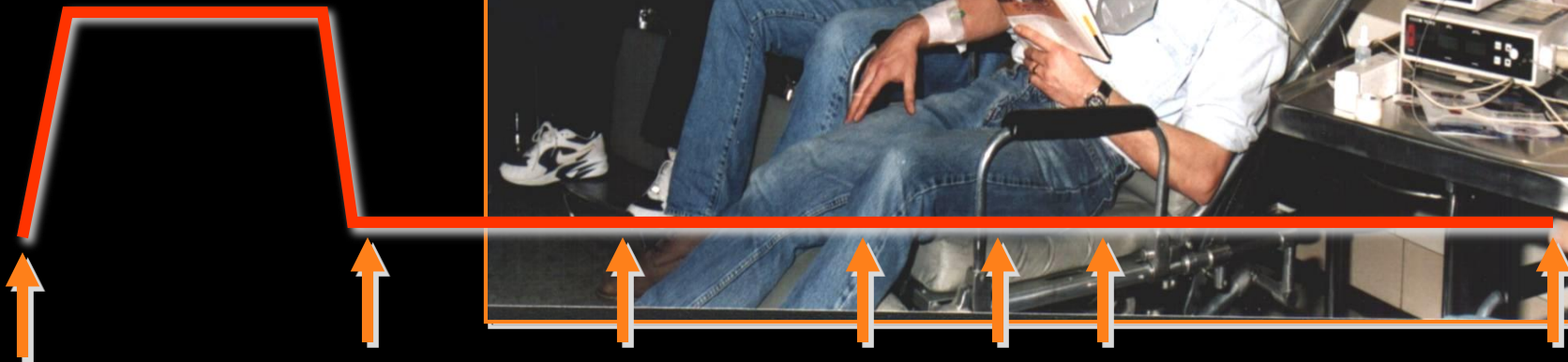
Normoxia

Hyperoxia

- 💡 **Tissue  $N_2$ - $O_2$  balance : increase in relative  $N_2$  content**
- 💡 **"Trigger time": at least 45 minutes = sustained change**

# NORMOBARIC HYPEROXIA

- 🐝 2cc blood samples
- 🐝 -20° C freezing of plasma
- 🐝 RIA assay < 24hrs



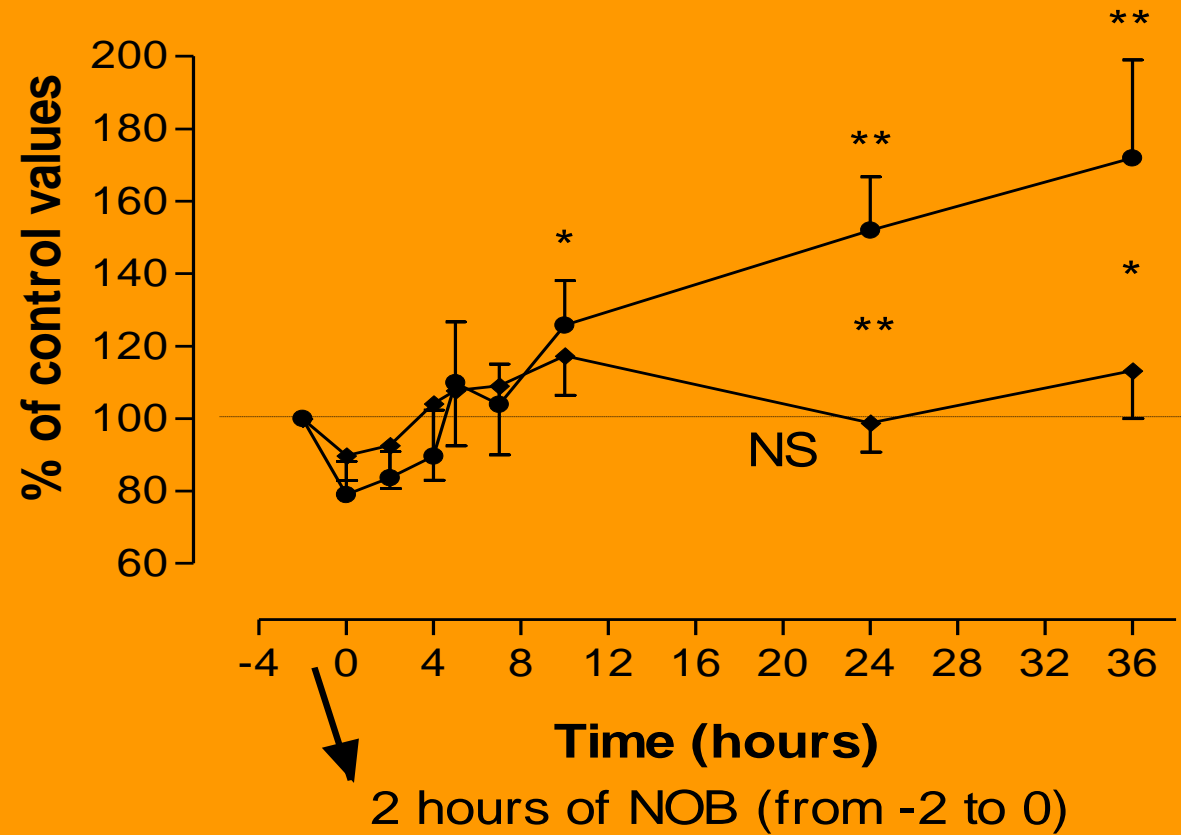


# HYPERBARIC HYPEROXIA

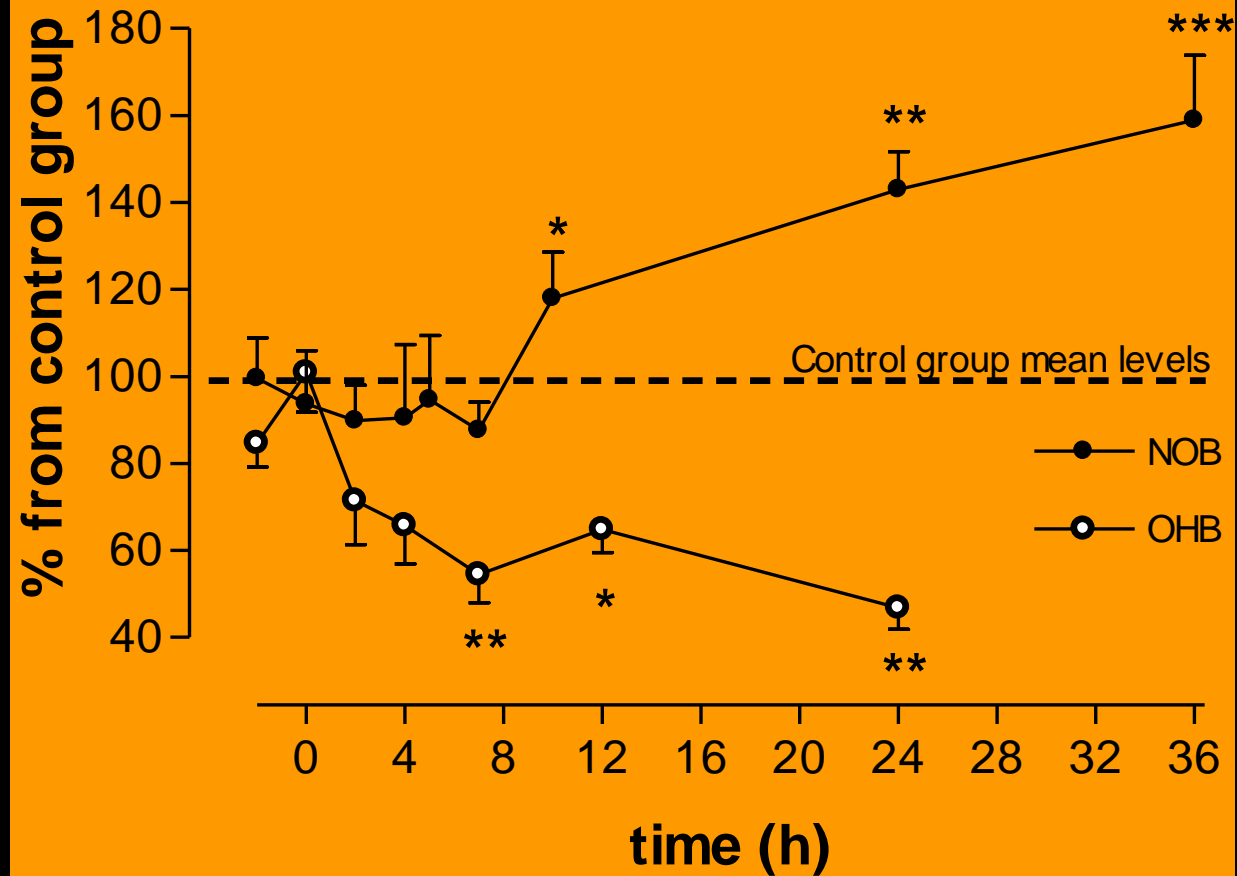
**Belgian ARMY Hyperbaric Chamber**



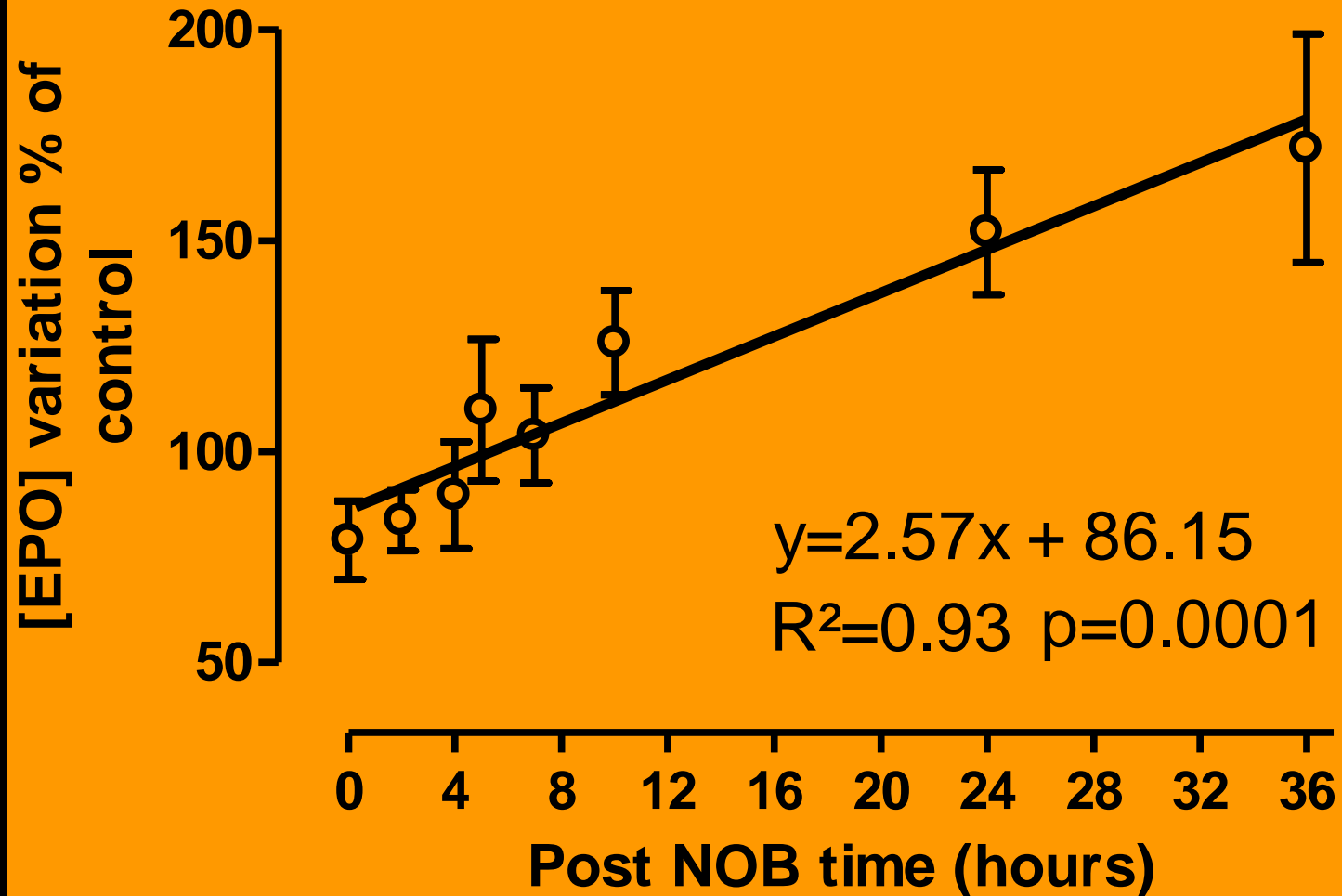
## Percentual variation of EPO plasmatic concentration



## Percentual variation from control group

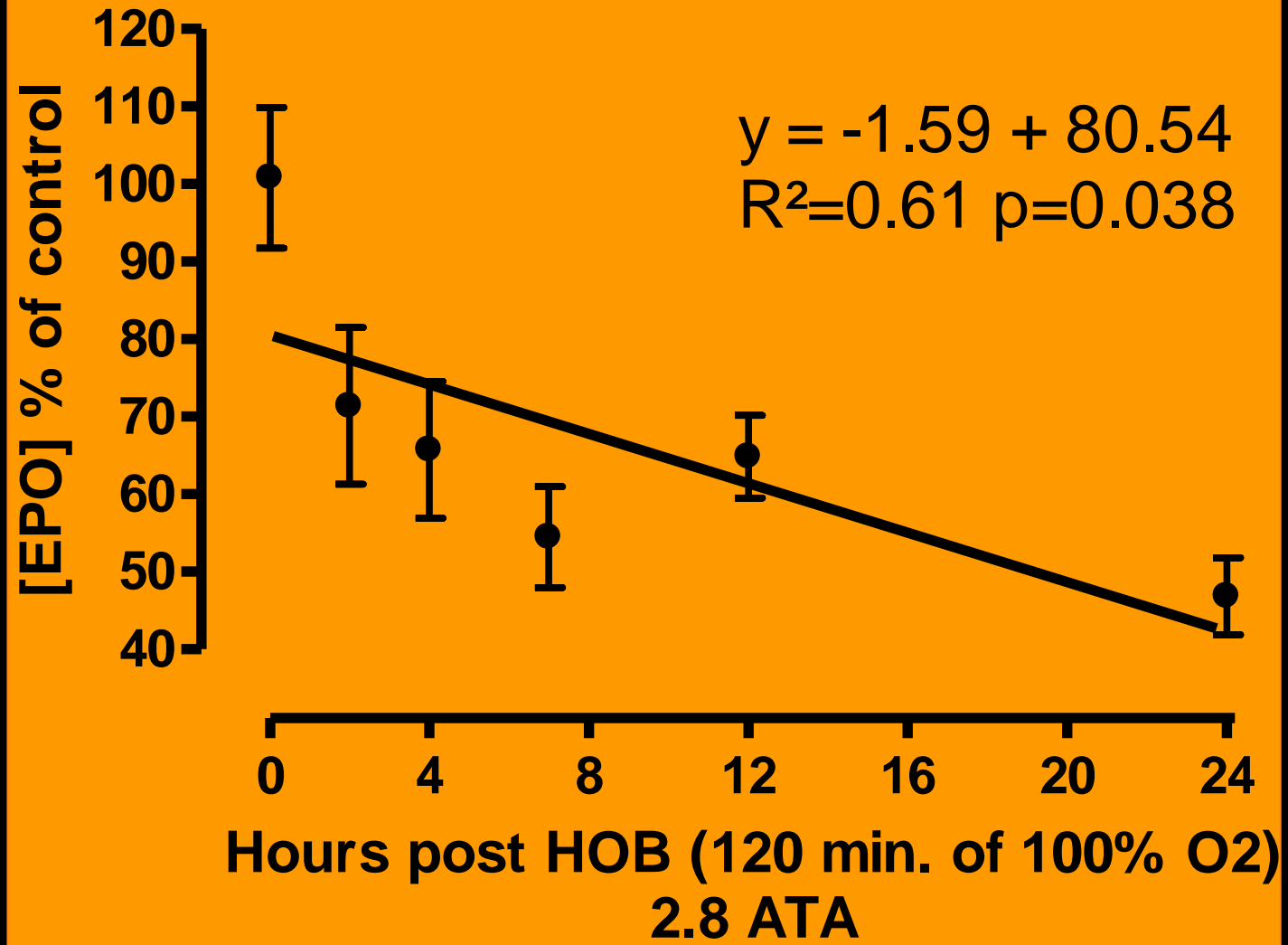


## Plasma [EPO] after 120 min. of normobaric 100% oxygen breathing





## [EPO] Plasma variation after HOB





## Serum erythropoietin levels in healthy humans after a short period of normobaric and hyperbaric oxygen breathing: the "normobaric oxygen paradox"

Costantino Balestra, Peter Germonpré, Jacques R. Poortmans and Alessandro Marroni

*Journal of Applied Physiology* 100:512-518, 2006. First published Oct 20, 2005;  
doi:10.1152/jappphysiol.00964.2005

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This information is current as of February 9, 2006 .

## ➤ HERE ARE THE ACTORS



ROS

HIF-1 $\alpha$

$\beta$

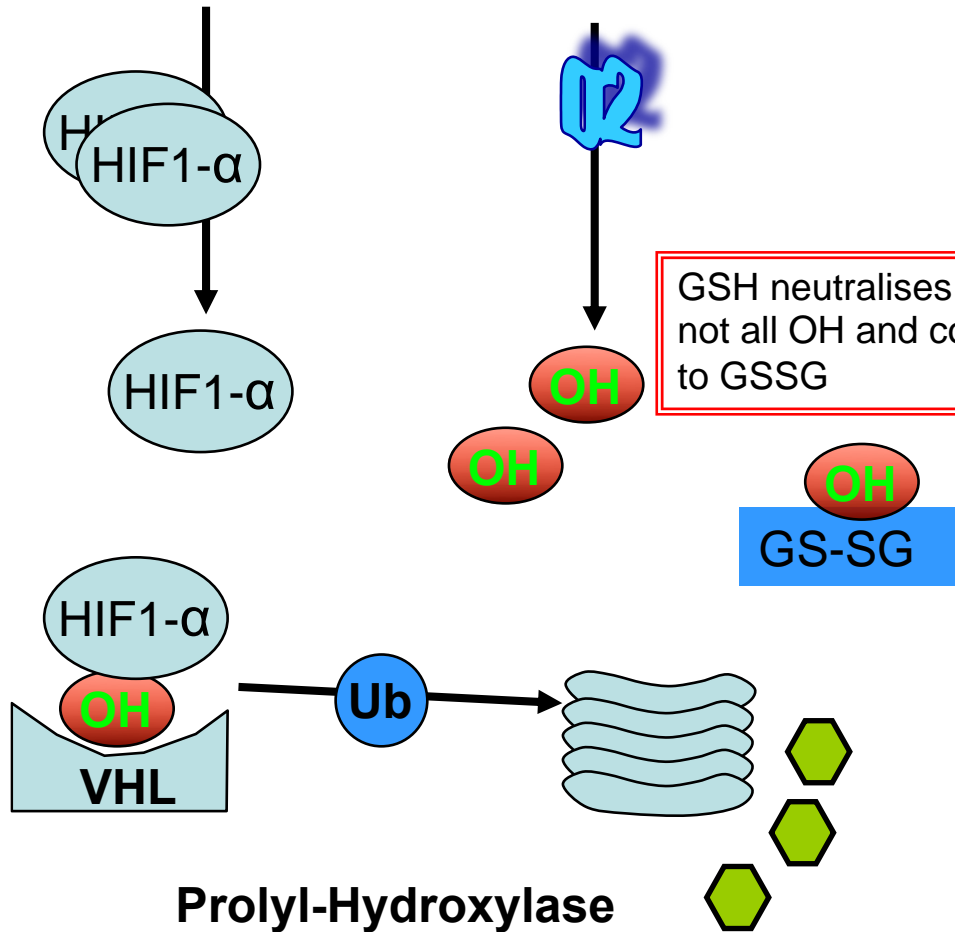
VHL

GSH

GSSG

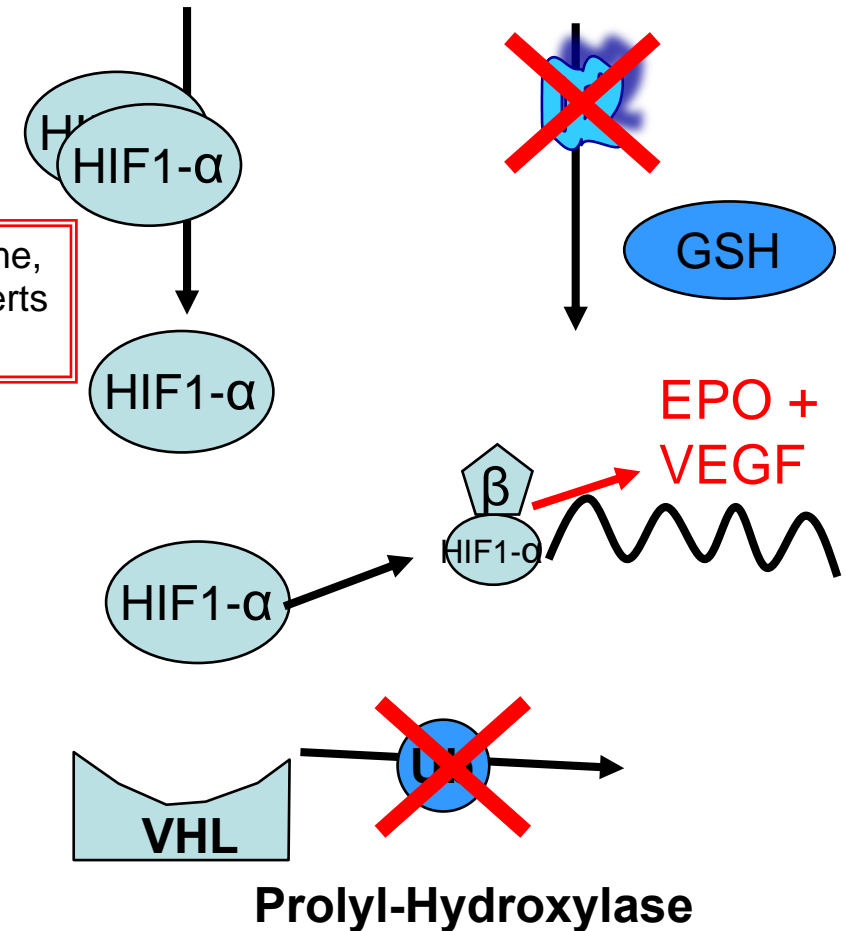
## Normal situations

Normoxia



Hypoxia

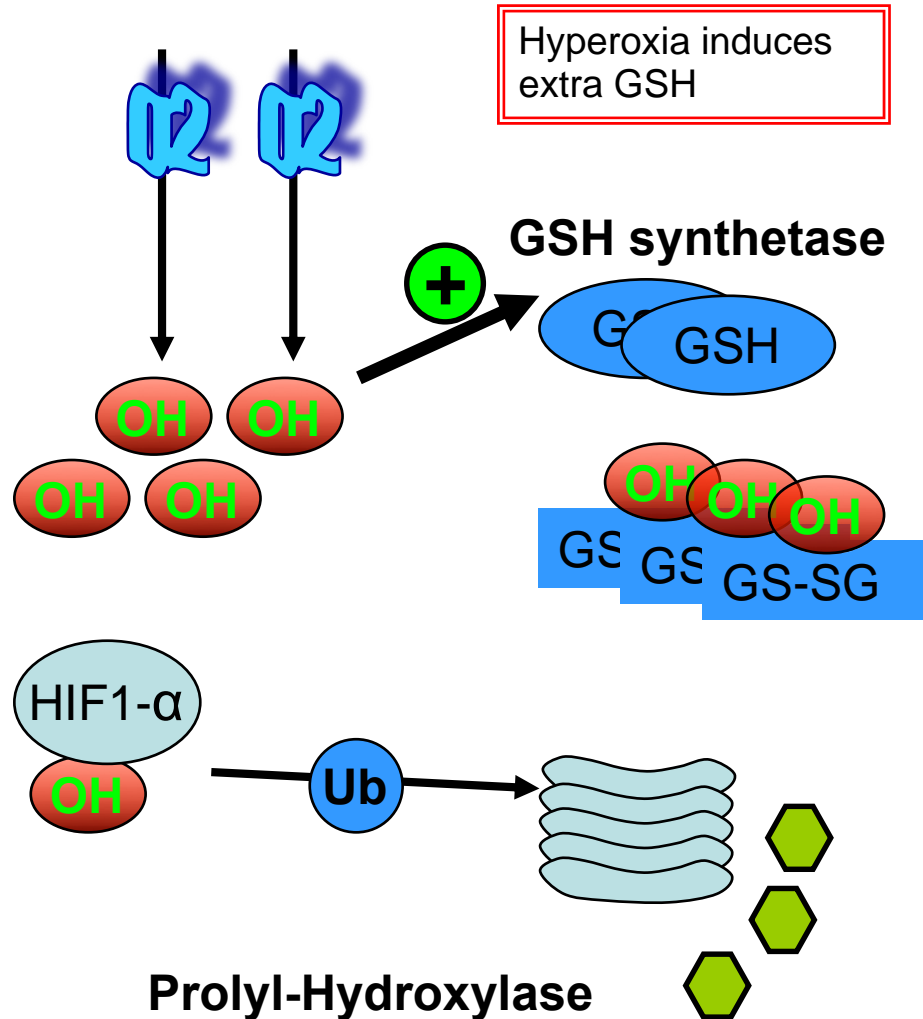
Extreme situation ! In less severe hypoxia, GSH neutralises all of the OH



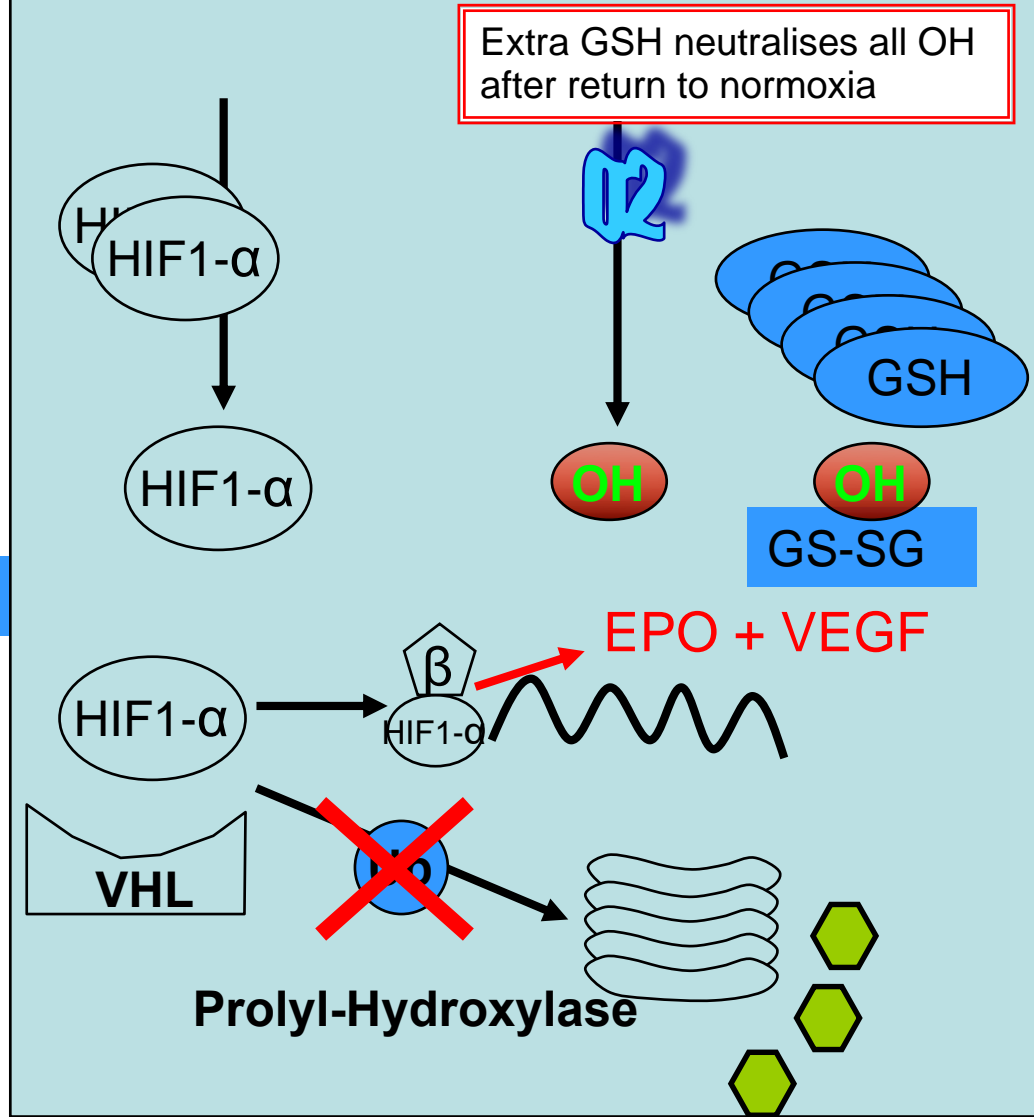


# Normobaric hyperoxia

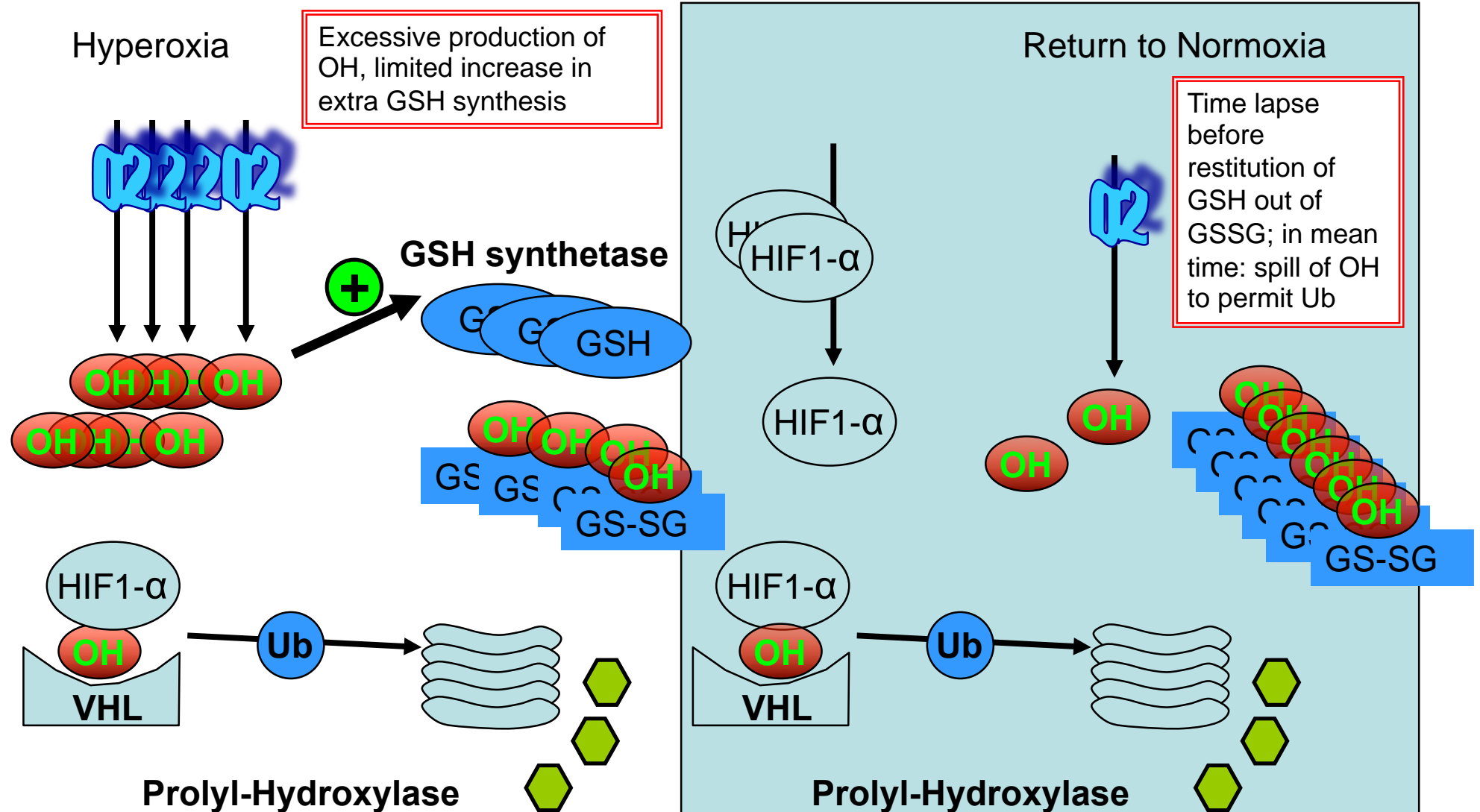
Hyperoxia



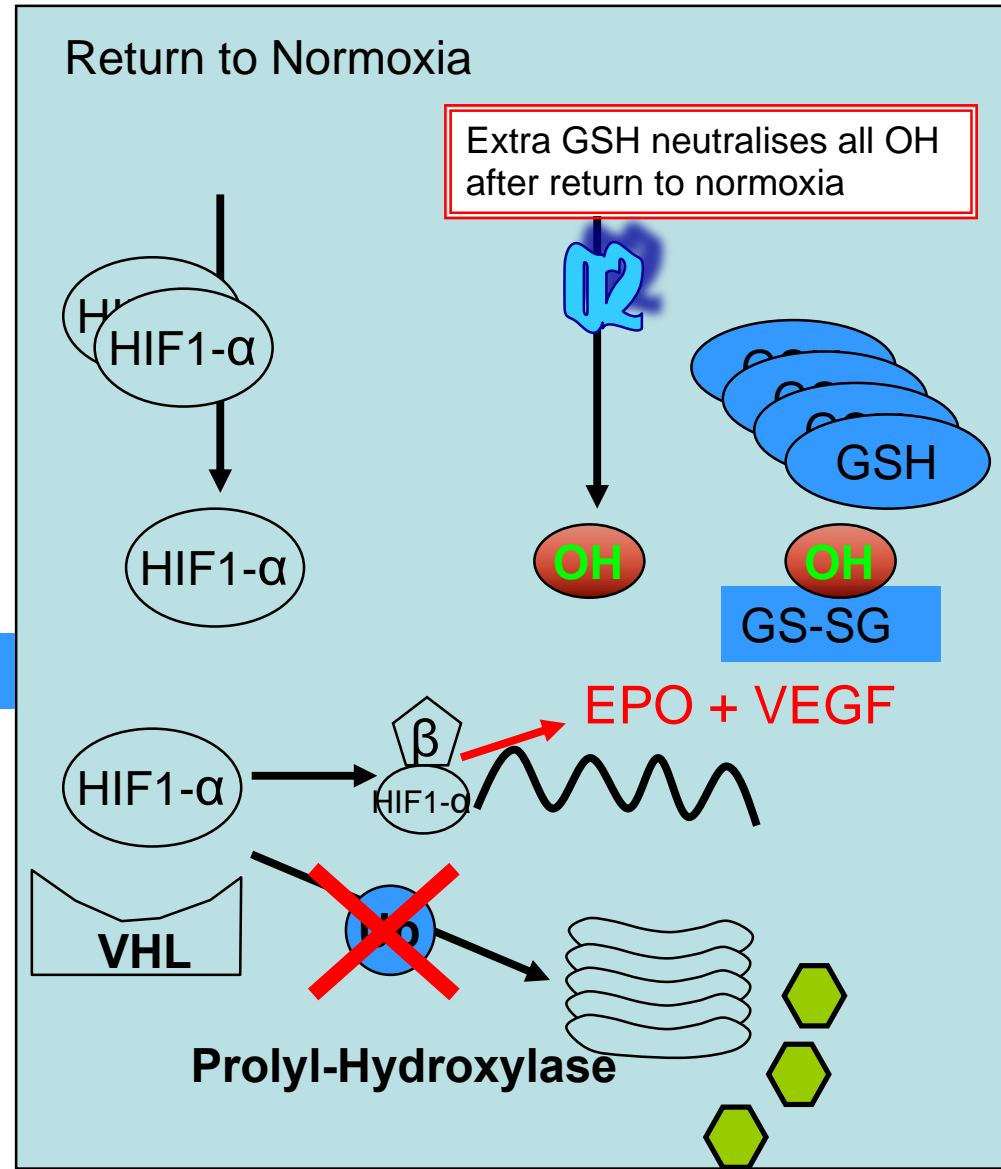
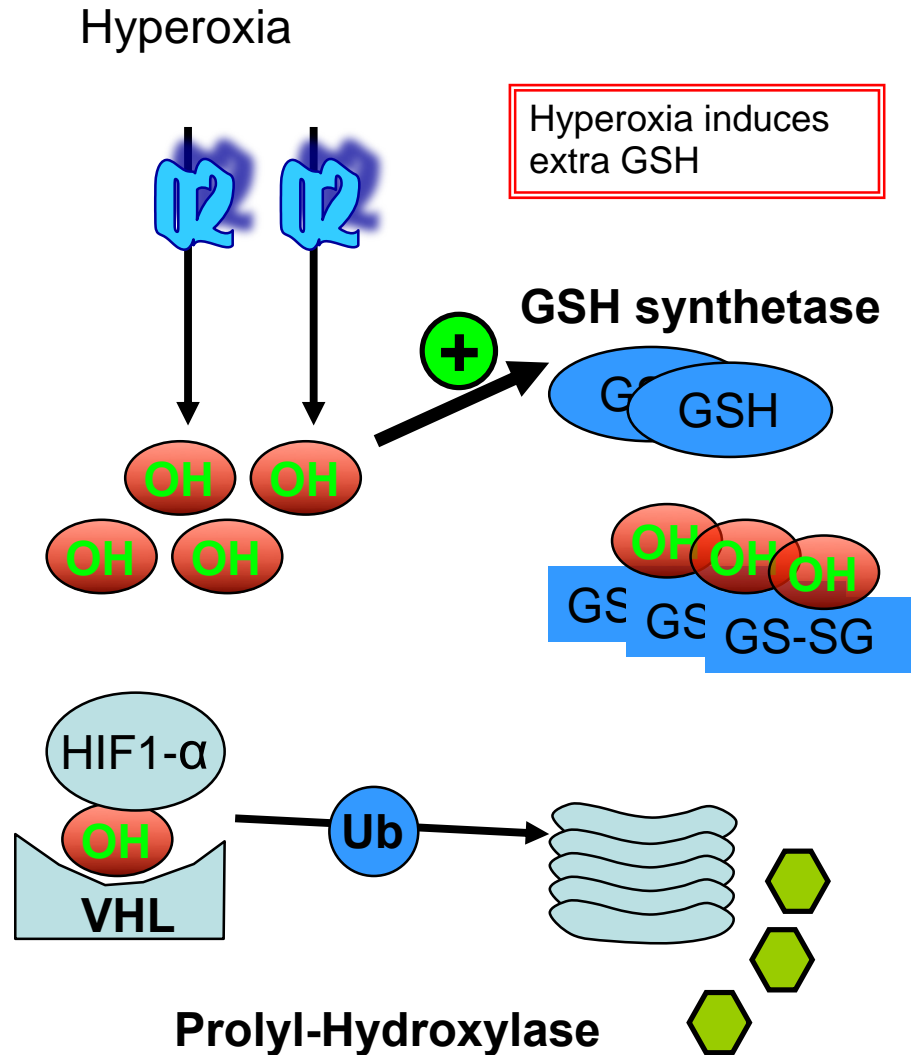
Return to Normoxia



# Hyperbaric hyperoxia



## Normobaric hyperoxia



# ERYTHROPOIETIN EPO





The background features a solid black field. A vibrant, multi-colored band curves across the top of the image, transitioning from yellow and orange on the left to green and blue on the right.

FOCUS?



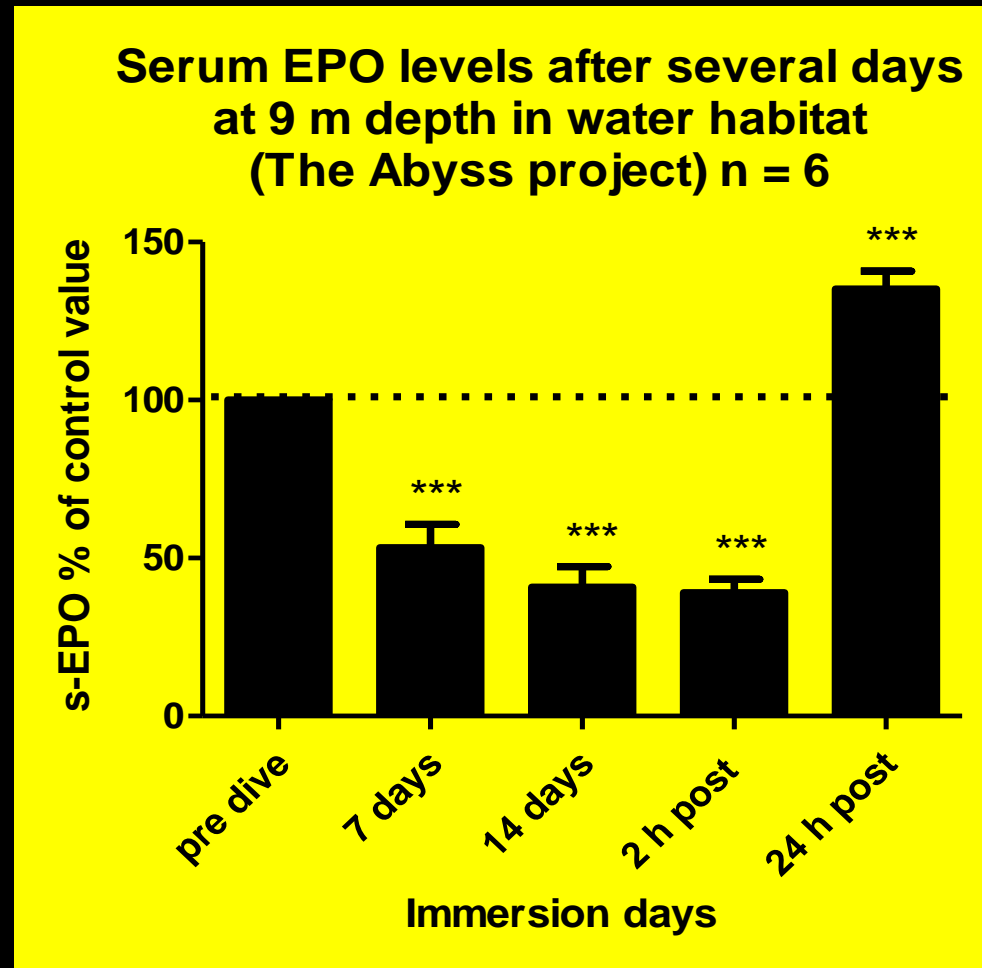


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# THE ABYSS PROJECT (PONZA ITALY)



Revelli L, Vagnoni S, D'Amore A, Di Stasio E, Lombardi CP, Storti G, Proietti R, Balestra C & Ricerca BM. (2013). EPO modulation in a 14-days undersea scuba dive. *Int J Sports Med* **34**, 856-860.



# EPO Modulation in a 14-Days Undersea Scuba Dive

## Authors

L. Revelli<sup>1</sup>, S. Vagnoni<sup>2</sup>, A. D'Amore<sup>1</sup>, E. D. Stasio<sup>3</sup>, C. P. Lombardi<sup>1</sup>, G. Storti<sup>4</sup>, R. Proietti<sup>2</sup>, C. Balestra<sup>5</sup>, B. M. Ricerca<sup>6</sup>

## Affiliations

Affiliation addresses are listed at the end of the article

## Key words

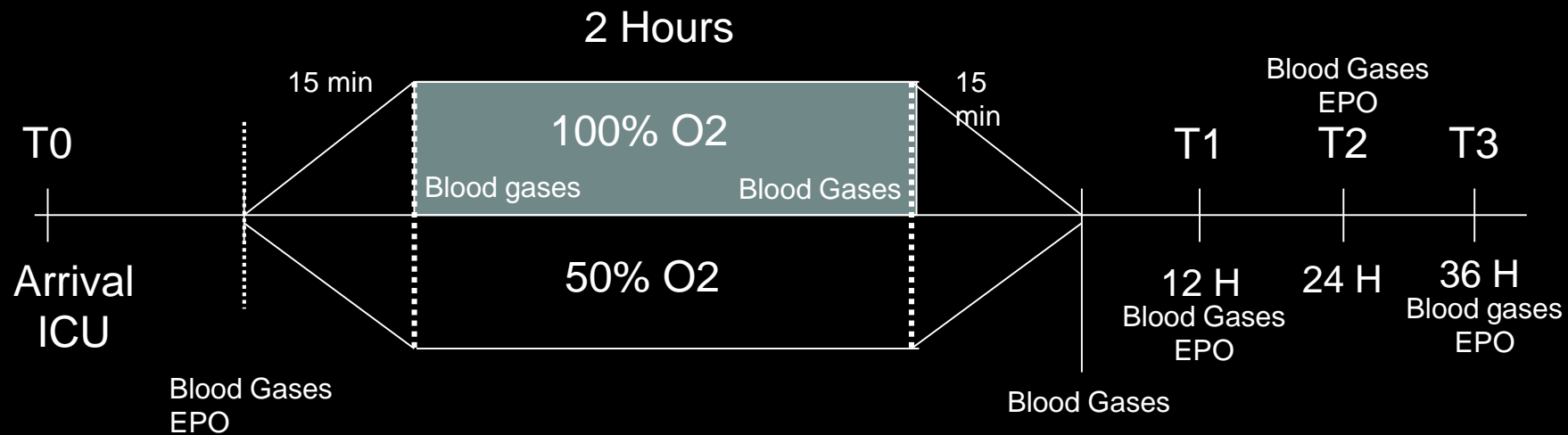
- erythropoietin (EPO)
- hyperbarism
- hyperoxia
- scuba dive
- reactive oxygen species (ROS)
- normobaric oxygen paradox (NOP)

## Abstract

Erythropoiesis is affected during deep saturation dives. The mechanism should be related to a downregulation of serum Erythropoietin (s-EPO) concentration or to a toxic effect of the hyperbaric hyperoxia. We evaluated s-EPO and other haematological parameters in 6 scuba divers before, during and after a 14-days Guinness saturation dive (8–10 m). Athletes were breathing air at 1.8–2 ATA, under the control of a team of physicians. Serum parameters were measured before diving (T0) and: 7 days (T1), 14 days (T2) after the beginning of the dive and 2 h (T3) and 24 h (T4) after resurfacing. Hgb, and many other haematological parameters did not change whereas Ht, s-EPO, the ratio between s-EPO predicted and that observed and reticulocytes

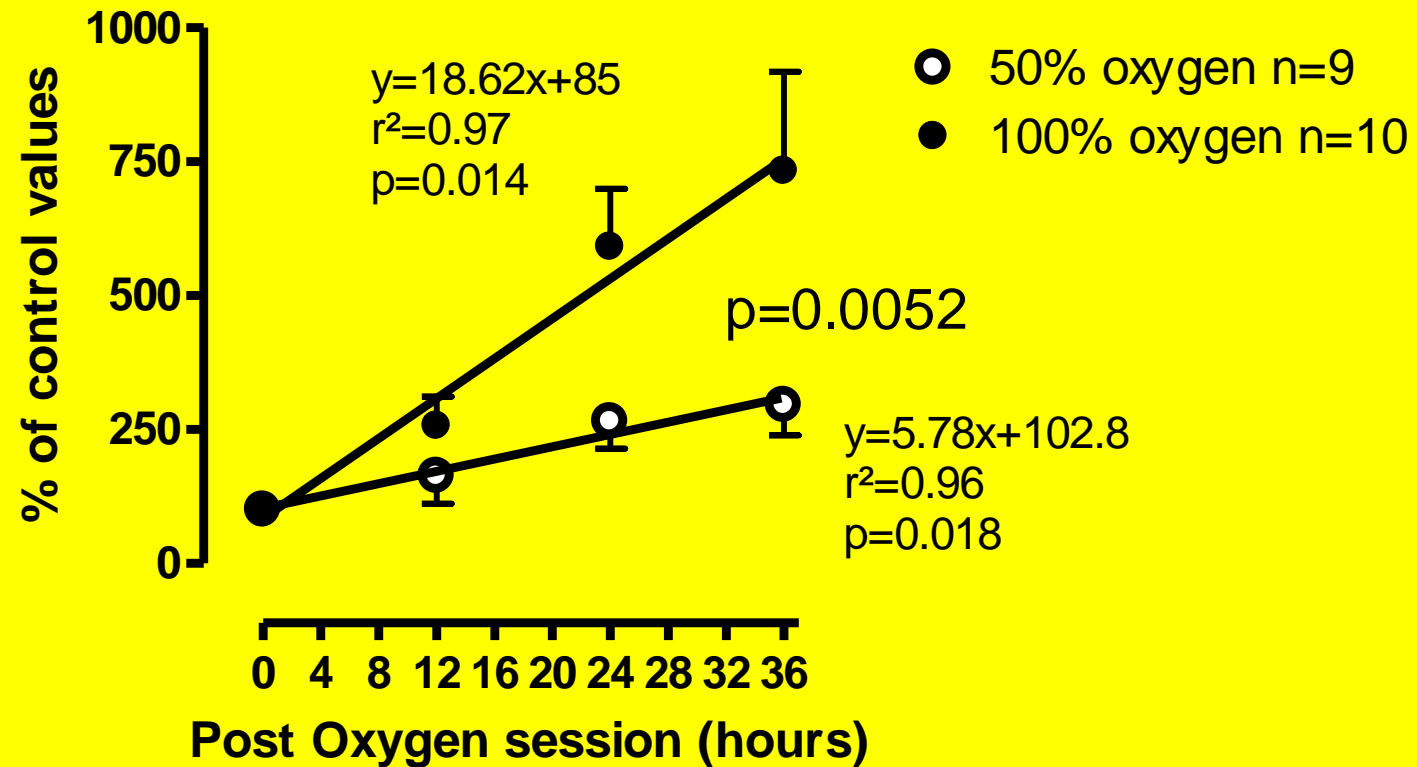
(absolute, percent) declined progressively from T0 to T3. At T4 a significant rise in s-EPO was observed. Hgb did not vary but erythropoiesis seemed to be affected as s-EPO and reticulocyte counts showed. All these changes were statistically significant. The experiment, conducted in realistic conditions of dive length, oxygen concentration and pressure, allows us to formulate some hypotheses about the role of prolonged hyperbarism on erythropoiesis. The s-EPO rise, 24 h after resurfacing, is clearly documented and related to the "Normobaric Oxygen Paradox". This evidence suggests interesting hypotheses for new clinical applications such as modulation of s-EPO production and Hgb content triggered by appropriate O<sub>2</sub> administration in pre-surgical patients or in some anemic disease.

# CLINICAL APPLICATION : METHODS



Two randomized groups of patients target number n=24  
Post cardiac surgery  
No hypoxia allowed  
No Haemoglobin levels drop

# EPO Level in Cardiac Patients post O2 administration (randomly assigned groups)



ion surgeries is clearly needed. Indeed, temperatures from 15°C to 25°C (for example) do not preclude identifying the role of fluid fluidity variation on clinical IRRSOL absorption while controlling other known variables such as hydrostatic pressure, irrigation flow, and types of surgery and tissue (fibroid, endometrium, uterine muscle, prostate, or bladder).

Urological procedures are not only different *per se*, but also sex and age illustrate temperature-related concerns. Young patients may just feel uncomfortably cold, but elderly patients

752

Correspondence

The normobaric oxygen paradox (NOP) is a new mechanism of triggering endogenous EPO production described in healthy human volunteers.<sup>4</sup> Participants were asked to breathe normobaric oxygen for 2 h at an inspired oxygen fraction ( $F_{I_{O_2}}$ ) of 100%. After 2 h in the hyperoxic state, participants were quickly switched back to normoxic conditions while breathing air. The authors found that EPO levels were significantly increased during the next 12–36 h compared with baseline levels. This study demonstrated that a sudden and sustained decrease in tissue oxygen tension, without a low level of tissue oxygen tension, could lead to an increase in serum EPO levels in these healthy individuals.<sup>4</sup>

We conducted a prospective, randomized, double-blind pilot trial at one university medical centre to assess the presence of this mechanism among patients undergoing cardiac surgery. After institutional Ethics Committee approval, 20 patients who gave written informed consent were studied. Inclusion criteria were age >18 yr, non-urgent valvular and/or coronary artery bypass graft surgery with the planned use of extracorporeal circulation, and expected postoperative mechanical ventilation in the intensive care unit (ICU). Exclusion criteria were severe preoperative renal disease, severe respiratory disease, and massive intraoperative bleeding. The protocol did not require any modification of the anaesthetic or the surgical routine management of the patient in the operating theatre.

At arrival in the ICU, patients were randomized to two

## Increase in endogenous erythropoietin synthesis through the normobaric oxygen paradox in cardiac surgery patients

Editor—Exogenous erythropoietins (EPOs) are used worldwide to reduce allogenic blood transfusion exposure. However, their use can be associated with some serious adverse effects<sup>1,2</sup> and has a significant economic impact on public health systems because of the high cost of these drugs.<sup>3</sup>

BJA

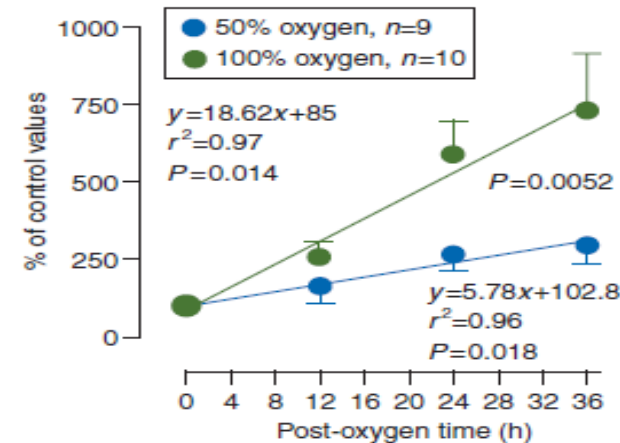


Fig 1 Linear regressions of EPO concentration variations.

cell progenitors, but also on other tissues such as the brain and the heart, where it may exert some protective effects against cellular damage.<sup>5</sup>

### Conflict of interest

None declared.





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medical  
hypotheses

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# Oxygen breathing may be a cheaper and safer alternative to exogenous erythropoietin (EPO) ☆

R. Burk \*

*Burk Labs, 9414 168th Place NE, Redmond, WA 98052, USA*

Received 22 March 2007; accepted 23 March 2007

mandated levels of

A recently discovered method of stimulating EPO production via a pattern of hypoxia and normoxia is based on the hypothesis that oxygen levels stimulate EPO production. Stimulating EPO production via a pattern of hypoxia and normoxia is based on the hypothesis that oxygen levels stimulate EPO production. Further, the use of oxygen therapy has been shown to reduce mortality, than the use of EPO.

A single patient study showed that oxygen therapy is an effective replacement for EPO in patients undergoing dose-dense chemotherapy. The use of simple oxygen therapy was found to be a cost-effective alternative to EPO. © 2007 Elsevier Ltd

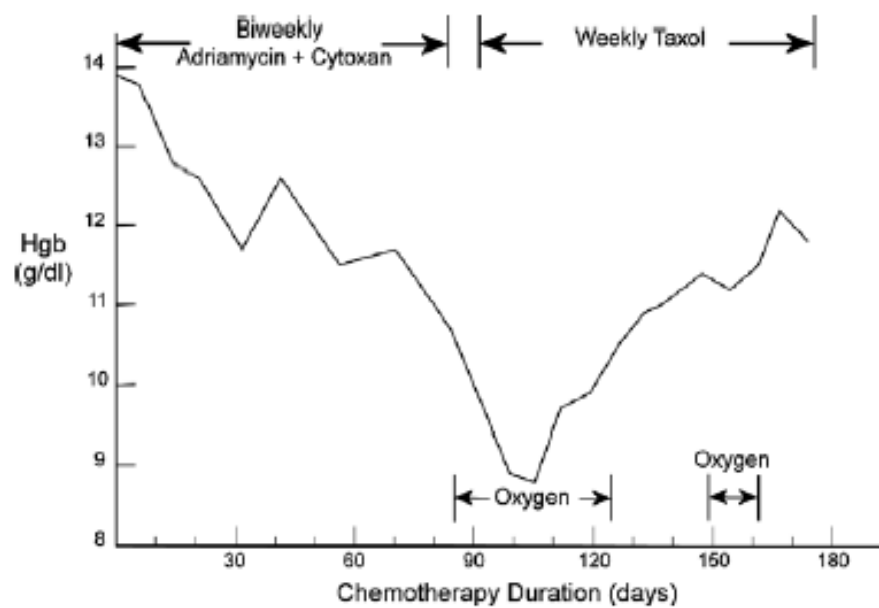
The Balestra study is clear evidence that oxygen breathing can stimulate endogenous EPO, but it is less clear whether this method can effectively treat anemia. A test that lends support to this hypothesis has been performed.

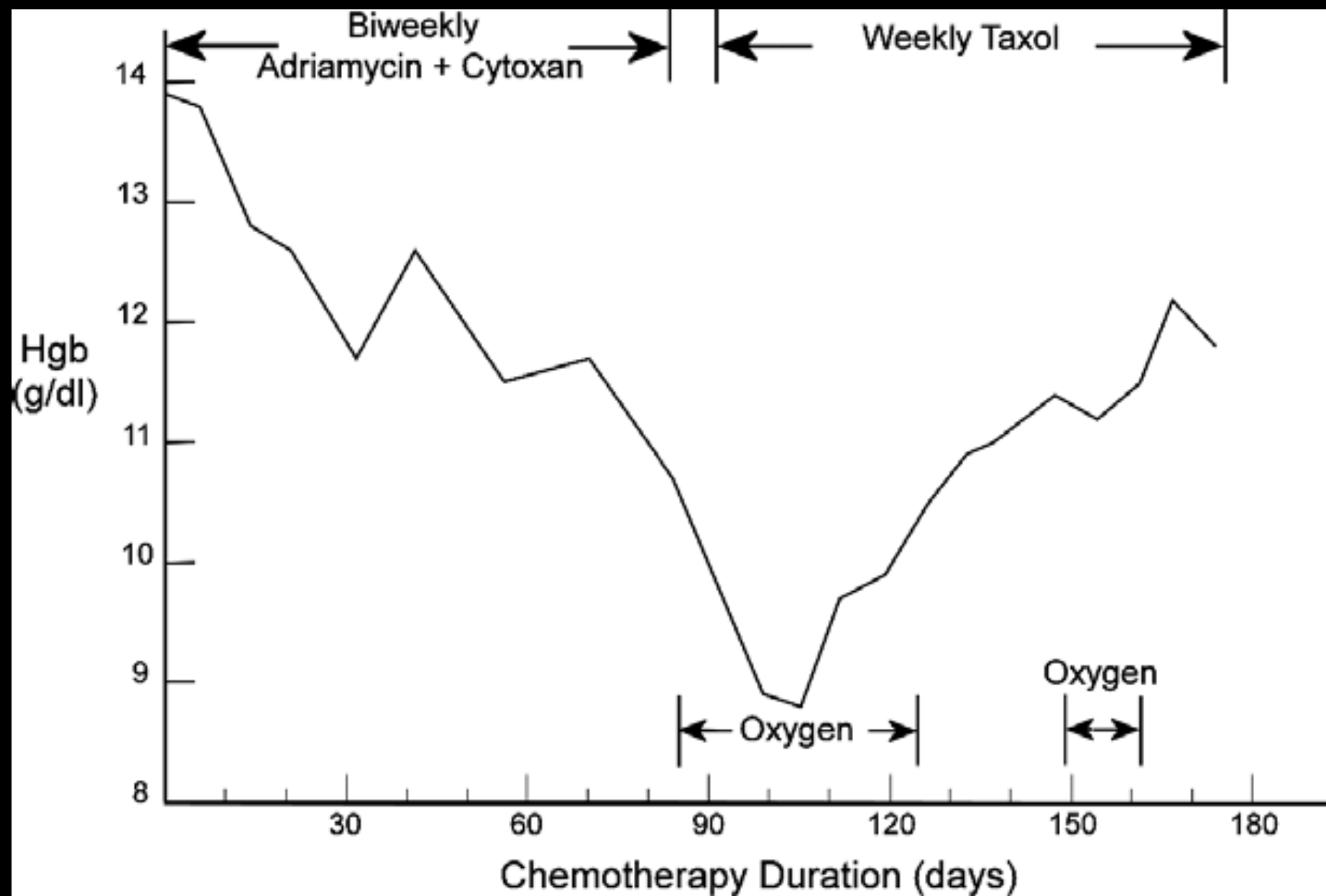
Fig. 1 is the hemoglobin chart of a 42-year old female patient undergoing chemotherapy for stage III breast cancer. The treatment consisted of Adriamycin and Cytosine, followed by Taxol. As the graph shows, an anemic trend commenced and worsened along with the beginning of chemotherapy treatment.

About 85 days into chemotherapy, treatment with oxygen breathing commenced. This consisted of breathing aviation oxygen (>99% pure oxygen) via a cannula for about 90 min, two to three times

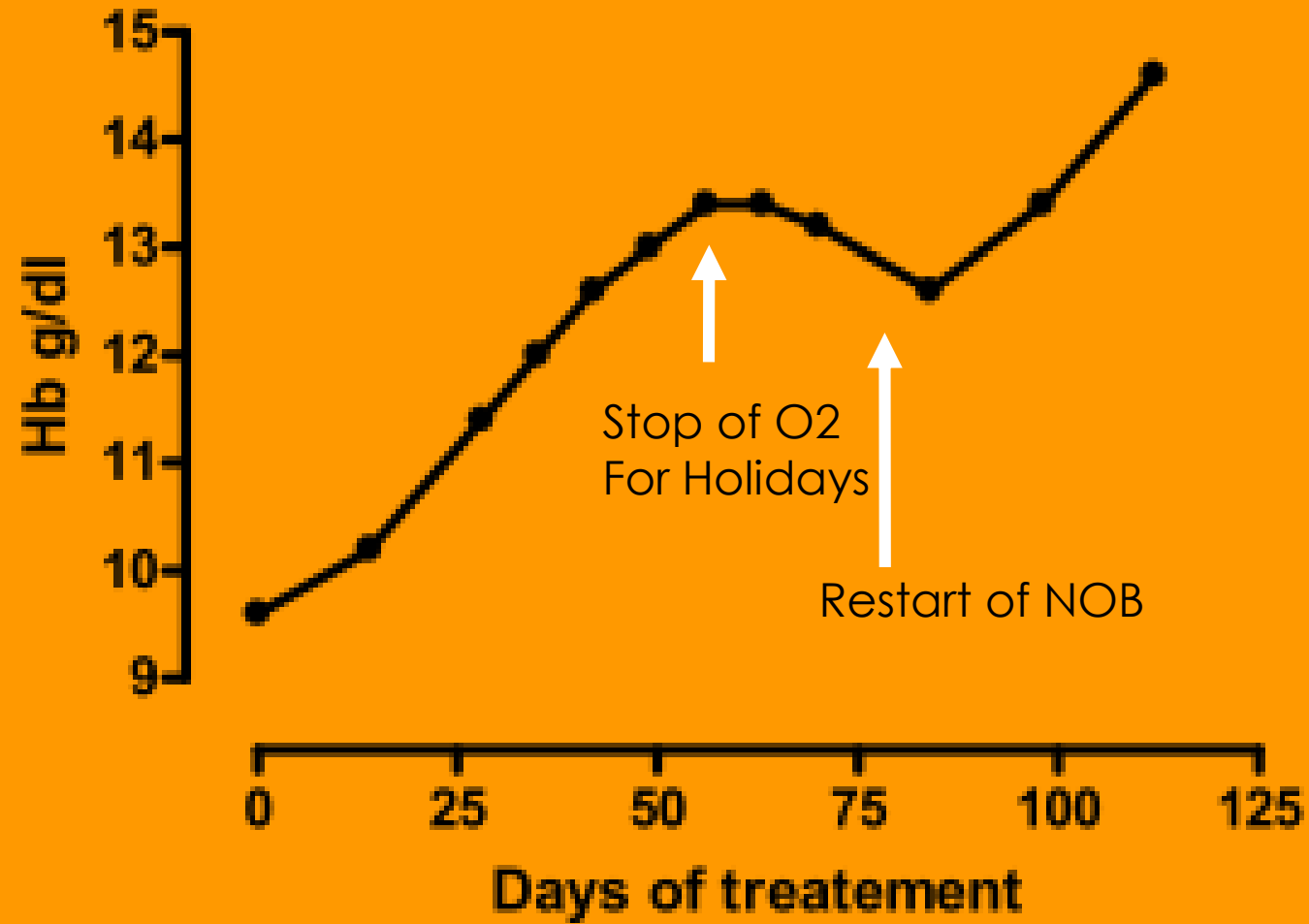
per week. This led to a significant increase in hemoglobin levels. This leads directly to the use of oxygen as a inexpensive alternative to EPO. The reaction associated with the use of oxygen therapy is a significant improvement in terms of cancer treatment.

Oxygen breathing can be an effective alternative to EPO in a stage III breast cancer patient undergoing chemotherapy. Treatment by using a pattern of hypoxia and normoxia is based on the hypothesis that oxygen levels stimulate EPO production.





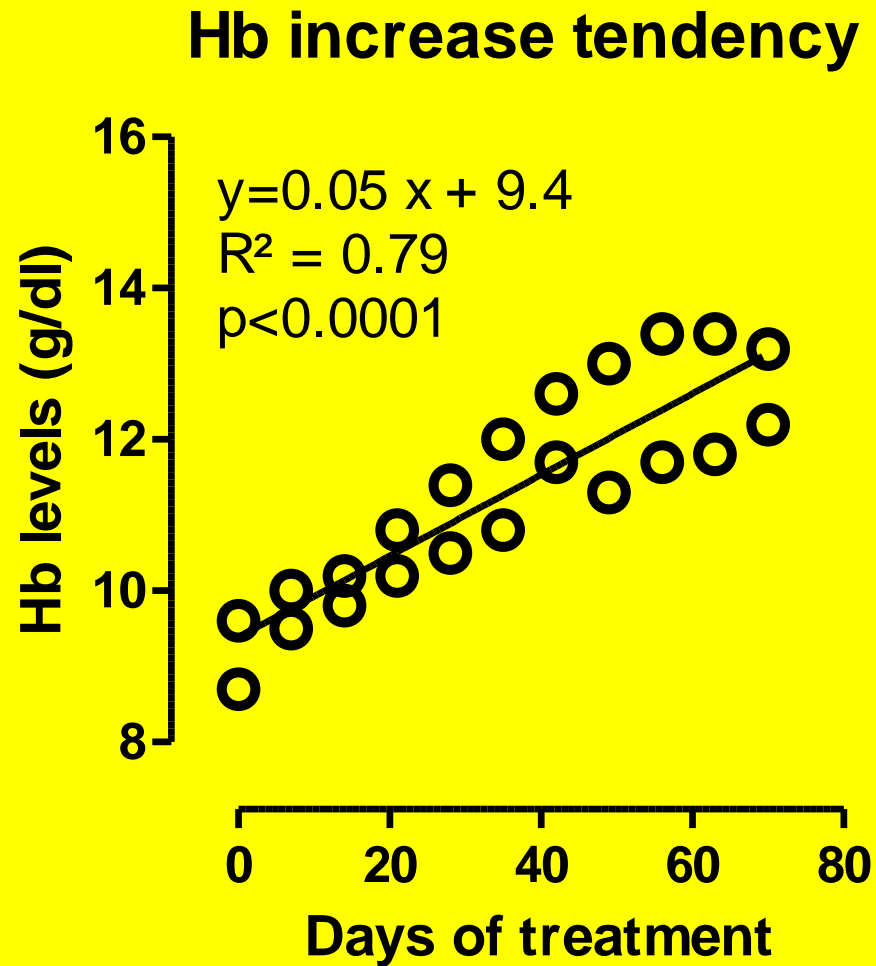
## Hemoglobin level





# PLOTTING THE 2 PATIENTS DATA

*Levels of Hb (g/dl) in two patients breathing oxygen. The first 100% O<sub>2</sub> every other day with adjuvant drug therapy (Darbepoetin Alpha + IV iron) (Myelofibrosis), The second breathing 40% O<sub>2</sub> 3 times per week with no other erythroid stimulating agents (Chemotherapy).*



# The 'normobaric oxygen paradox': a simple way to induce endogenous erythropoietin production and concomitantly raise hemoglobin levels in anemic patients

COSTANTINO BALESTRA\*, PhD, PETER GERMONPRÉ\*,†, MD, PIERRE LAFERE\*,†, MD, YANNICK CICCARELLA\*, MD & PHILIPPE VAN DER LINDEN\*, MD, PhD

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## EPO and doping

Costantino Balestra · Peter Germonpré

Accepted: 21 February 2010  
© Springer-Verlag 2010

To the Editor,

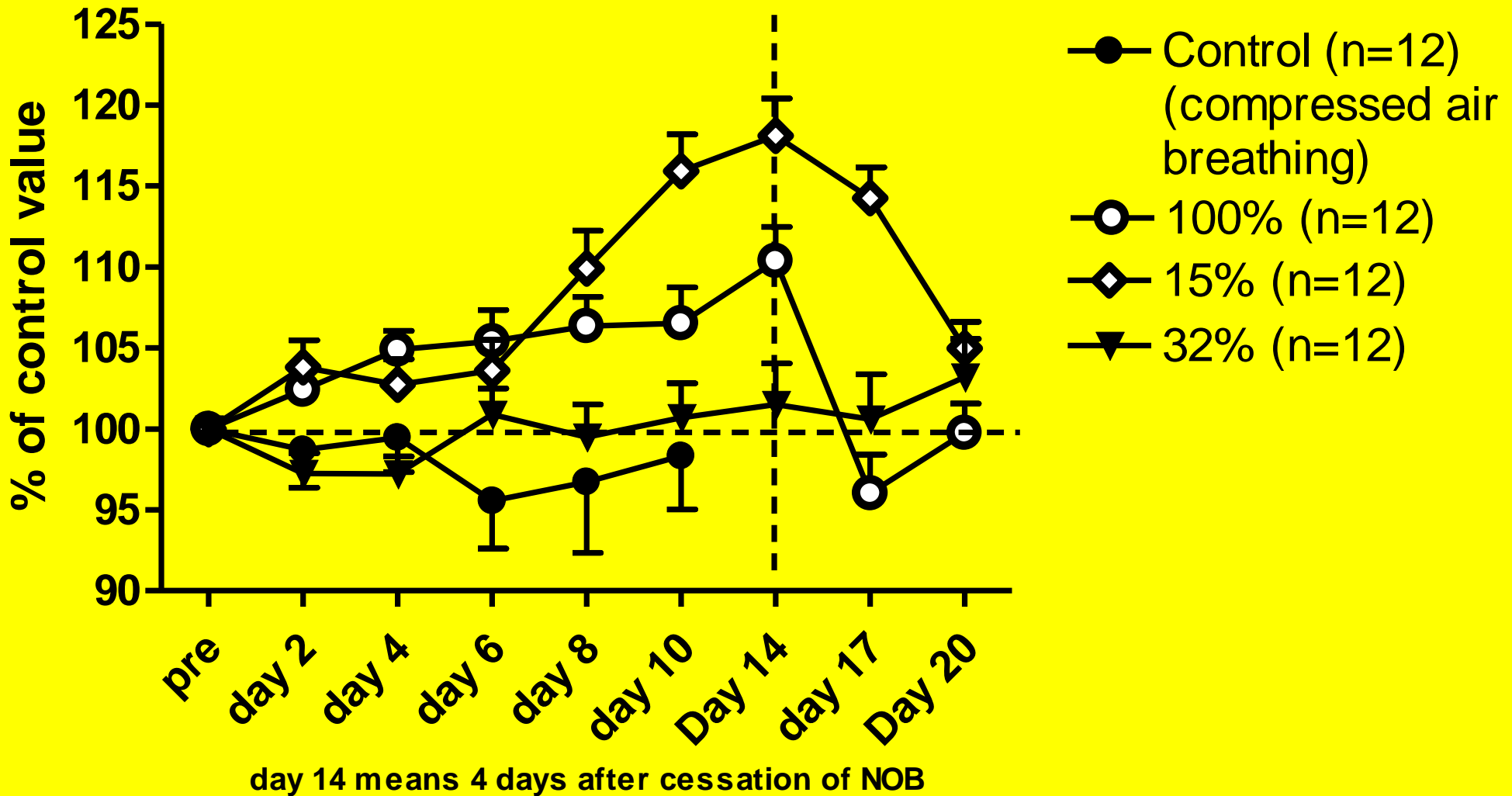
It is with great interest that we read exchanges on intermittent hypoxic (Boning 2009; Ferretti 2009) exposure as a potential doping procedure. Erythropoietin (EPO) does introduce potential concerns in terms of doping, both due to its effects and side effects. An earlier publication on exogenous EPO (Noakes 2008) clearly showed an increase of sub-maximal performance with a significant increase of time to exhaustion after prolonged rHuEPO administration. It is clear that in healthy subjects, at sub-maximal effort levels, cardiac output is sufficient to maintain adequate oxygen delivery to active muscles. Accordingly, it appears that EPO achieves more than increasing blood oxygen carrying capacity alone. Being both a hormone and a cytokine, the actual actions of EPO are complex: EPO is neuroprotective and even neuroregenerative in both the peripheral and central nervous system. Moreover, EPO also has antiapoptotic effects that may be coupled to antioxidant activity.

Exercise-induced plasma volume contraction is linked to

Urine testing for exogenous EPO has been available for several years. It has been used in international cycling events. However, this does not exclude the possibility of endogenous EPO induction. Intermittent hypoxia (Sanchis-Gomar et al. 2009) is the natural trigger for EPO production, and is widely used by (endurance) athletes since many years. We have recently described the use of intermittent hyperoxia to stimulate EPO production (Balestra et al. 2006). This has subsequently been shown to be capable of increasing haemoglobin levels in a chronically anaemic patient (Burk 2007).

The mechanism proposed to explain this “normobaric oxygen paradox” involves a complex play of oxygen-free radicals (OFRs) presence and their “scavenging” enzymes in the cell. In the presence of OFR, the continuously produced hypoxia-inducible factor alpha (HIF-1 $\alpha$ ) is instantly linked to the (tumour suppressing) Von Hippel Lindau Protein (VHLp). This complex is subsequently ubiquitinated in the proteasome pathway and finally recycled in the proteasome. In a hypoxic state, the absence of OFR prevents

## Hb Variation after 100% O<sub>2</sub> breathing (30 minutes every other day)





## **Pulsed high oxygen induces a hypoxic-like response in human umbilical endothelial cells and in humans**

**F. Cimino, C. Balestra, P. Germonpré, D. De Bels, F. Tillmans, A. Saija, A. Speciale and F. Virgili**

*J Appl Physiol* 113:1684-1689, 2012. First published 4 October 2012;  
doi: 10.1152/jappphysiol.00922.2012

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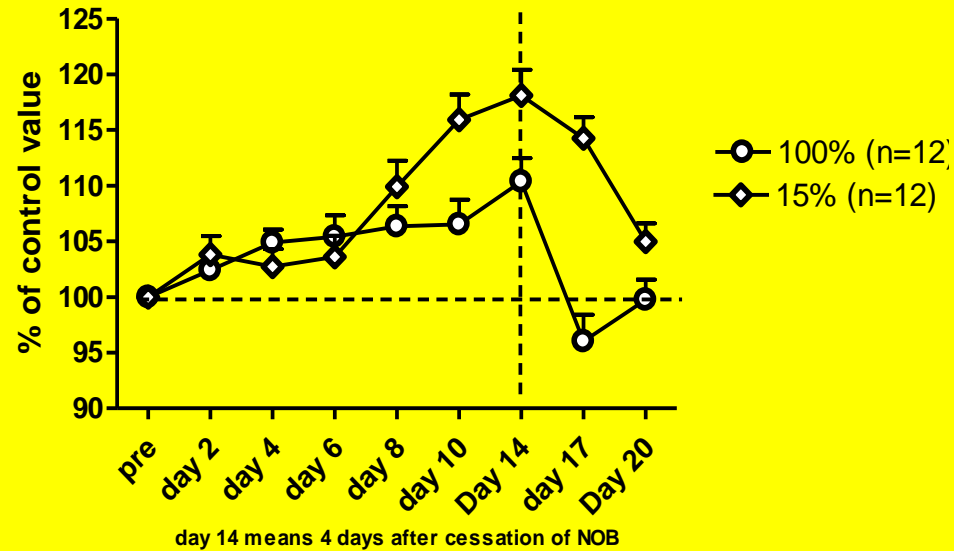
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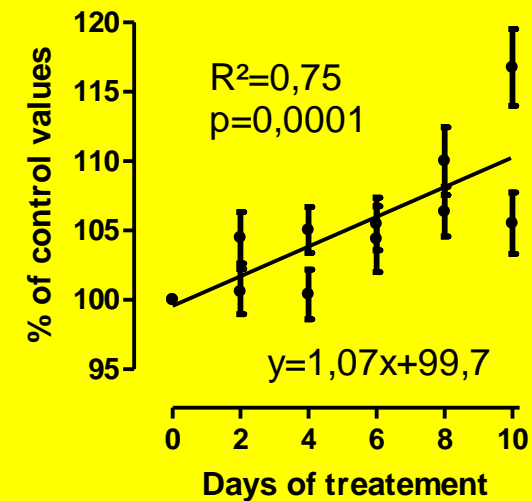
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# HYPOXIA = HYPEROXIA

Hb Variation after O<sub>2</sub> breathing  
(30 minutes every other day)

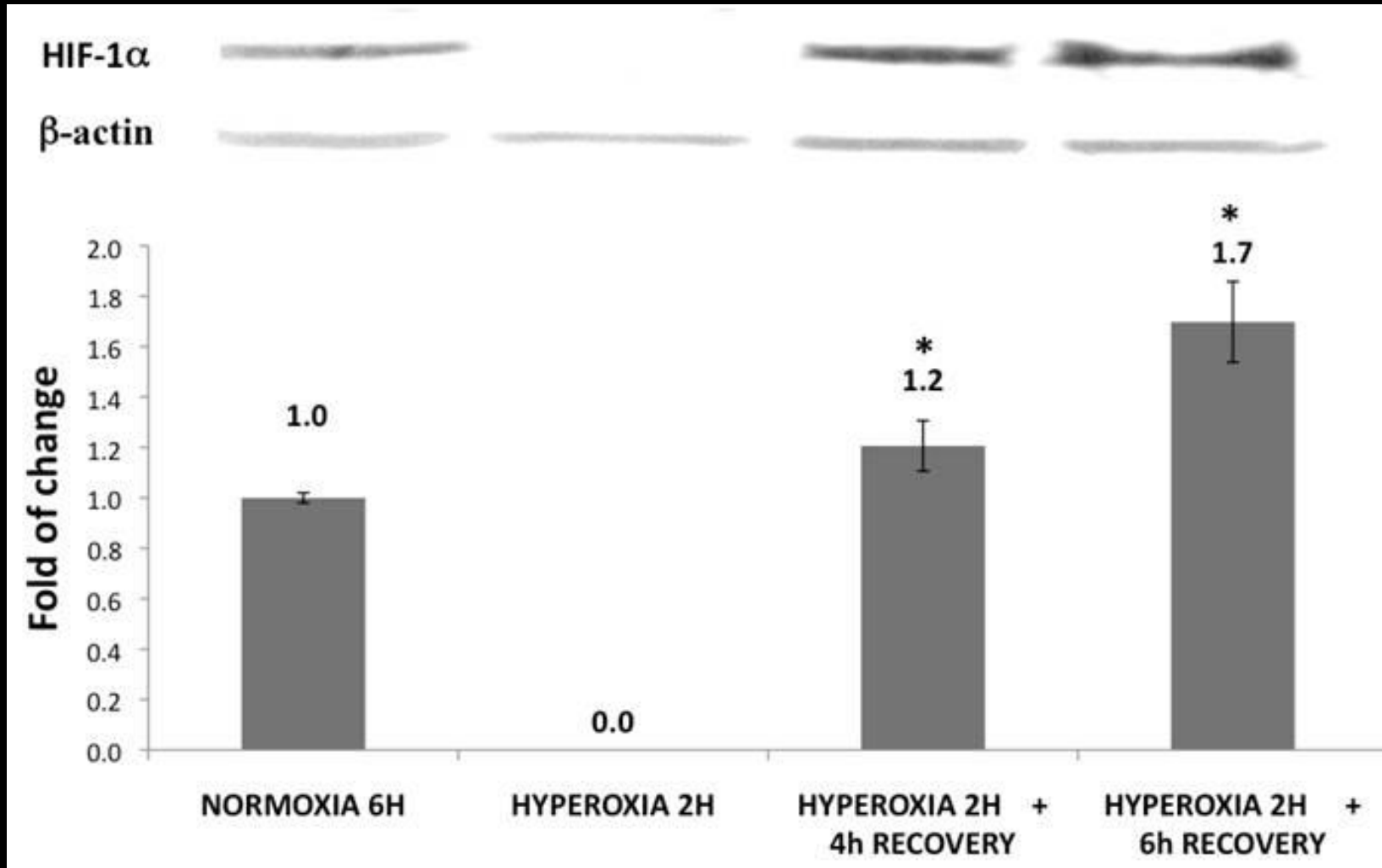


Hb variation after different PO<sub>2</sub> breathing  
(30 min every other day)  
(100% and 15%)



# HUVEC CELLS

CIMINO F, BALESTRA C, GERMONPRE P, DE BELS D, TILLMANS F, SAIJA A, SPECIALE A & VIRGILI F. (2012). PULSED HIGH OXYGEN INDUCES A HYPOXIC-LIKE RESPONSE IN HUMAN UMBILICAL ENDOTHELIAL CELLS (HUVECS) AND IN HUMANS. *JOURNAL OF APPLIED PHYSIOLOGY*.



# INTERMITTENT OXYGEN HYPOXIA-HYPEROXIA

## Pulsed high oxygen induces a hypoxic-like response in human umbilical endothelial cells and in humans

F. Cimino,<sup>1\*</sup> C. Balestra,<sup>2\*</sup> P. Germonpré,<sup>2,4</sup> D. De Bels,<sup>2,3</sup> F. Tillmans,<sup>2</sup> A. Saija,<sup>1</sup> A. Speciale,<sup>1</sup> and F. Virgili<sup>5</sup>

<sup>1</sup>Department Farmaco-Biologico, School of Pharmacy, University of Messina, Messina, Italy; <sup>2</sup>Environmental and Occupational Physiology Laboratory, Haute Ecole Paul Henri Spaak, Brussels, Belgium; <sup>3</sup>Intensive Care Department, Brugmann University Hospital, Brussels, Belgium; <sup>4</sup>Hyperbaric Centre, Queen Astrid Military Hospital, Brussels, Belgium; and <sup>5</sup>National Research Institute for Food and Nutrition, Rome, Italy

Submitted 27 July 2012; accepted in final form 1 October 2012

**Cimino F, Balestra C, Germonpré P, De Bels D, Tillmans F, Saija A, Speciale A, Virgili F.** Pulsed high oxygen induces a hypoxic-like response in human umbilical endothelial cells and in humans. *J Appl Physiol* 113: 1684–1689, 2012. First published October 4, 2012; doi:10.1152/jappphysiol.00922.2012.—It has been proposed that relative changes of oxygen availability, rather than steady-state hypoxic or hyperoxic conditions, play an important role in hypoxia-inducible factor (HIF) transcriptional effects. According to this hypothesis describing the “normobaric oxygen paradox”, normoxia following a hyperoxic event is sensed by tissues as an oxygen shortage, upregulating HIF-1 activity. With the aim of confirming, at cellular and at functional level, that normoxia following a hyperoxic event is “interpreted” as a hypoxic event, we report a combination of

transfer, angiogenesis, glycolytic metabolism, proliferation, and apoptosis (17).

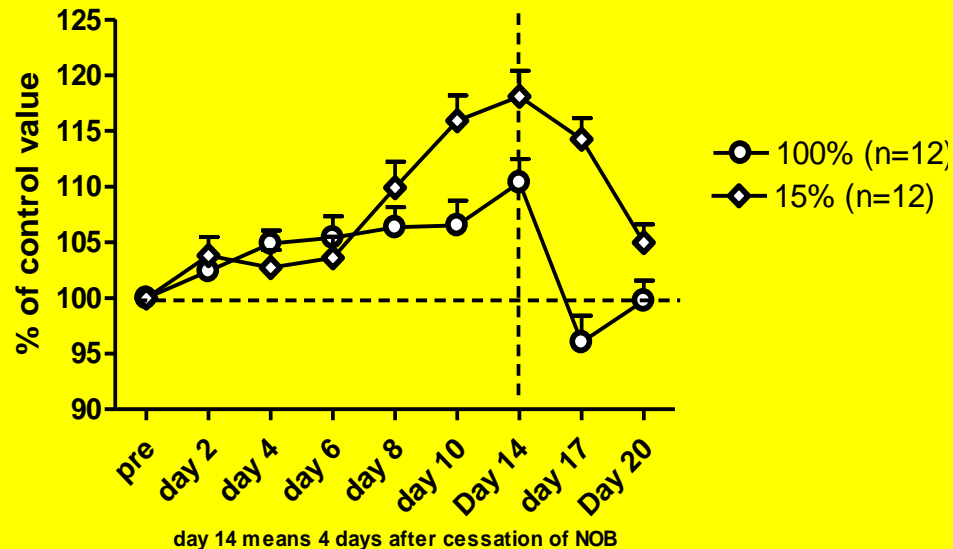
The importance of oxygen concentration sensing by cells in a wide range of cellular responses renders the full understanding of HIF activity an attractive tool to open new avenues in the development of therapeutics able to target HIF pathway, either repressing or activating the expression of a large spectrum of genes in turn involved in a wide spectrum of diseases (29, 30).

According to this pivotal role in metabolism regulation, in the last decade, HIF has been the object of a large number of investigations, which addressed the basis of its mechanism of action. It is established that HIF-1 acts as a heterodimer, consisting of HIF-1 $\alpha$  and HIF-1 $\beta$  subunits. HIF-1 $\alpha$  represents the regulatory subunit that is primarily activated under conditions of oxygen deprivation, when hydroxylation by prolyl and asparaginyl hydroxylases is inhibited. This results in stabilization and transactivation of HIF-1 $\alpha$ , which induces the expression of ~100 target genes by binding to the hypoxia-responsive element (HRE) located in the regulatory DNA sequence (30).

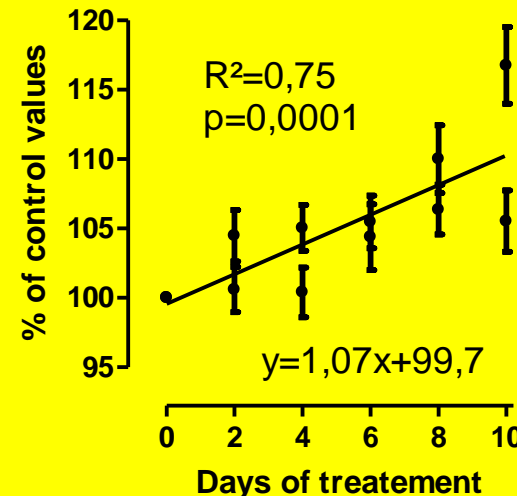
Despite such an established understanding of the basic mechanism of action, some aspects of HIF modulation are still unrevealed. A few years ago, our laboratory proposed a novel mechanism of regulation of HIF activity based on relative changes of oxygen availability rather than on steady-state hypoxic or hyperoxic conditions (5).

On the basis of our experimental observations addressing the effect of rebound relative hypoxia after hyperoxia obtained by normo- and hyperbaric oxygen breathing conditions, we hypothesized that the expression of one of the HIF target genes, erythropoietin (EPO), is modulated by the cellular availability

**Hb Variation after O<sub>2</sub> breathing  
(30 minutes every other day)**



**Hb variation after different PO<sub>2</sub> breathing  
(30 min every other day)  
(100% and 15%)**





# ORTHOPAEDIC PATIENTS

- Traumatic orthopaedic patients (n=80)
- Followed after surgery for 7 days
- Mean age +/- 80
- Randomized in two groups
  - 1 h/day Oxygen
  - 1 h/day Air
- Blinded, no therapeutic change

	Oxygen Group (n=40)	Air Group (n=40)	P
Sex ratio ♀/♂	32/8 (80% of ♀)	30/10 (75% of ♀)	
Age (Years)	80.4 ± 7.43	81.7 ± 8.41	0.4832
Delay to intervention (Days)	2.03 ± 1.03	2.28 ± 0.96	0.2638
Side ratio (Left/Right)	19/21	22/18	
Type of intervention			
Proximal femoral intramedullar nail	31	28	
Hemiarthroplasty	9	12	
Perioperative blood loss (ml)	473 ± 282	424 ± 378	0.3862
Renal function			
Creatinin	52.6 ± 16.5	47.1 ± 21.7	0.1007
Urea	0.853 ± 0.234	0.763 ± 0.237	0.0556
Glomerular filtration (ml/min)	88.4 ± 22.1	80.3 ± 24.8	0.0512
Associated pathologies			
Hypertension	16	19	
Neurologic diseases	14	14	
Cardiopathy	5	6	
Diabetes	8	7	
COPD	4	5	
Transfusion	3	14	0.0052
No transfusion	37	26	

# TRANSFUSION RATE

	Transfusion	No transfusion	Total
Oxygen	3	37	40
Air	14	26	40
Total	17	63	80

$p = 0.019$ , two tailed Fischer's exact test

- $1,2 \pm 0,7$  RBC/patient Air
  - $0,12 \pm 0,44$  RBC/patient  $O_2$
- }  $<0.01$



Original Contribution

## Can the normobaric oxygen paradox (NOP) increase reticulocyte count after traumatic hip surgery?

Pierre Lafère MD (Staff Anesthesiologist)<sup>a,d</sup>, Thomas Schubert MD (Staff Surgeon)<sup>b</sup>,  
David De Bels MD (Assistant Professor of Critical Care Medicine)<sup>c,e,\*</sup>,  
Peter Germonpré MD (Head, Center for Hyperbaric Oxygen Therapy)<sup>d,e</sup>,  
Costantino Balestra PhD (Head, Environmental and Occupational  
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### Keywords:

Allogeneic red blood cell  
transfusion;  
Erythropoietin;  
Hip surgery/Traumatic;  
Normobaric oxygen  
paradox;  
Reticulocytes;  
Transfusion;

### Abstract

**Study Objective:** To determine if the normobaric oxygen paradox (NOP) was effective in increasing reticulocyte count and reducing postoperative requirements for allogeneic red blood cell transfusion after traumatic hip surgery.

**Design:** Prospective, randomized, double blinded, multi-center study.

**Setting:** Surgical wards of two academic hospitals.

**Patients:** 85 ASA physical status 1 and 2 patients undergoing surgery for traumatic hip fracture.

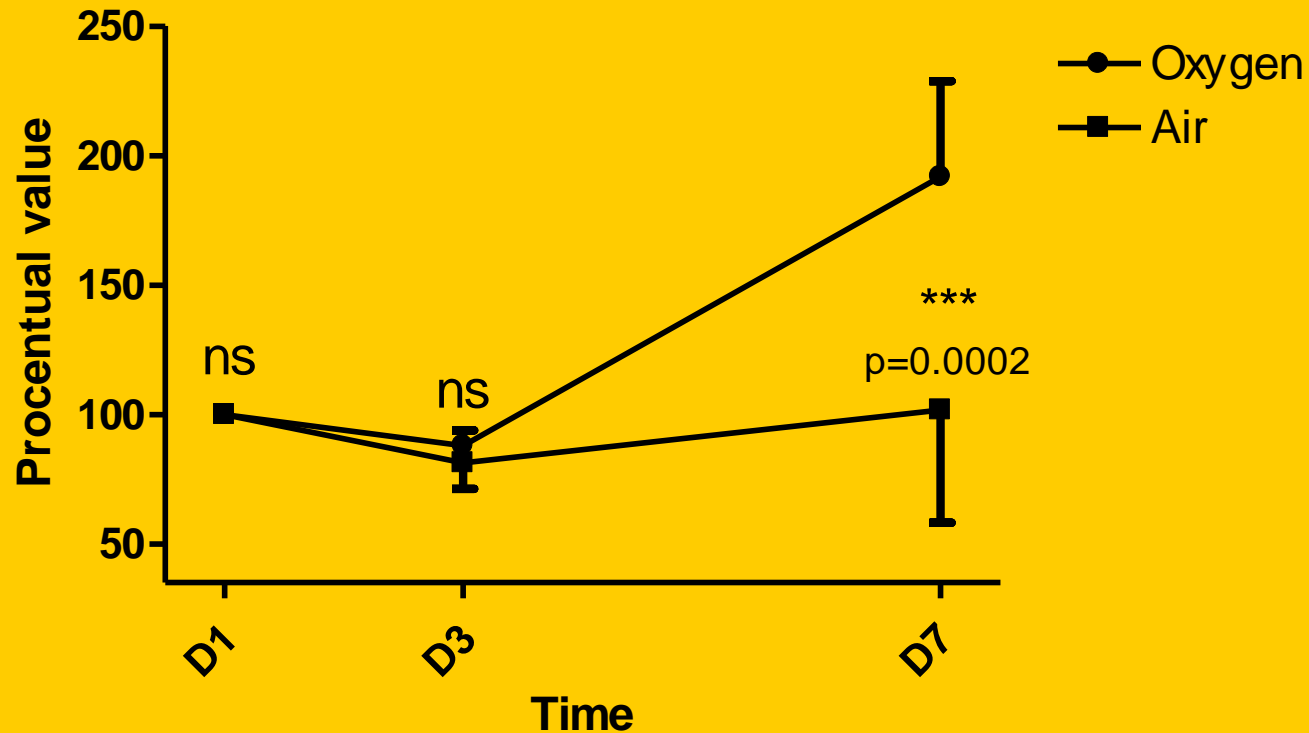
**Interventions:** Patients were randomly assigned to receive 30 minutes of air [air group (control); n = 40] or 30 minutes of 100% oxygen (O<sub>2</sub> group; n = 14) at 15 L/min every day from the first postoperative day (POD 1) until discharge.

**Measurements:** Venous blood samples were taken at admission and after surgery on POD 1, POD 3, and POD 7. Hemoglobin (Hb), hematocrit (Hct), reticulocytes, hemodynamic variables, and transfusion requirements were recorded, as were hospital length of stay (LOS) and mortality.

**Main Results:** Full analysis was obtained for 80 patients. On hospital discharge, the mean increase in reticulocyte count was significantly higher in the O<sub>2</sub> group than the air group. Percent variation also increased: 184.9% ± 41.4% vs 104.7% ± 32.6%, respectively;  $P < 0.001$ . No difference in Hb or Hct levels was noted at discharge. Allogeneic red blood cell transfusion was 7.5% in the O<sub>2</sub> group versus 35% in the air group ( $P = 0.0052$ ). Hospital LOS was significantly shorter in the O<sub>2</sub> group than the air group (7.2 ± 0.7 days vs 7.8 ± 1.6 days, respectively;  $P < 0.05$ ).



## RETICULOCYTE PROCENTUAL COMPARISON



Both Surgeons received  
Congratulations From the  
Blood bank one year after the  
Experiment.....Guess Why??

# Hyperoxia may be beneficial

Enrico Calzia, MD, PhD; Pierre Asfar, MD, PhD; Balász Hauser, MD, PhD; Martin Matejovic, MD, PhD; Costantino Ballestra, PhD; Peter Radermacher, MD, PhD; Michael Georgieff, MD, PhD

The current practice of mechanical ventilation comprises the use of the least inspiratory O<sub>2</sub> fraction associated with an arterial O<sub>2</sub> tension of 55 to 80 mm Hg or an arterial hemoglobin O<sub>2</sub> saturation of 88% to 95%. Early goal-directed therapy for septic shock, however, attempts to balance O<sub>2</sub> delivery and demand by optimizing cardiac function and hemoglobin concentration, without making use of hyperoxia. Clearly, it has been well-established for more than a century that long-term exposure to pure O<sub>2</sub> results in pulmonary and, under hyperbaric conditions, central nervous O<sub>2</sub> toxicity. Nevertheless, several arguments support the use of ventilation with 100% O<sub>2</sub> as a supportive measure during the first 12 to 24 hrs of septic shock. In contrast to patients without lung disease undergoing anesthesia, ventilation with 100% O<sub>2</sub> does not worsen intrapulmonary shunt under conditions of hyperinflammation, particularly when low tidal volume–high positive end-expiratory pressure ventilation is used. In healthy volunteers and experimental animals, exposure to hyperoxia may cause pulmonary inflammation, enhanced oxidative stress, and tissue apoptosis. This, however, requires long-term exposure or injurious tidal volumes. In contrast, within the timeframe of a peri-

operative administration, direct O<sub>2</sub> toxicity only plays a negligible role. Pure O<sub>2</sub> ventilation induces peripheral vasoconstriction and thus may counteract shock-induced hypotension and reduce vasopressor requirements. Furthermore, in experimental animals, a redistribution of cardiac output toward the kidney and the hepato-splanchnic organs was observed. Hyperoxia not only reverses the anesthesia-related impairment of the host defense but also is an antibiotic. In fact, perioperative hyperoxia significantly reduced wound infections, and this effect was directly related to the tissue O<sub>2</sub> tension. Therefore, we advocate mechanical ventilation with 100% O<sub>2</sub> during the first 12 to 24 hrs of septic shock. However, controlled clinical trials are mandatory to test the safety and efficacy of this approach. (Crit Care Med 2010; 38[Suppl.]:S559–S568)

**KEY WORDS:** early goal-directed therapy; septic shock; vasoconstrictor; intrapulmonary shunt; hypoxic pulmonary vasoconstriction; oxygen radicals; oxidative stress; oxygen toxicity; nitric oxide; apoptosis; leukocyte endothelial interaction; normobaric oxygen paradox; erythropoietin

## Original articles

### The 'normobaric oxygen paradox': does it increase haemoglobin?

David De Bels, Sigrid Theunissen, Jacques Devriendt, Peter Germonpré, Pierre Lafere, Joseph Valsamis, Thyl Snoeck, Philippe Meeus and Costantino Balestra

#### Abstract

(De Bels D, Theunissen S, Devriendt J, Germonpré P, Lafere P, Valsamis J, Snoeck T, Meeus P, Balestra C. The 'normobaric oxygen paradox': does it increase haemoglobin? *Diving and Hyperbaric Medicine*. 2012;42(2):67-71.)

**Background:** A novel approach to increasing erythropoietin (EPO) using oxygen (O<sub>2</sub>) (the 'normobaric oxygen paradox') has been reported in healthy volunteers. We investigated whether the EPO increase is sufficient to induce erythropoiesis by comparing two protocols of O<sub>2</sub> administration.

**Methods:** We compared the effect of daily versus alternate days 100% O<sub>2</sub>, breathed for 30 minutes, on haemoglobin concentrations during a 12-day period. Nine subjects underwent the two protocols six weeks apart.

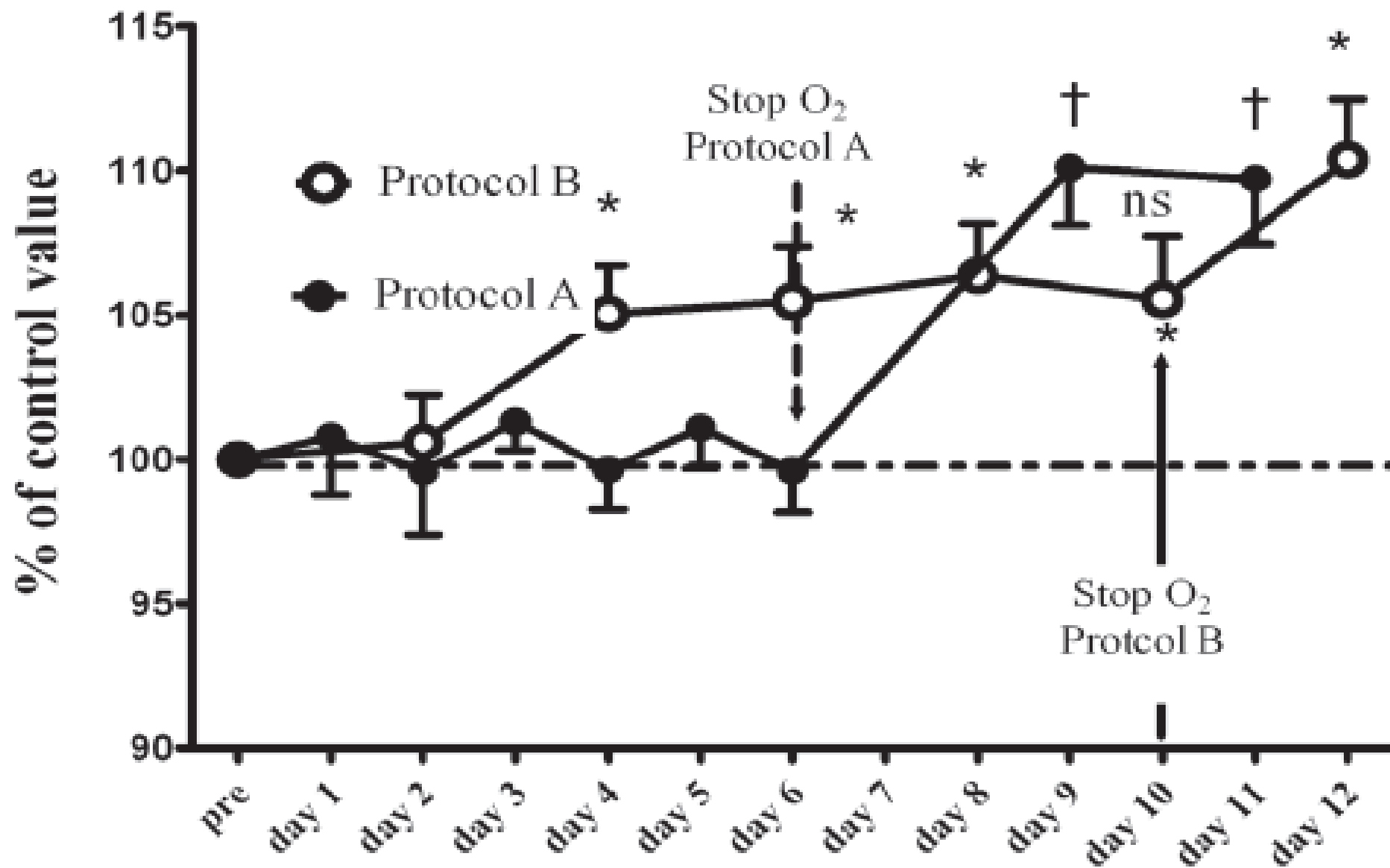
**Results:** We observed a significant increase in haemoglobin (as a percentage of baseline) in the alternate-days group compared to the daily group and to baseline after four days ( $105.5 \pm 5.7\%$  vs.  $99.6 \pm 3.3\%$  difference from baseline;  $P < 0.01$ ). At the end of the experimental period, haemoglobin values increased significantly compared to baseline in both groups. There was a significant percentage rise in reticulocyte count in the alternate-days group compared to the daily group ( $182 \pm 94\%$  vs.  $93 \pm 34\%$ ;  $P < 0.001$ ).

**Conclusion:** The normobaric oxygen paradox seems effective in increasing haemoglobin in non-anaemic, healthy volunteers, providing sufficient time is allowed between O<sub>2</sub> applications. The exact time interval is not clearly defined by this study but should probably be at least or greater than two days. Further studies are needed to define more precisely clinical applications in the use of O<sub>2</sub> as a pharmaceutical agent.

#### Key words

Oxygen, haematology, reactive oxygen species (ROS), physiology

Comparison baseline haemoglobin after 30 minutes of 100% O<sub>2</sub> breathing daily (protocol A; †  $P < 0.01$ ) or on alternate days (protocol B; \*  $P < 0.01$ ); ns – not significant





# Oxygen Sensing, Homeostasis, and Disease

**TO THE EDITOR:** Two points could have been added to the review article by Semenza (Aug. 11 issue)<sup>1</sup> on a fundamental mechanism in cell, tissue, and organ metabolism. First, considering the metabolic signals modifying signal transduction, the author mentions hypoxia as the sole trigger to expression of hypoxia-inducible factor 1 (HIF-1). One study<sup>2</sup> pointed out the possible role of nonhypoxic partial pressure of oxygen (PO<sub>2</sub>) variation as a trigger to HIF-1 $\alpha$ . In this model, transient normobaric hyperoxia followed by a return to normoxia led to a significant increase in serum erythropoietin concentration. This increase began 8 hours after the return to normoxia, which is consistent with the time frame of gene expression.

Second, in the section on co-opted adaptation to hypoxia in cancer, the author points out that intratumoral hypoxia is associated with increased risks of metastasis and death. This finding has been the trigger for research on the consequences of hyperoxia on cancer-cell survival. A recent hypothesis<sup>3</sup> is that transient normobaric hyperoxia could be deleterious for tumor cells and, in parallel, beneficial (cytoprotective) for normal cells; this could open interesting therapeutic options in adjuvant treatments against cancer.

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No potential conflict of interest relevant to this letter was reported.

1. Semenza GL. Oxygen sensing, homeostasis, and disease. *N Engl J Med* 2011;365:537-47. [Erratum, *N Engl J Med* 2011; 365:968.]

2. Balestra C, Germonpré R, Poortmans JR, Marroun A. Serum erythropoietin levels in healthy humans after a short period of normobaric and hyperbaric oxygen breathing: the "normobaric oxygen paradox." *J Appl Physiol* 2006;100:S12-8.

3. De Bels D, Corazza F, Germonpré R, Balestra C. The normobaric oxygen paradox: a novel way to administer oxygen as an adjuvant treatment for cancer? *Med Hypotheses* 2011;76:467-70.

**TO THE EDITOR:** The review by Semenza examines the role of HIF-1 $\alpha$  in the context of hypoxia and ischemia. However, we think that the role of HIF-1 $\alpha$  can be seen from another point of view. HIF-1 $\alpha$  does not appear to be the clear hypoxic marker.<sup>1</sup> It is stabilized not only because of hypoxia but also by metabolic imbalances, induced growth, and lower intracellular pH values. The curious association of metabolic pathways and different metabolites<sup>2</sup> with this marker causes doubt about the function of HIF-1 as a molecular regulator to oxygen deprivation. The fact that hypoxia always decreases the intracellular pH value<sup>3</sup> discloses another face of HIF-1 beyond the phenomenon called hypoxia. The biologic function of this protein is rather that of a sensitive controller and regulator of an adaptive metabolic response to alterations of the pH value. That this function is independent from the oxygen status of cells is reflected by a multitude of HIF-1 detections at normoxia.<sup>4</sup> Altogether, we suggest that HIF-1 is mainly a sensor and regulator for the intracellular pH value.

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EXPERT OPINION

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## The “Normobaric Oxygen Paradox”: a new tool for the anesthetist?

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### ABSTRACT

Hypoxia is the natural trigger for endogenous EPO production but recently the use of intermittent hyperoxia to stimulate EPO has been postulated and this phenomenon has been called the “normobaric oxygen paradox” (NOP). The “NOP” is a mechanism by which oxygen regulates the expression of the Hypoxia Inducible Factor 1 alpha (HIF-1 $\alpha$ ). The HIF-1 $\alpha$ -depending gene regulation is responsible for many different genetic expressions including EPO and VEGF. It has been proposed that relative changes of oxygen availability rather than steady state hypoxic or hyperoxic conditions, play an important role in HIF transcriptional effects. According to this hypothesis, the cell interprets the return to normoxia after a hyperoxic event as an oxygen shortage, and induces HIF-1-regulated gene synthesis, including EPO. Being both a hormone and a cytokine, the actual actions of EPO are complex; its clinical utility has been postulated for neuroprotection and cardioprotection. The precise level of inspired oxygen and the exact time-frame for its iterative administration are not totally known. N-Acetyl-L-Cysteine (NAC) supplementation has been shown to help. All the reported data demonstrate how hyperoxic and hypoxic states can potentially be manipulated if oxygen is been considered as a multifaceted molecule more than just a gas.

(*Minerva Anestesiol* 2014;80:366-72)

**Key words:** Oxygen - Hyperoxia - Erythropoietin.

NAC - I - O<sub>2</sub>



**Dico ergo** ad qñem q  
qz pluralitas  
non est ponenda sine necessitate ⁊ non  
ē necessitas quare debeat poni tpus di-  
cretum mensurās motum angeli. naz

« *Pluralitas non  
est ponenda sine  
necessitate* »





## The normobaric oxygen paradox: A novel way to administer oxygen as an adjuvant treatment for cancer?

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### ARTICLE INFO

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### ABSTRACT

The “normobaric oxygen paradox” is a dual mechanism by which oxygen regulates the expression of the Hypoxia Inducible Factor 1 alpha (HIF-1 $\alpha$ ). The HIF-1 $\alpha$ -depending gene regulation is responsible for many different genetic expressions including EPO and VEGF that are usually expressed in parallel.

First, VEGF under-expression could decrease tumor angiogenesis leading to a decrease in tumor growth or even apoptosis of cancer cells.

Second, induction of EPO-expression can provide cytoprotection.

Altogether, this could be deleterious for cancer cells while helping non-malignant cells (at least neural and cardiac) cells to be protected from the side effects of chemotherapy. Eventually, HIF induction could boost immune response by inflammatory cells, increasing their antitumor activity.

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### Introduction

Cancer still remains one of the leading causes of death worldwide. The therapeutic arsenal is regularly increasing and most chemotherapeutic agents aim at inducing selective apoptosis of cancer cells. A deficit in the apoptotic machinery can be seen in more than 50% of cancers [1]. The “normobaric oxygen paradox” seems to have a beneficial effect on chemotherapy-induced anemia [2,3] while inducing modifications in the cellular mechanisms of adaptation to hypoxia.

### Hypothesis

hypoxia. This latter depends on oxygen free radicals availability. In fact, in presence of reactive oxygen species (ROS) and under normoxic conditions, the hypoxia Inducible Factor 1 alpha, (HIF-1 $\alpha$ ) is hydroxylated by prolyl-hydroxylase. This results in ubiquitylation by the Von Hippel Lindau tumor suppressing Protein (VHLp) and finally in the degradation of HIF-1 $\alpha$  in the proteasome (see Fig. 1). In case of limited availability or absence of reactive oxygen species, the HIF-1 $\alpha$  will not link with VHLp and thus can be dimerized with the HIF-1 $\beta$ . This HIF complex can thus bind to target promoters known as hypoxia responsive elements leading to the transcription of the erythropoietin gene as well as many other genes involved in cellular metabolism [5].

## Research article

## Open Access

**Hyperoxia retards growth and induces apoptosis and loss of glands and blood vessels in DMBA-induced rat mammary tumors**

Anette Raa<sup>1</sup>, Christine Stansberg<sup>2,3</sup>, Vidar M Steen<sup>2,3</sup>, Rolf Bjerkvig<sup>1,4</sup>,  
Rolf K Reed<sup>1</sup> and Linda EB Stuhr<sup>\*1</sup>

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Email: Anette Raa - [anette.raa@biomed.uib.no](mailto:anette.raa@biomed.uib.no); Christine Stansberg - [christine.stansberg@helse-bergen.no](mailto:christine.stansberg@helse-bergen.no);  
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Published: 30 January 2007

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RAA A, STANSBERG C, STEEN VM, BJERKVIG R, REED RK & STUHR LE. (2007).  
HYPEROXIA RETARDS GROWTH AND INDUCES APOPTOSIS AND LOSS OF GLANDS AND  
BLOOD VESSELS IN DMBA-INDUCED RAT MAMMARY TUMORS. *BMC CANCER* 7, 23.

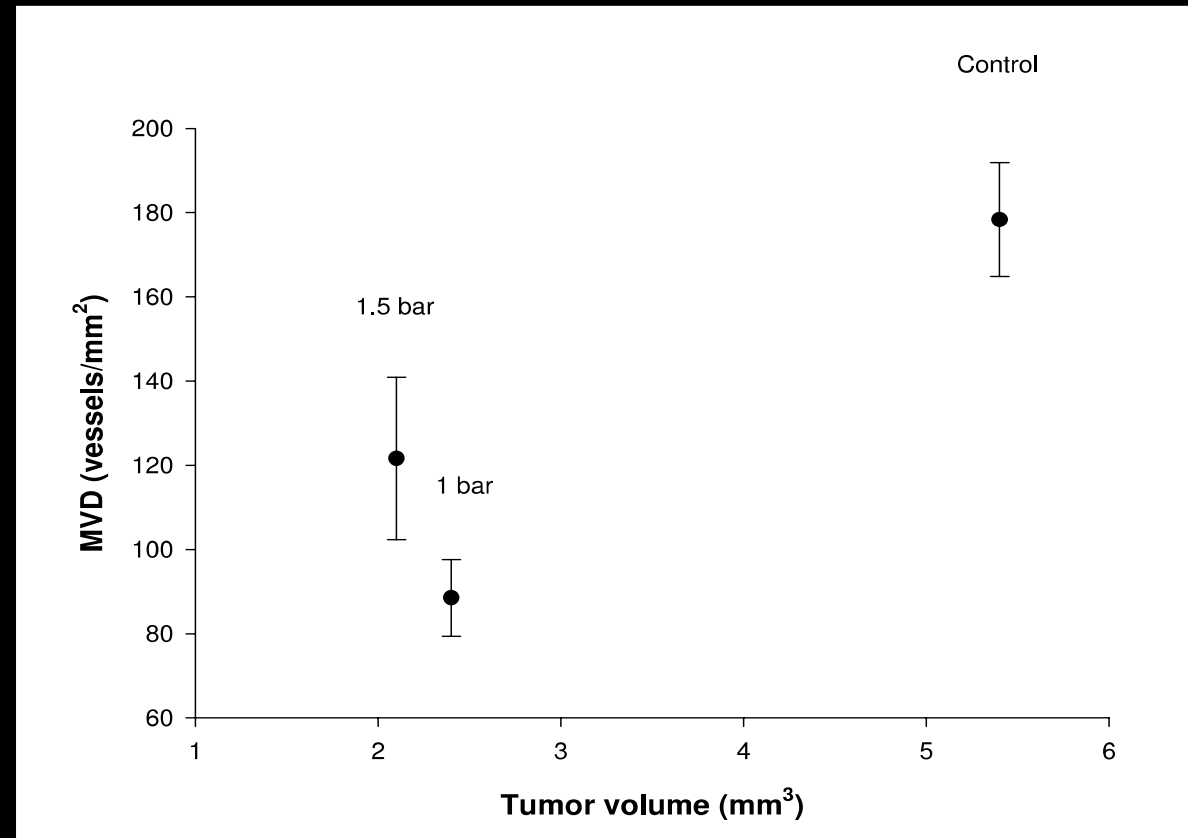
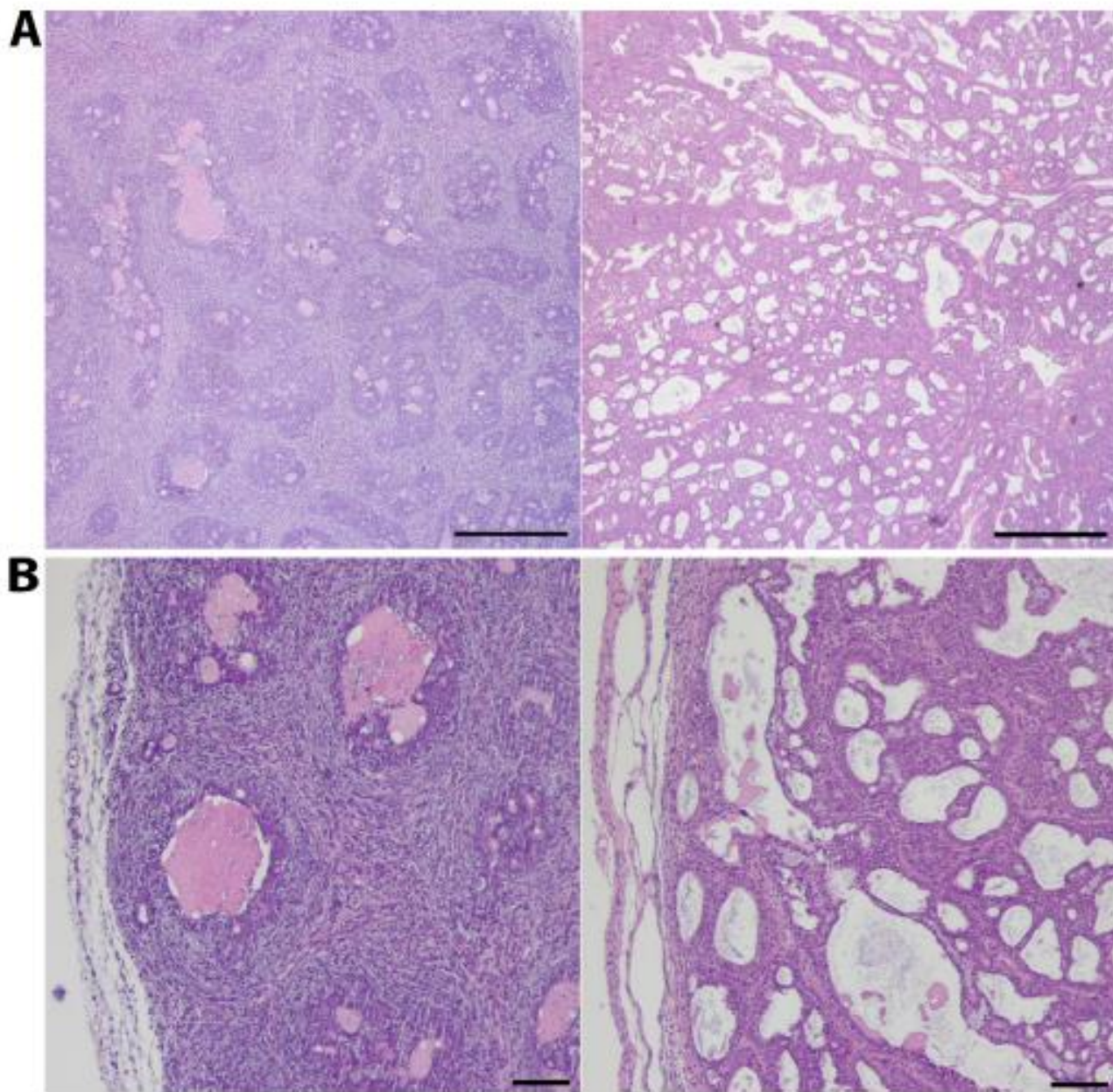
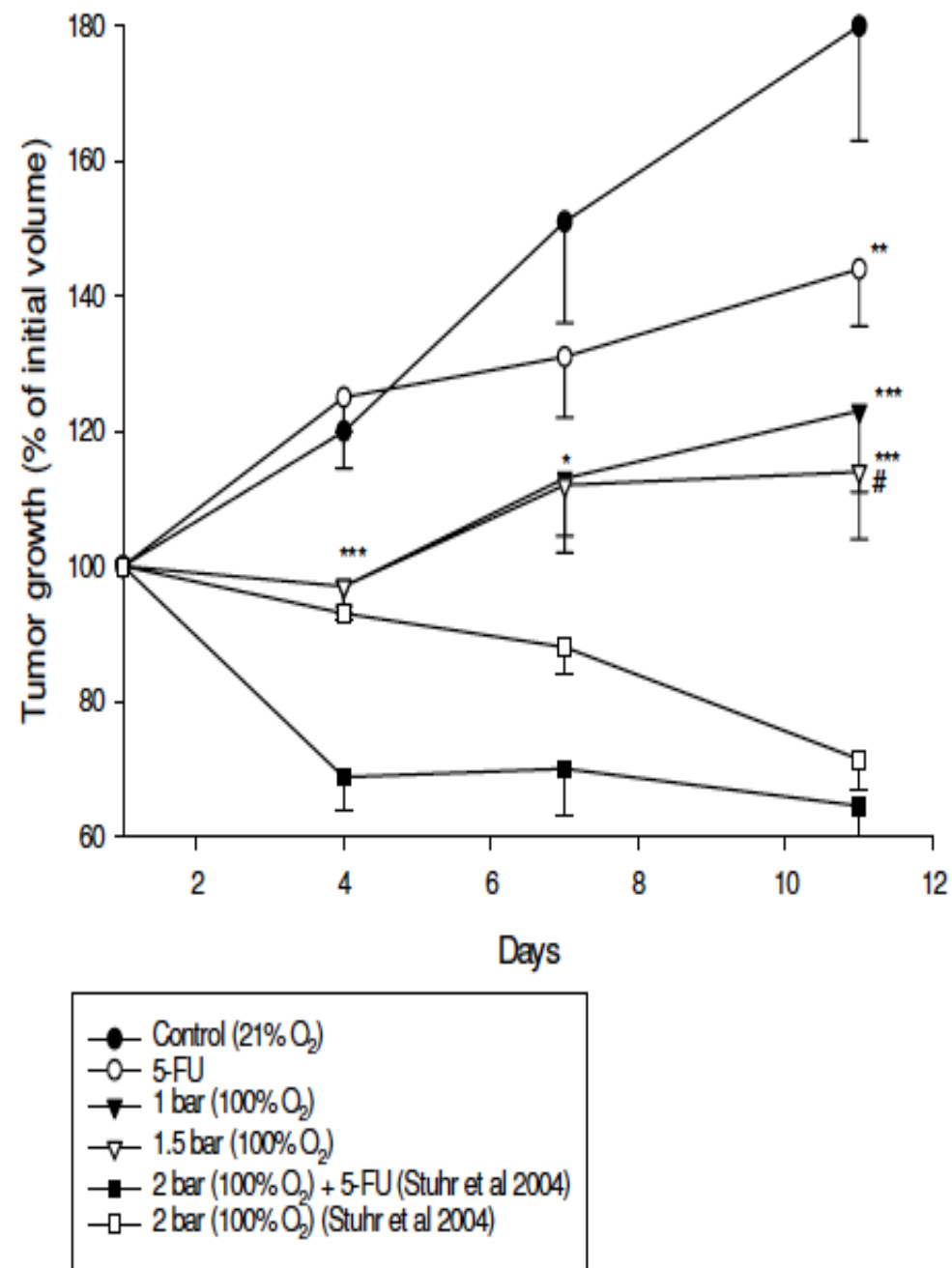


Figure 2 Relationship between tumor size and blood vessels per





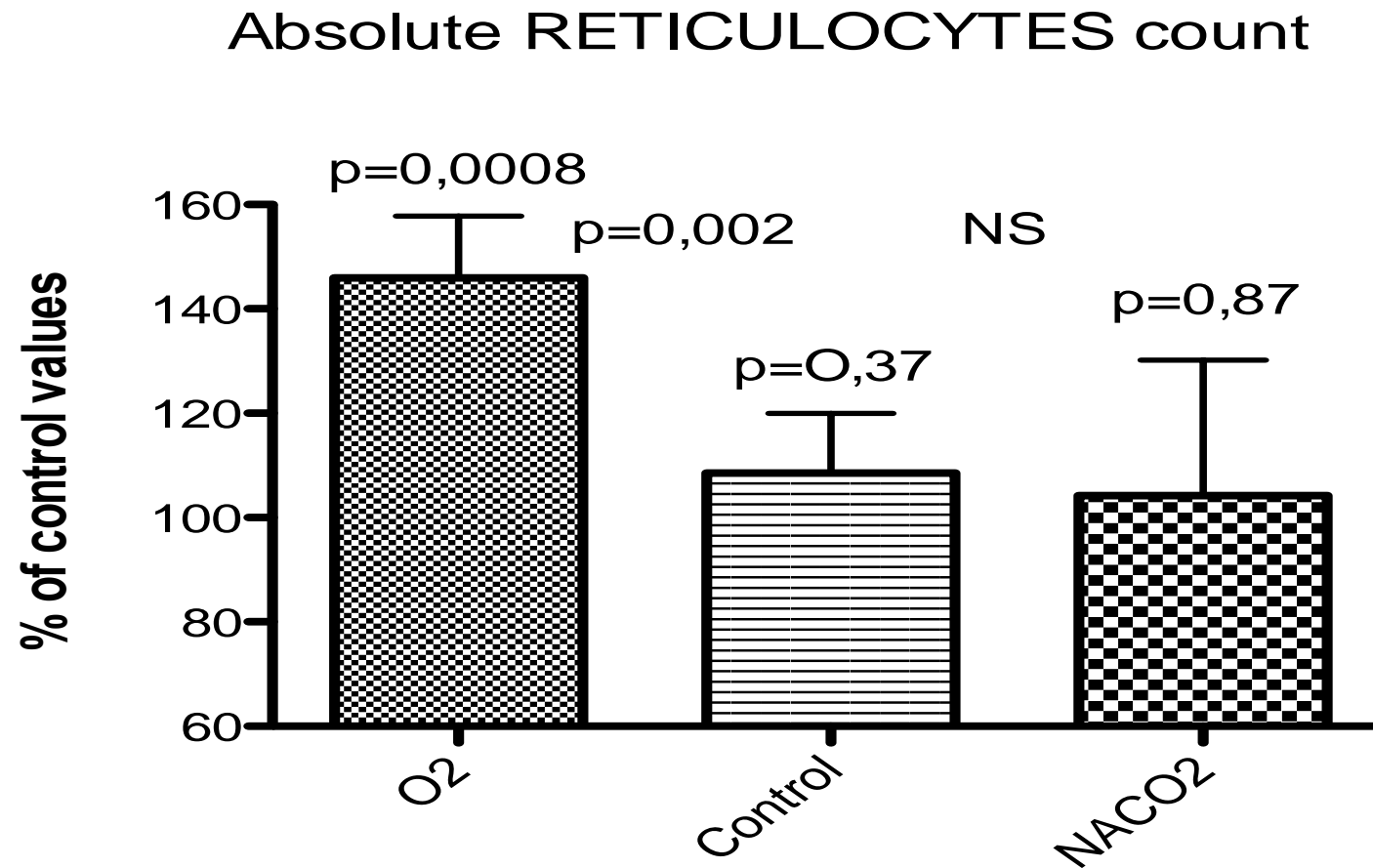
**Figure 2**  
Examples of eosin-hematoxylin stained tumor-tissue of the central (A) and peripheral (B) part of the mammary tumor in control (left) and during hyperoxic treatment (right, 1 bar,  $pO_2 = 1.0$ ). The images under A are scaled to the same magnification ( $\times 4$ ) and the images under B to the same magnification ( $\times 10$ ). Scale bar indicate 500  $\mu m$  (A) and 100  $\mu m$  (B).





# ABDOMINAL ONCOLOGIC PATIENTS

N=60



# FRACTAL DIMENSION OF LEUKEMIC CELLS AFTER HYPEROXIA

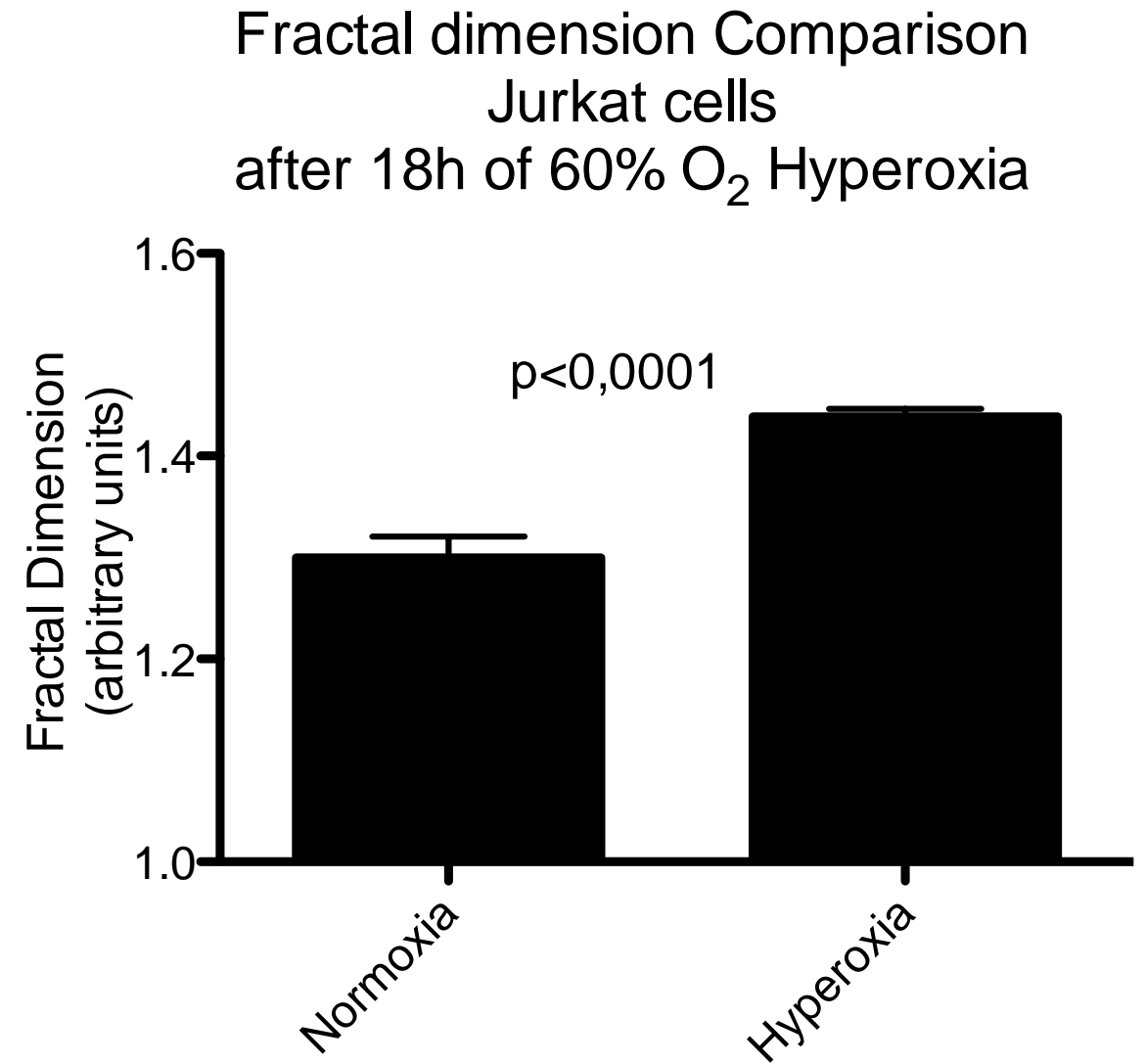
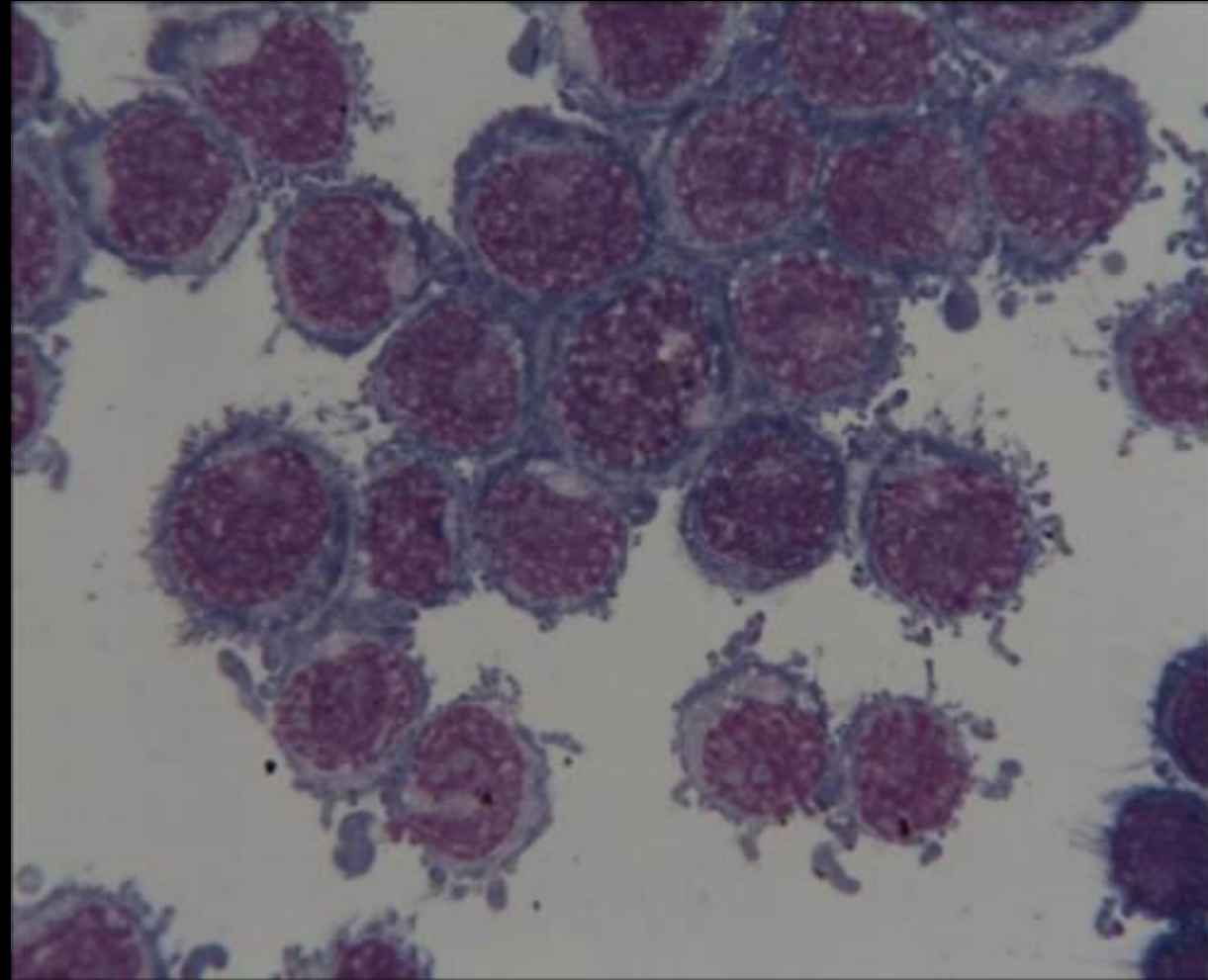
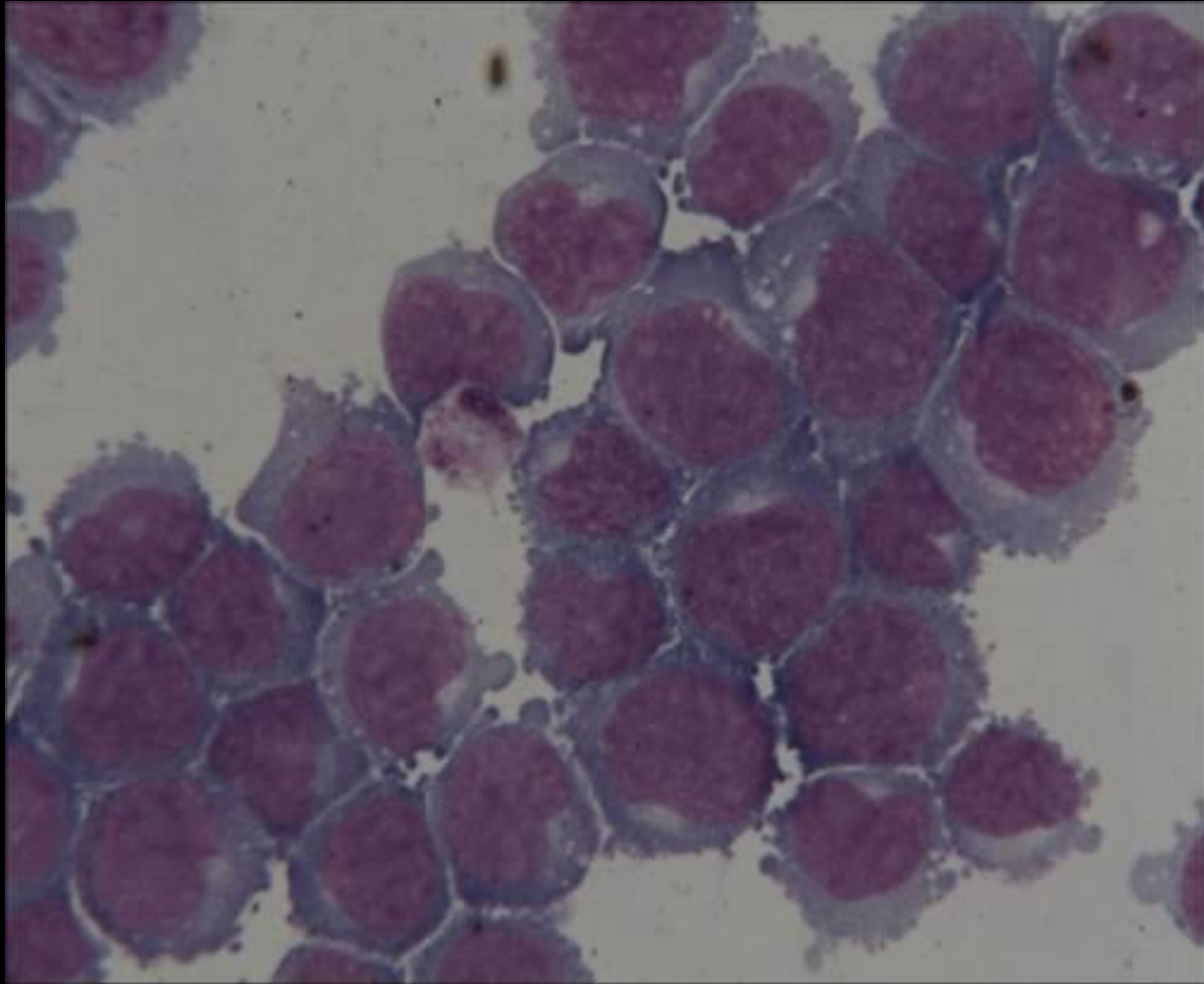
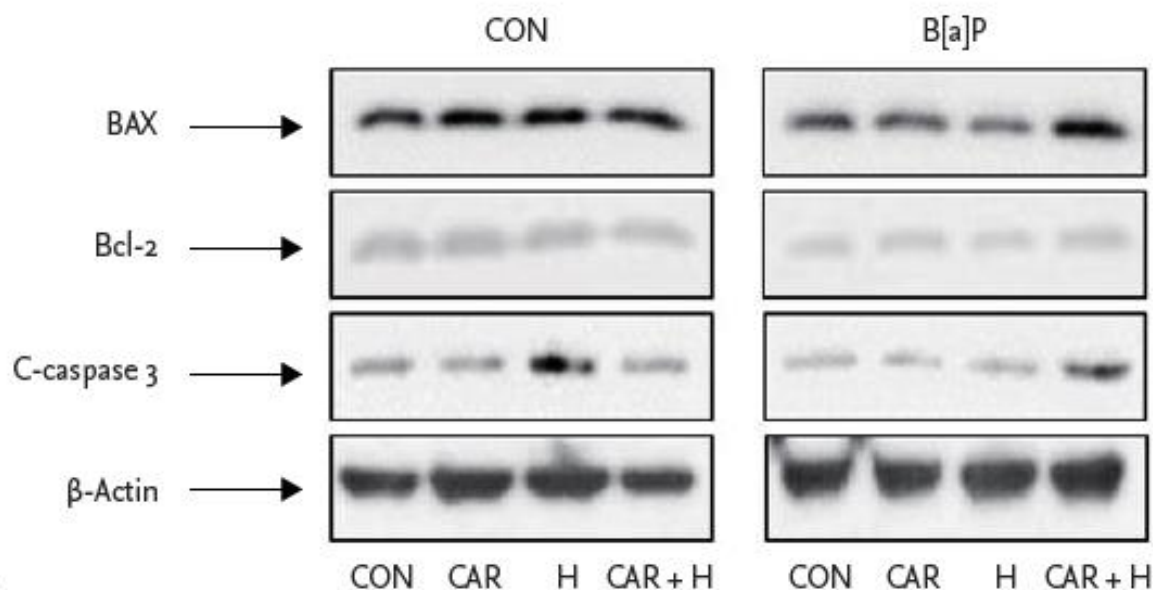


Figure 4



50x



## KEY MESSAGE

1. Intermittent normobaric hyperoxia with carboplatin displays a synergistic tumoricidal effect in a mouse lung cancer model.
2. Addition of hyperoxia to chemotherapy enhanced oxidative stress, which is considered to induce cell death mainly via apoptosis.
3. Intermittent normobaric hyperoxia may be a useful adjuvant therapy for lung cancer.

## ORIGINAL ARTICLE

Korean J Intern Med 2018;33:541-551  
<https://doi.org/10.3904/kjim.2016.334>



KJIM

# Combination of carboplatin and intermittent normobaric hyperoxia synergistically suppresses benzo[a]pyrene-induced lung cancer

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**Background/Aims:** We explored the effects of intermittent normobaric hyperoxia alone or combined with chemotherapy on the growth, general morphology, oxidative stress, and apoptosis of benzo[a]pyrene (B[a]P)-induced lung tumors in mice.

**Methods:** Female A/J mice were given a single dose of B[a]P and randomized into four groups: control, carboplatin (50 mg/kg intraperitoneally), hyperoxia (95% fraction of inspired oxygen), and carboplatin and hyperoxia. Normobaric hyperoxia (95%) was applied for 3 hours each day from weeks 21 to 28. Tumor load was determined as the average total tumor numbers and volumes. Several markers of oxidative stress and apoptosis were evaluated.

**Results:** Intermittent normobaric hyperoxia combined with chemotherapy reduced the tumor number by 59% and the load by 72% compared with the control B[a]P group. Intermittent normobaric hyperoxia, either alone or combined with chemotherapy, decreased the levels of superoxide dismutase and glutathione and increased the levels of catalase and 8-hydroxydeoxyguanosine. The Bax/Bcl-2 mRNA ratio, caspase 3 level, and number of transferase-mediated dUTP nick end-labeling positive cells increased following treatment with hyperoxia with or without chemotherapy.

**Conclusions:** Intermittent normobaric hyperoxia was found to be tumoricidal and thus may serve as an adjuvant therapy for lung cancer. Oxidative stress and its effects on DNA are increased following exposure to hyperoxia and even more with chemotherapy, and this may lead to apoptosis of lung tumors.

**Keywords:** Apoptosis; Carboplatin; Hyperoxia; Lung neoplasms; Oxidative stress

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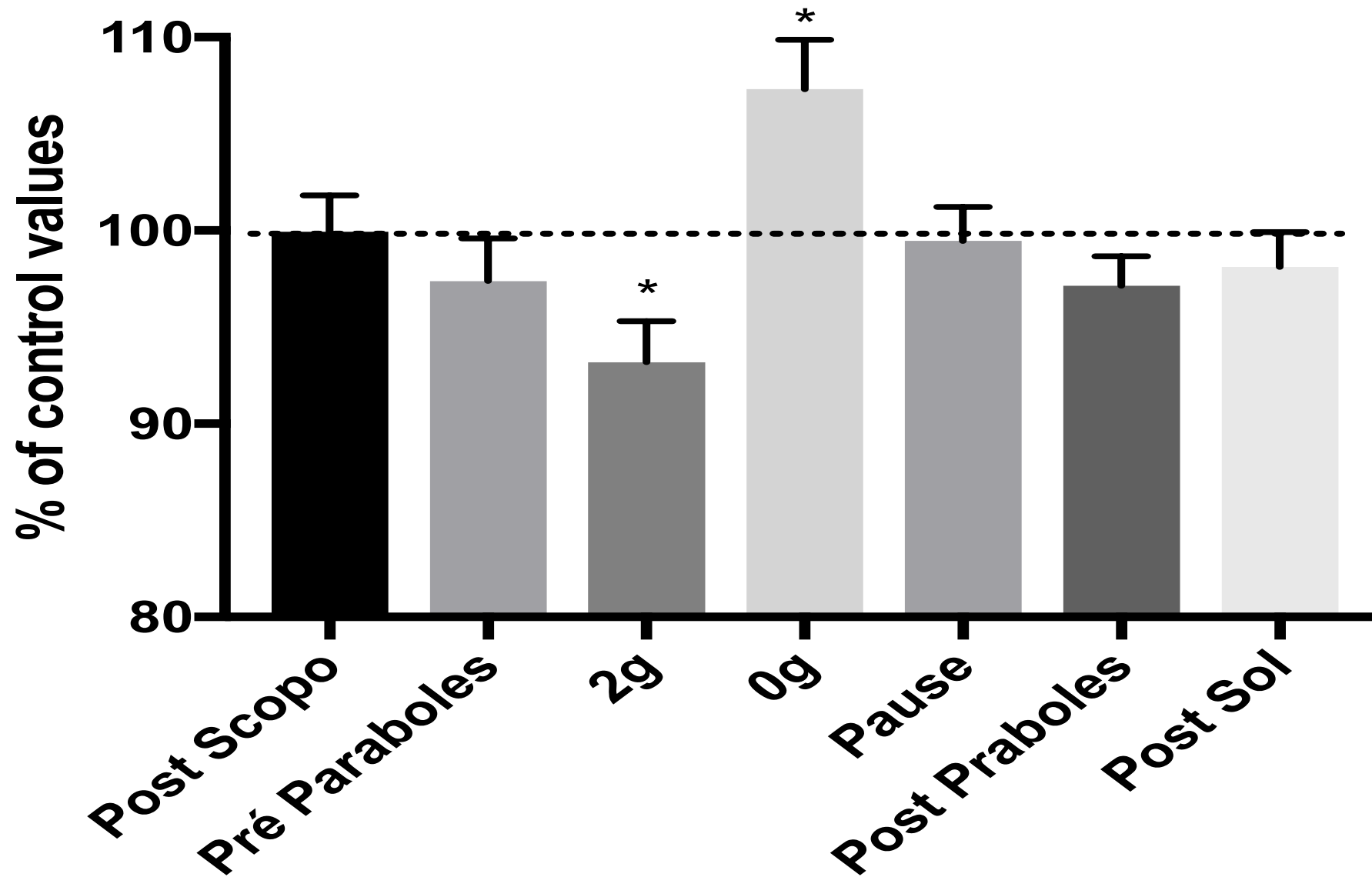








# Parabolic flights



# Functional comparison between critical flicker fusion frequency and simple cognitive tests in subjects breathing air or oxygen in normobaria

Walter Hemelryck, Miroslav Rozloznik, Peter Germonpré, Costantino Balestra and Pierre Lafère

## Abstract

(Hemelryck W, Rozloznik M, Germonpré P, Balestra C, Lafère P. Functional comparison between critical flicker fusion frequency and simple cognitive tests in subjects breathing air or oxygen in normobaria. *Diving and Hyperbaric Medicine*. 2013 September;43(3):138-142.)

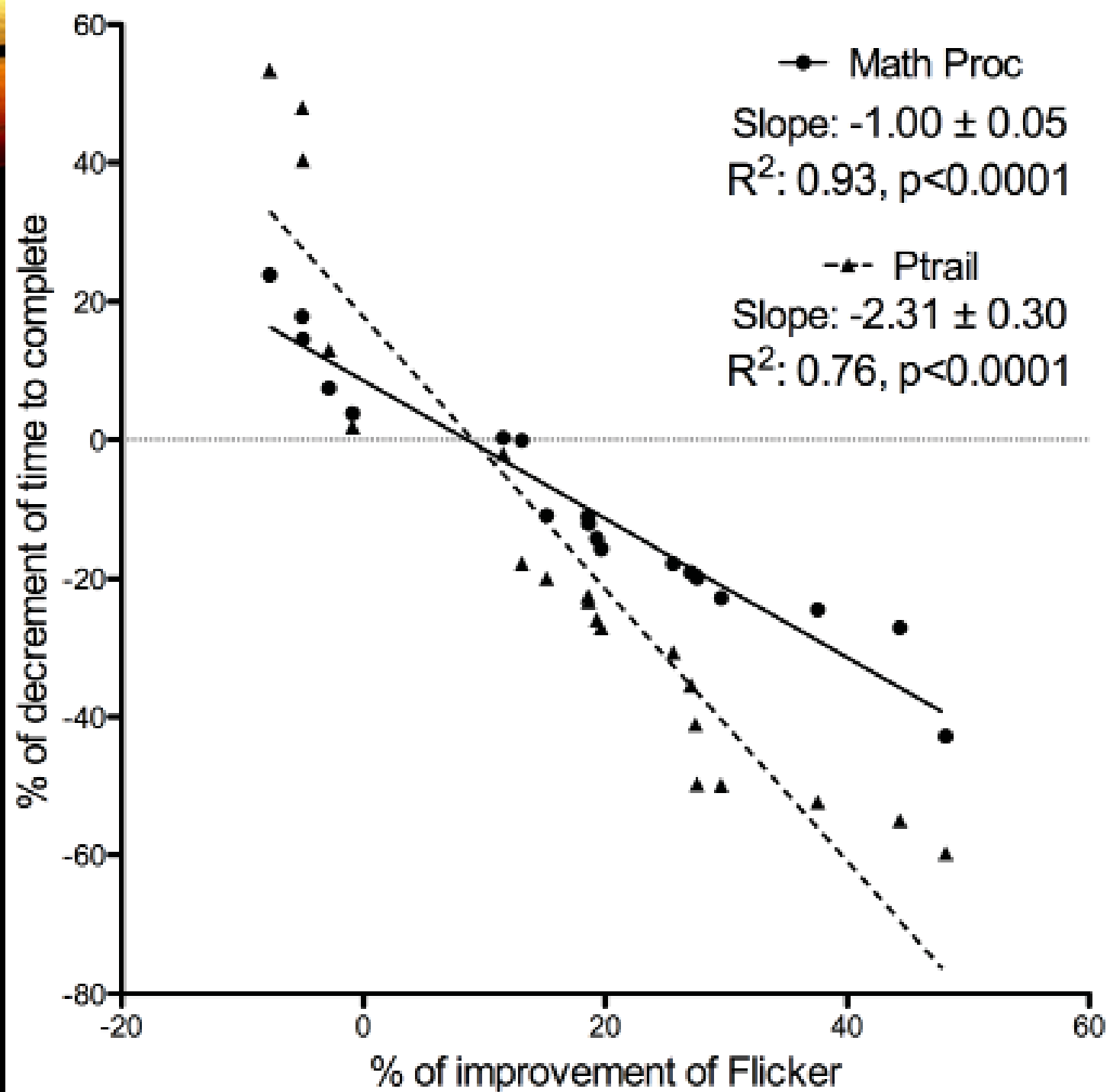
**Introduction:** Measurement of inert gas narcosis and its degree is difficult during operational circumstances, hence the need for a reliable, reproducible and adaptable tool. Although being an indirect measure of brain function, if reliable, critical flicker fusion frequency (CFFF) could address this need and be used for longitudinal studies on cortical arousal in humans.

**Methods:** To test the reliability of this method, the comparison between CFFF and three tests (Math-Processing Task, Trail-Making Task, and Perceptual Vigilance Task) from the Psychology Experiment Building Language battery (PEBL) were used to evaluate the effect of 10 minutes of 100% normobaric oxygen breathing on mental performance in 20 healthy male volunteers.

**Results:** Breathing normobaric oxygen significantly improved all but one of the measured parameters, with an increase of CFFF ( $117.3 \pm 10.04\%$  of baseline,  $P < 0.0001$ ) and a significant reduction of time to complete in both the math-processing ( $2,103 \pm 432.1$  ms to  $1,879 \pm 417.5$  ms,  $P = 0.0091$ ) and trail-making tasks ( $1,992 \pm 715.3$  to  $1,524 \pm 527.8$  ms,  $P = 0.0241$ ). The magnitude of CFFF change and time to completion of both tests were inversely correlated (Pearson  $r = -0.9695$  and  $-0.8731$  respectively,  $P < 0.0001$ ). The perceptual vigilance task did not show a difference between air and  $O_2$  ( $P > 0.4$ ).

**Conclusions:** The CFFF test provides an assessment of cognitive function that is similar to some tests from PEBL, but requires a less complicated set up and could be used under various environmental conditions including diving. Further research is needed to assess the combined effects of increased pressure and variations in inspired gas mixtures during diving.





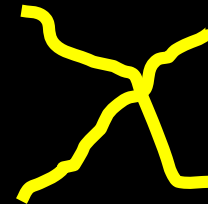
# NARCOSIS TEST

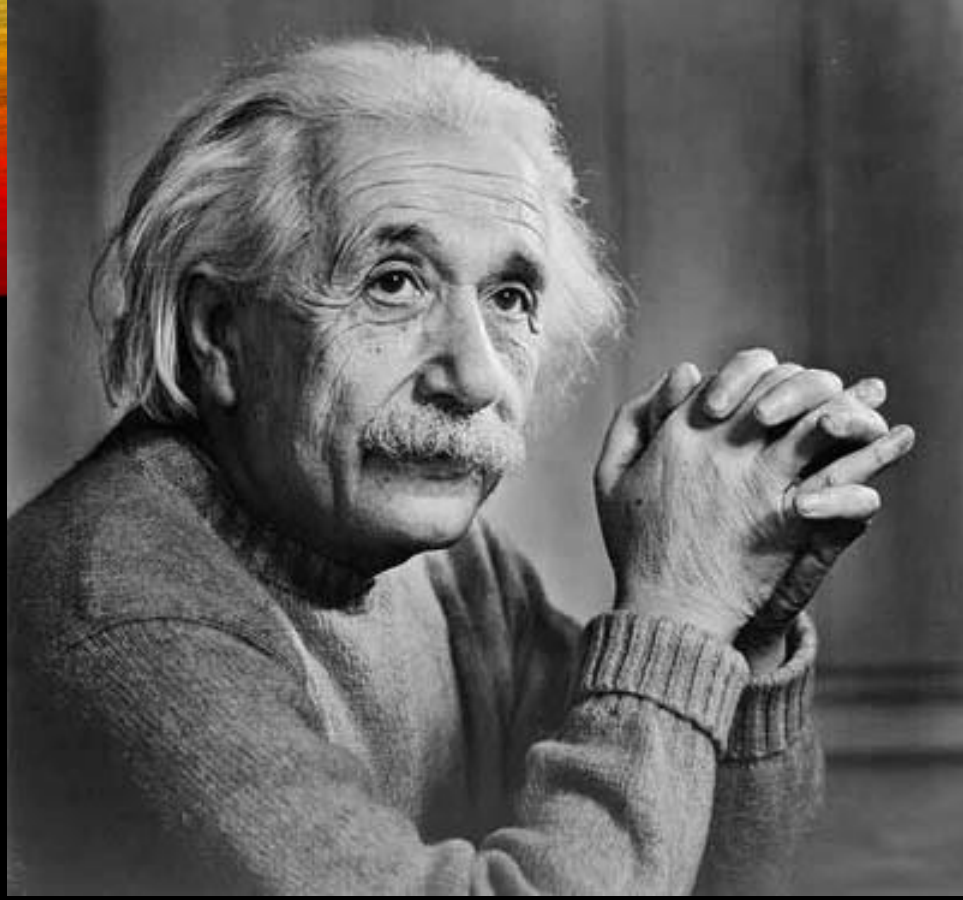
Are You Drunk ?

YES

☐

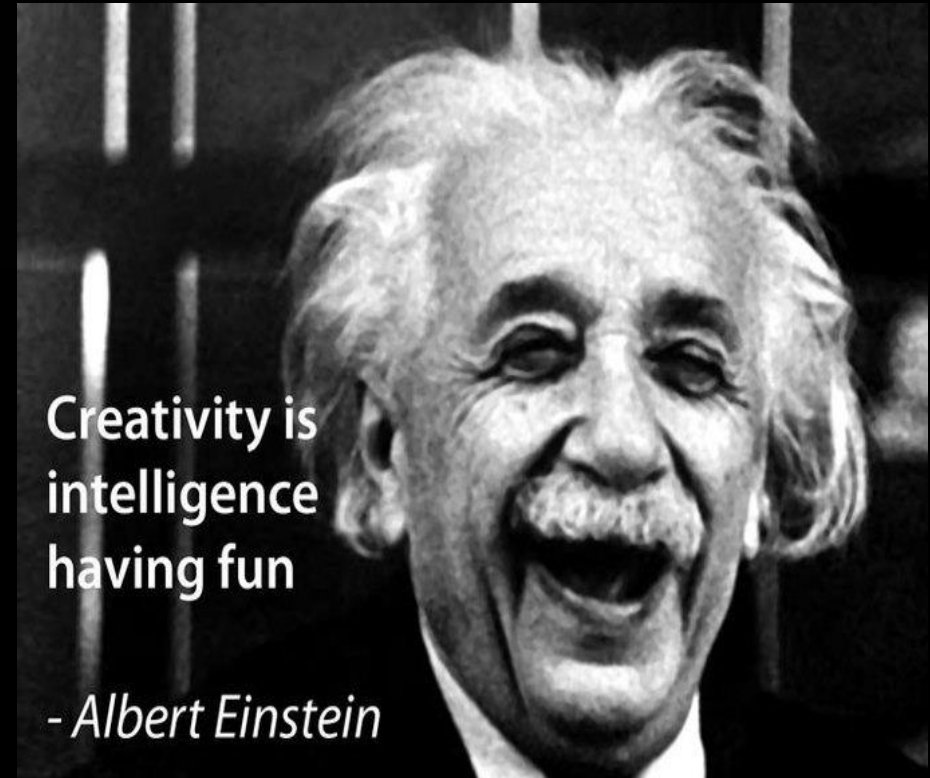
NO

☐



**"Creativity is contagious,  
pass it on"**

**- Albert Einstein**



**Creativity is  
intelligence  
having fun**

**- Albert Einstein**

THINGS CAN ALWAYS GO WRONG  
EVEN WITH FORESEEN EVENTS....







**YOUR ATTENTION**

**I THANK YOU  
FOR**