Kenneth Goldsmith Award

Playing with fire

Red cells, Anaemia and Transfusion

Jonathan Wallis

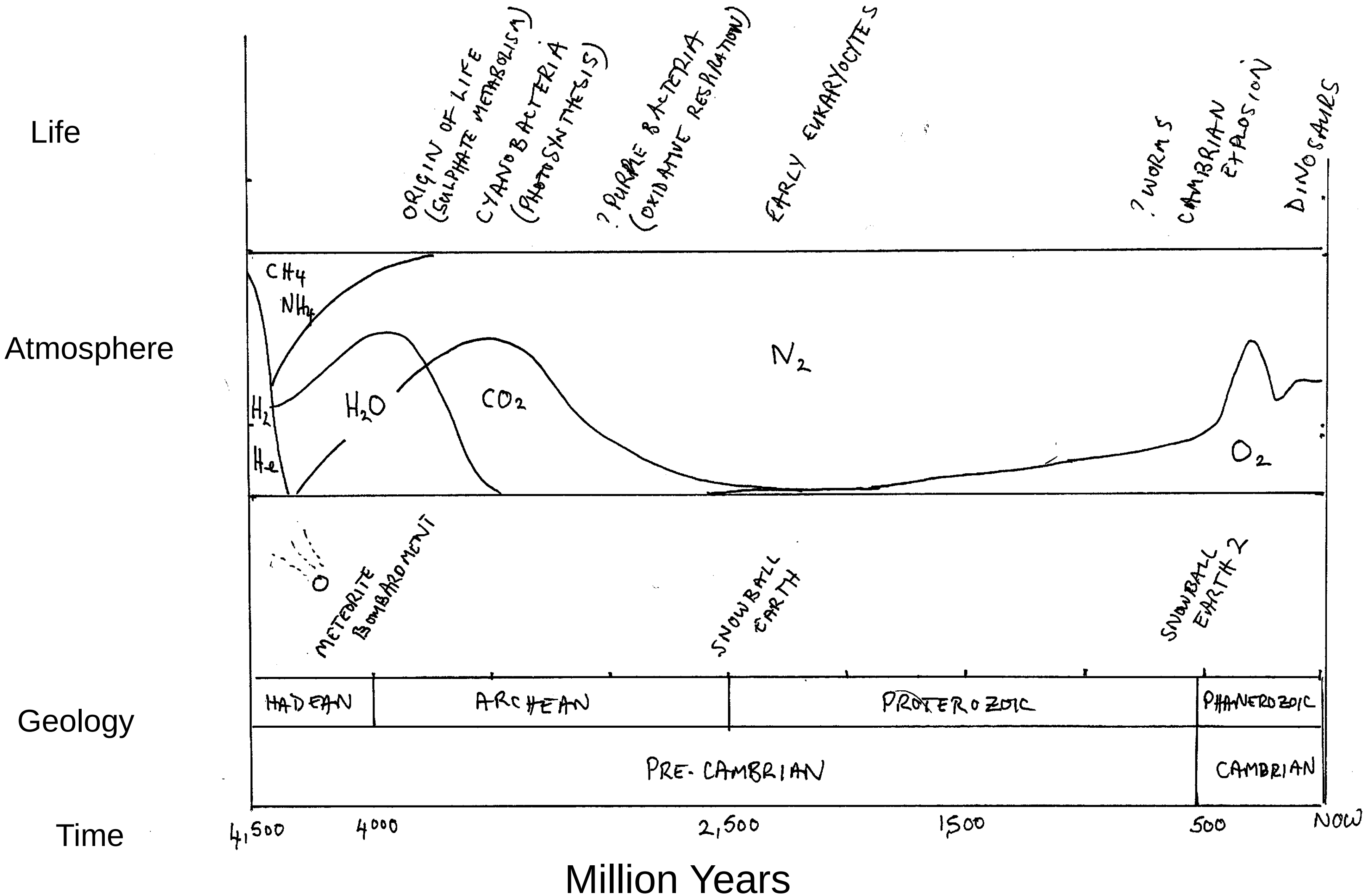
Freeman Hospital

Newcastle upon Tyne

Hadean eon: 4-4.5 billion years ago

Oxygen and Environment

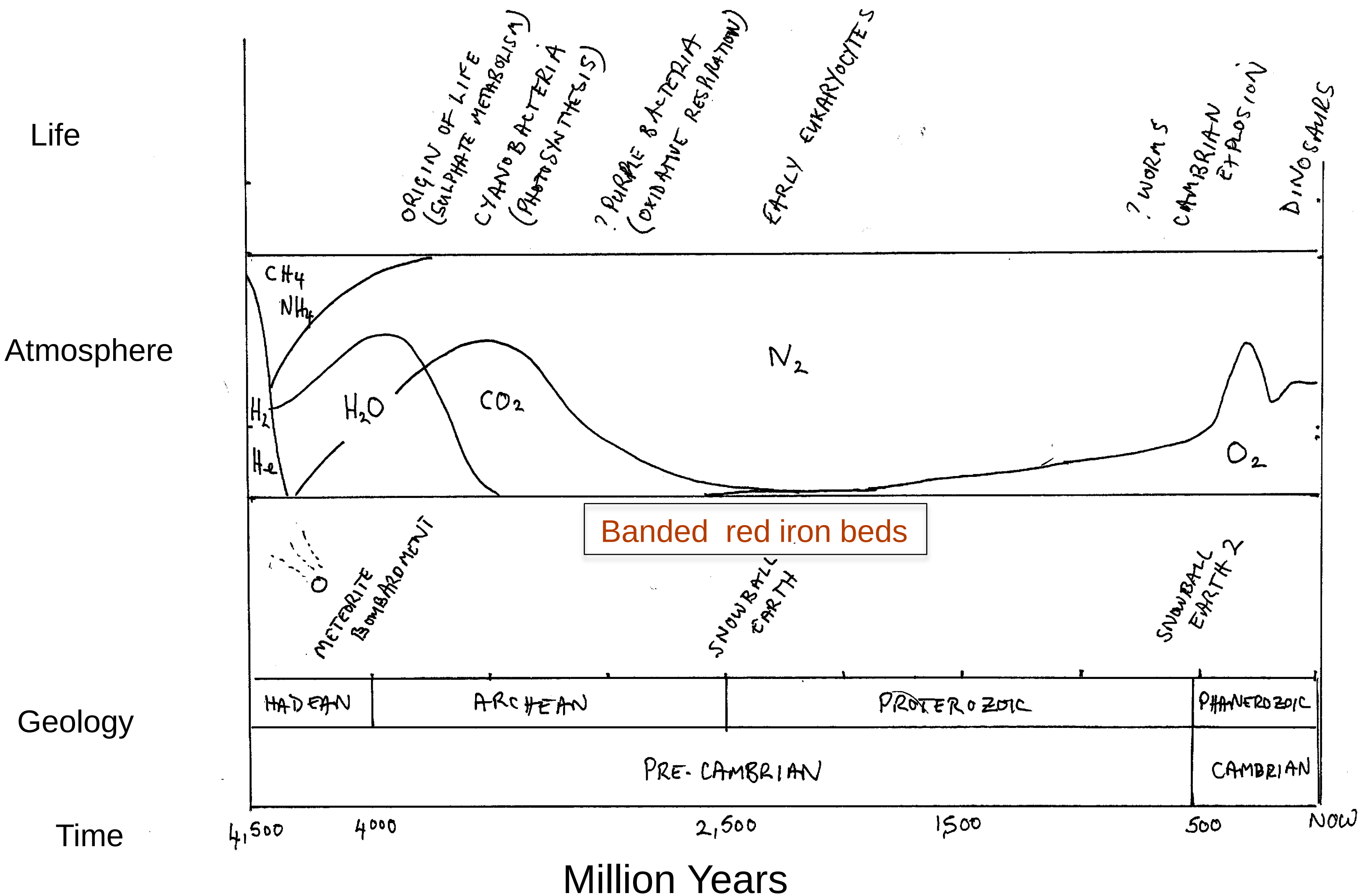
from ‘Oxygen’ by Nick Lane, pub OUP 2002



Banded iron deposits in Western Australia

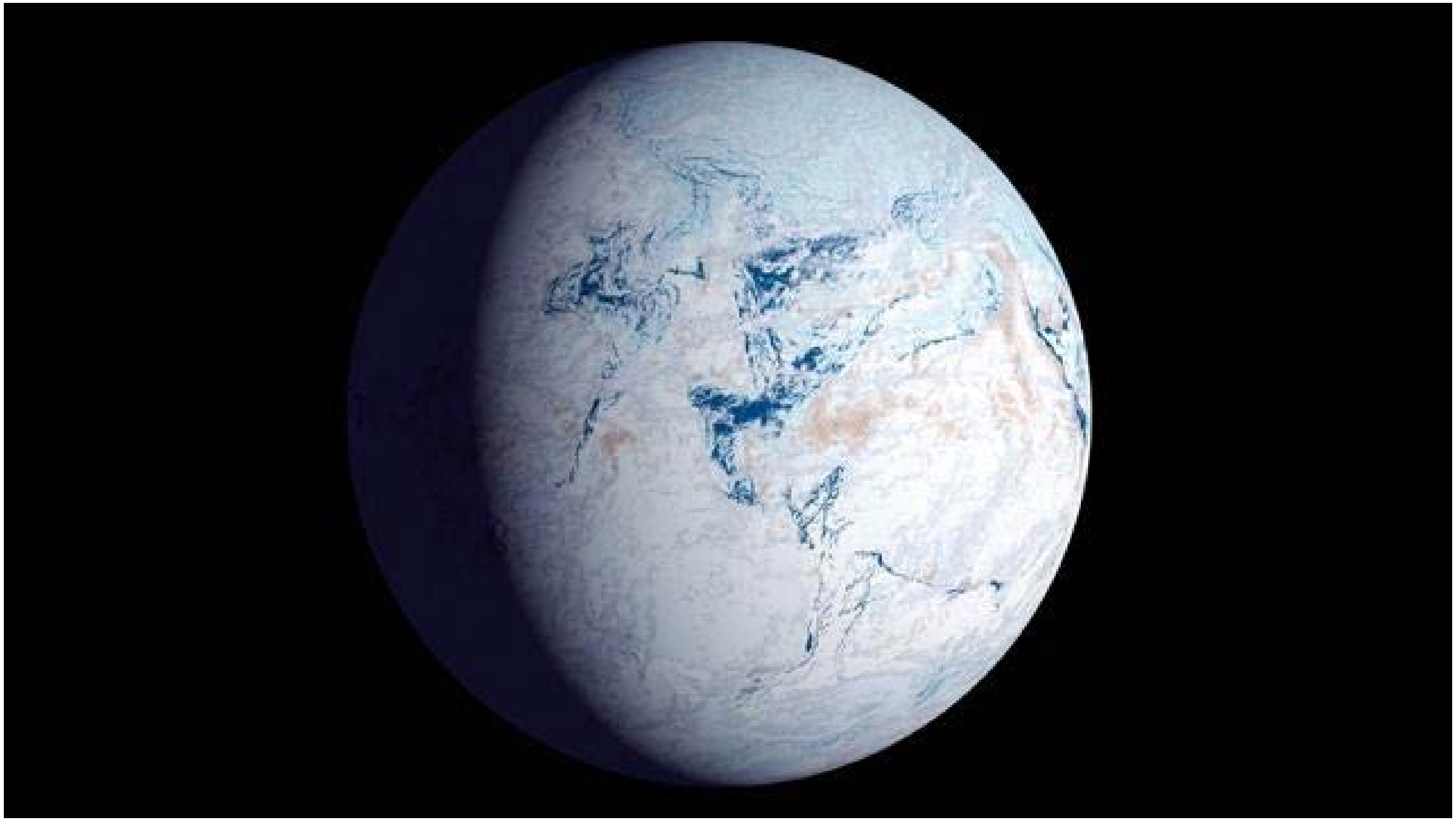
Oxygen and Environment

from 'Oxygen' by Nick Lane, pub OUP 2002



Snowball earth: 2.5 billion years ago

Atmosphere = Nitrogen and small amounts of Oxygen and CO_2



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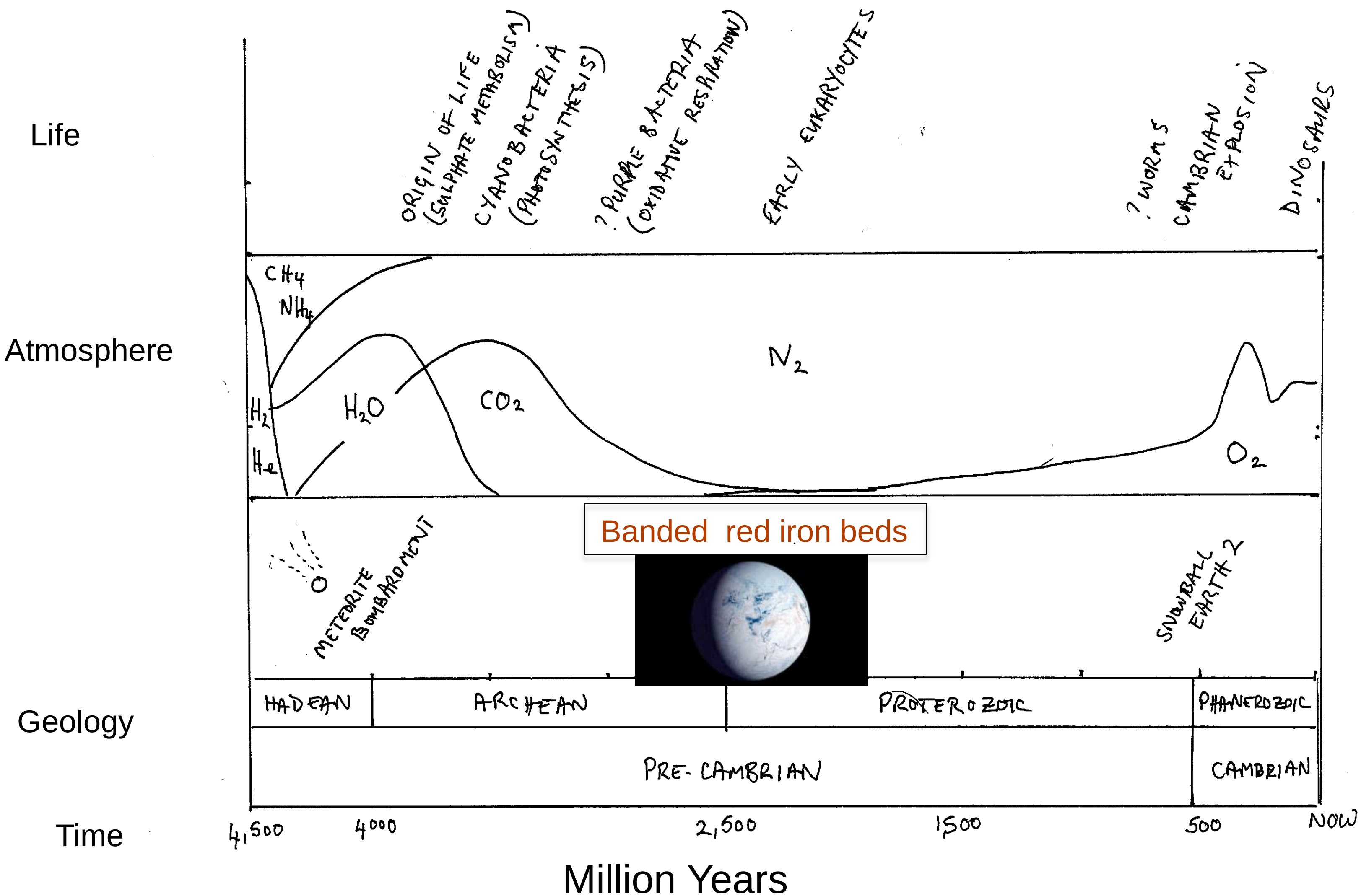
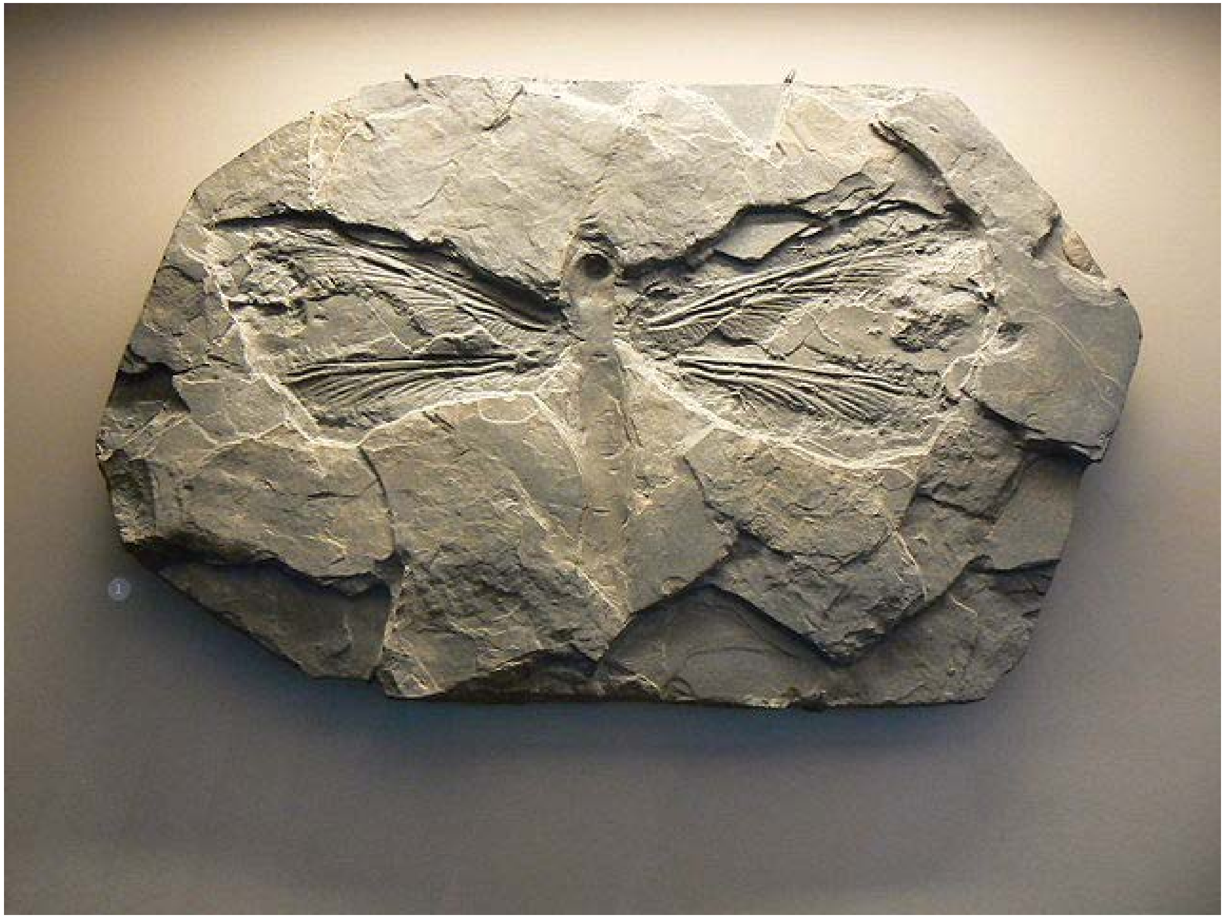


1979 Bolsover dragonfly
25 inch wingspan



"Meganeuradae" by Hcrepin - Own work. Licensed under Creative Commons Attribution-Share Alike 3.0-2.5-2.0-1.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Meganeuradae.jpg#mediaviewer/File:Meganeuradae.jpg

Oxygen and Environment

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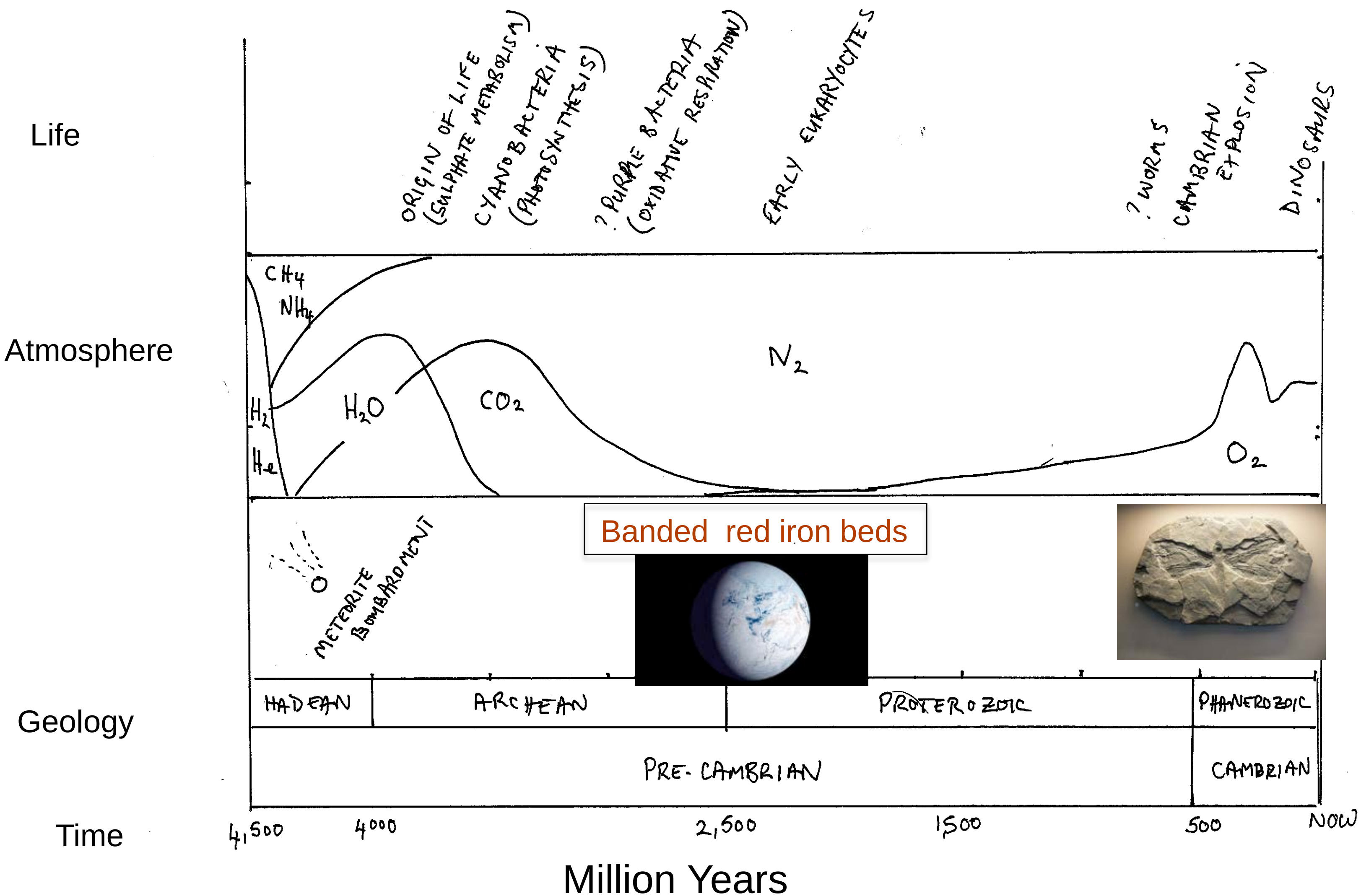



Dr. OXYGEN
Portable Oxygen Generator
Dr. OXYGEN
"Better life with Fresh Oxygen"

Hindenburg disaster

~~Hydrogen is dangerous !~~

Oxygen is dangerous!

Oxygen is dangerous!

Oxygen strips electrons from other elements

Anaerobic bacteria are killed by oxygen

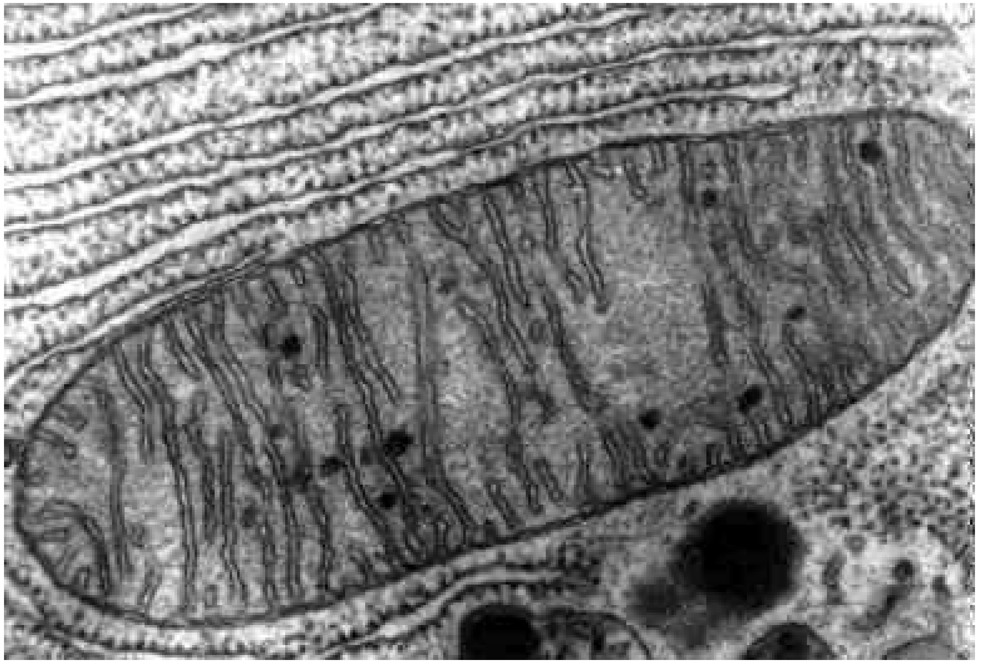
Radiation damage is via oxygen radicals

Hyperoxia causes brain, lung and retinal damage

O2 plus Carbon = forest fires

O2 plus H = Hindenburg disaster

Mitochondria (‘purple bacteria’)



Nucleated cells (eukaryocytes) nearly all contain mitochondria

Atmospheric pO2 is 150mm Hg

Arterial pO2 is 100 mmHg

Venous mixed pO2 is 35mm Hg

Mitochondria can operate maximally at pO2 << 5mmHg

Oxygen damages other molecules

Aerobic respiration is much more efficient than anaerobic respiration

Complex life is a balance between efficient metabolism and tissue damage

Managing oxygen delivery

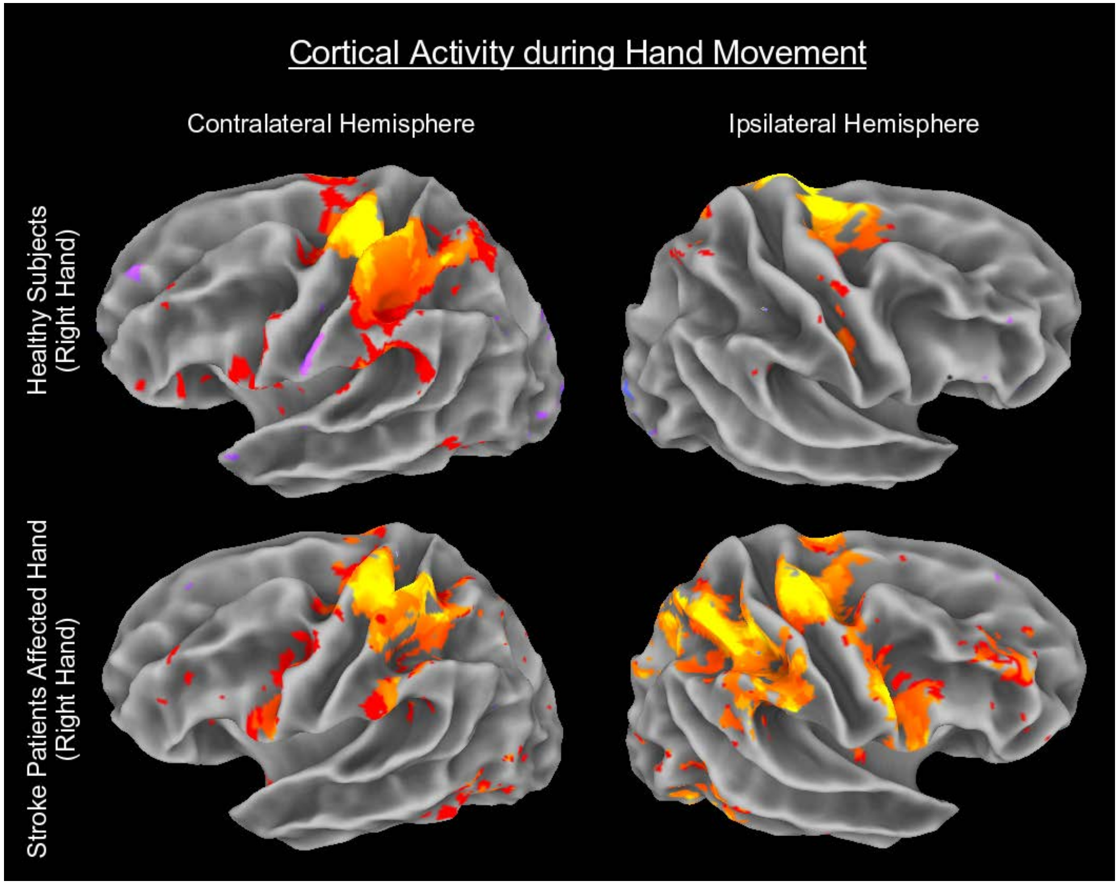
Pulmonary Oxygenation

Cardiac output

Blood Haemoglobin concentration

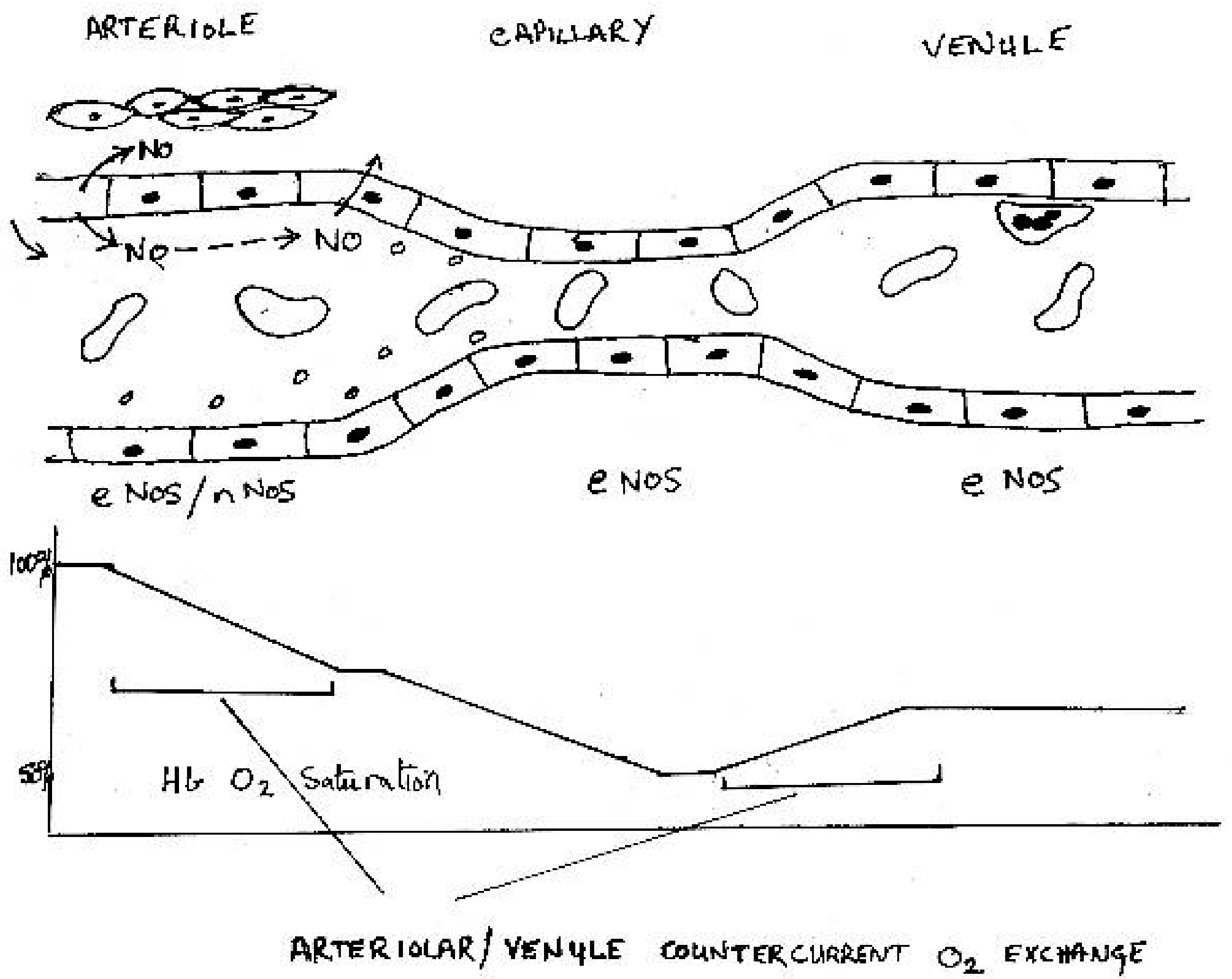
Microcirculatory control

Functional MRI maps oxygenation levels in the brain
Increased blood flow in active areas leads to higher O2 levels



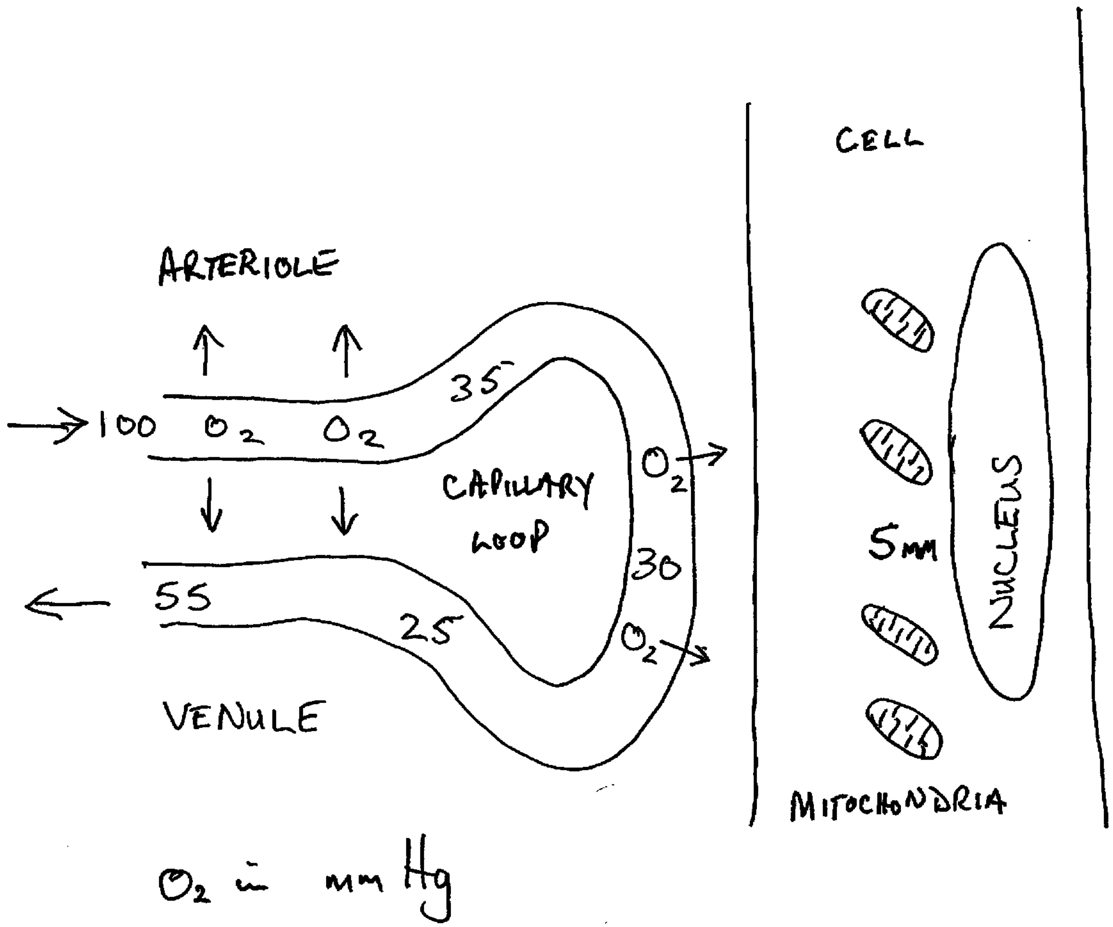
Longitudinal gradients in peri-arteriolar O_2 concentration

Dulling & Berne 1970 Circ Res 27; 669-78



Counter current exchange may occur in some tissues

Tsai et al 2003 Phys Rev 83; 933-63



Microcirculatory control

Feed-forward control systems

Local networks

Sympathetic nervous control

Adrenergic hormonal control

Feedback control

Hypoxic vasodilation

Hypoxic vasodilation

Low oxygen tension without red cells

No vasodilation

Low pO2 levels in the presence of red cells

Vasodilation

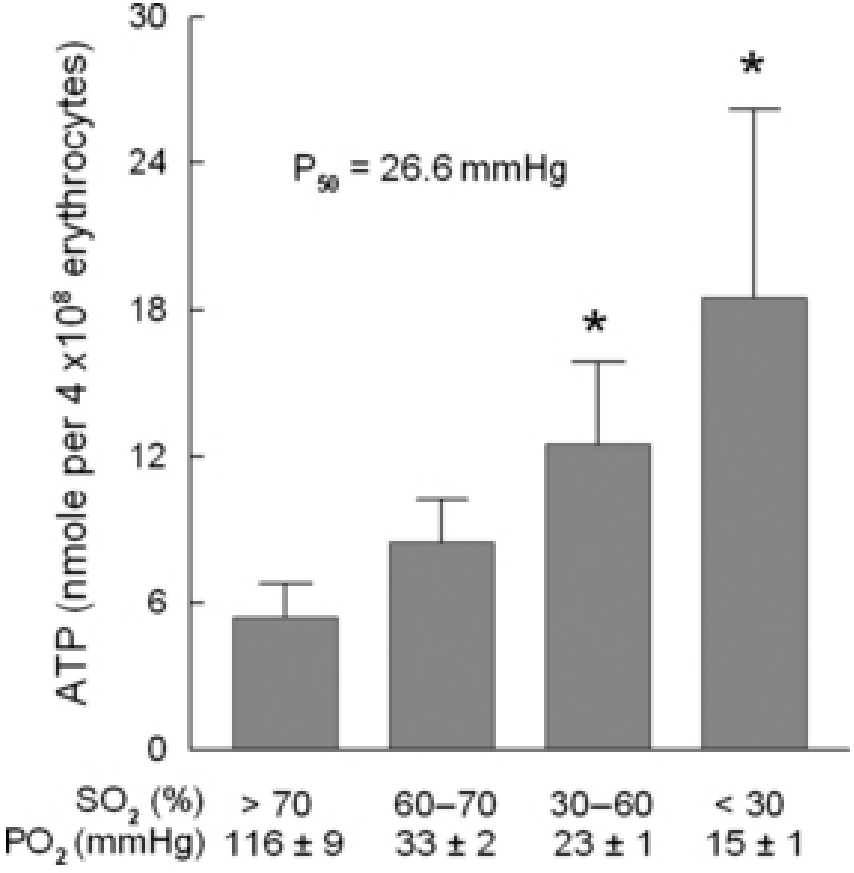
Desaturation of Hb may play a role in vascular control

Stein & Ellsworth 1993 J Appl Physiol 75; 1601-7

Mechanisms by which Hb O2 desaturation may cause vasodilation

- Release of NO or nitrosothiols from red cells
  - Stamler et al. 1997 Science 276 2034-7
- Production of NO from NO_3 by deoxyHb
  - Patel et al 2011 Cardiovasc Res 89; 507-15
- Release of erythrocyte ATP
  - Ellsworth & Sprague 2009. Physiology 24; 107-16

Erythrocyte-derived ATP and Perfusion Distribution: Role of Intracellular and Intercellular Communication. Ellsworth et al.



Microcirculation

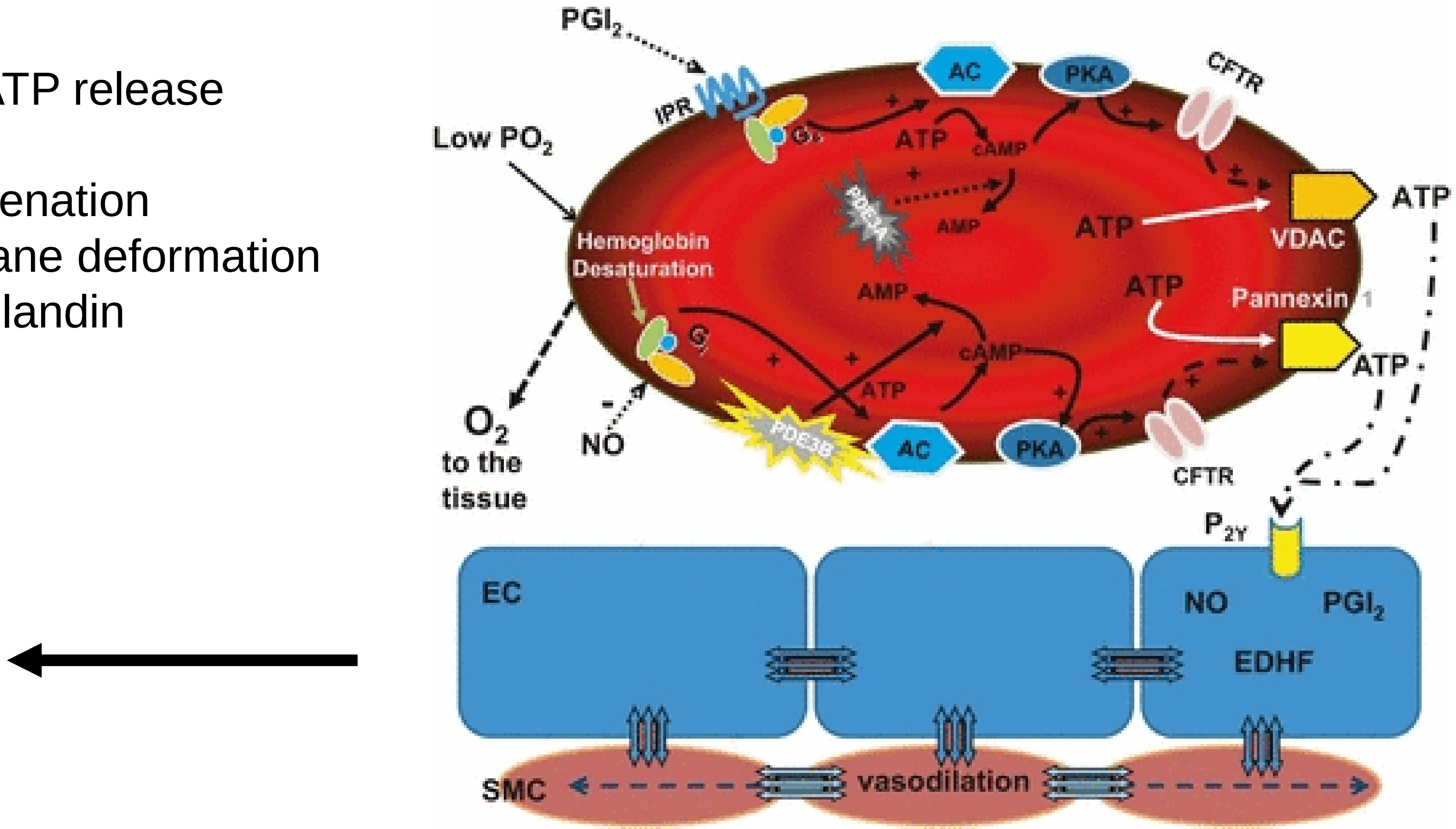
Volume 19, Issue 5, pages 430-439, 8 JUL 2012 DOI: 10.1111/j.1549-8719.2011.00158.x

http://onlinelibrary.wiley.com/doi/10.1111/j.1549-8719.2011.00158.x/full#f1

Erythrocyte-derived ATP and Perfusion Distribution: Role of Intracellular and Intercellular Communication

Stimuli to ATP release

1. Deoxygenation
2. Membrane deformation
3. Prostaglandin



Vasodilation signal may pass upstream to a distance of 1200um

Microcirculation
Volume 19, Issue 5, pages 430-439, 8 JUL 2012 DOI: 10.1111/j.1549-8719.2011.00158.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1549-8719.2011.00158.x/full#f4

Am J Physiol Heart Circ Physiol 299: H1981–H1989, 2010.
First published September 17, 2010; doi:10.1152/ajpheart.00611.2010.

Adenine nucleotide control of coronary blood flow during exercise

Mark W. Gorman,[1] G. Alec Rooke,[2] Margaret V. Savage,[1] M. P. Suresh Jayasekara,[3] Kenneth A. Jacobson,[3] and Eric O. Feigl[1]

Departments of [1]Physiology and Biophysics and [2]Anesthesiology, University of Washington, Seattle, Washington; [3]Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

Submitted 22 June 2010; accepted in final form 17 September 2010

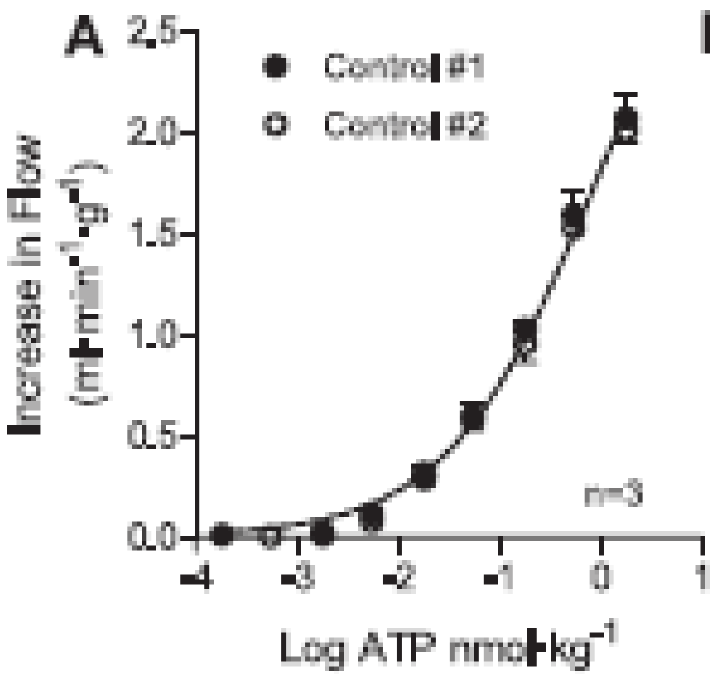


Table 2. *Hemodynamic and metabolic variables at rest and during graded treadmill exercise*

	Rest	Rest + Drug	Exercise Level 1	Exercise Level 2
Coronary Blood Flow, $ml \cdot min^{-1} \cdot g^{-1}$				
Vehicle	0.55 ± 0.05	0.54 ± 0.05	0.87 ± 0.07	1.11 ± 0.11
Purinergic Blockade	0.58 ± 0.05	0.44 ± 0.05	0.83 ± 0.07	1.04 ± 0.09
Myocardial O_2 Consumption, $\mu l\ O_2 \cdot min^{-1} \cdot g^{-1}$				
Vehicle	76 ± 9	68 ± 6	124 ± 10	169 ± 14
Purinergic Blockade	79 ± 7	66 ± 6	130 ± 9	169 ± 14
Mean Aortic Pressure, mmHg				
Vehicle	108 ± 6	108 ± 6	112 ± 11	140 ± 29
Purinergic Blockade	105 ± 6	123 ± 5	116 ± 4	123 ± 7
Heart Rate, beats/min				
Vehicle	106 ± 8	111 ± 6	167 ± 5	199 ± 4
Purinergic Blockade	119 ± 4	74 ± 4	138 ± 6	170 ± 8
Arterial Hydrogen Ion Concentration, nM				
Vehicle	38 ± 1.6	40 ± 0.9	38 ± 0.8	38 ± 1.1
Purinergic Blockade	39 ± 0.7	33 ± 0.5	31 ± 1.3	33 ± 1.0
Coronary Venous Hydrogen Ion Concentration, nM				
Vehicle	43 ± 0.9	44 ± 0.7	42 ± 0.8	44 ± 1.2
Purinergic Blockade	42 ± 0.5	38 ± 0.4	37 ± 0.9	39 ± 0.9
Arterial Carbon Dioxide Tension, mmHg				
Vehicle	33 ± 1	34 ± 1	31 ± 1	30 ± 1
Purinergic Blockade	35 ± 1	26 ± 1	24 ± 2	26 ± 1
Coronary Venous Carbon Dioxide Tension, mmHg				
Vehicle	45 ± 1	46 ± 1	42 ± 1	45 ± 1
Purinergic Blockade	45 ± 1	38 ± 1	38 ± 1	39 ± 1
Arterial Oxygen Tension, mmHg				
Vehicle	90 ± 2	88 ± 1	87 ± 3	90 ± 5
Purinergic Blockade	86 ± 1	99 ± 2	97 ± 3	89 ± 2
Arterial Hemoglobin Saturation, %				
Vehicle	95 ± 0.4	95 ± 0.2	95 ± 0.5	95 ± 0.7
Purinergic Blockade	95 ± 0.5	97 ± 0.3	97 ± 0.2	96 ± 0.3
Coronary Venous Oxygen Tension, mmHg				
Vehicle	16 ± 2	17 ± 2	14 ± 2	13 ± 1
Purinergic Blockade	15 ± 1	12 ± 1	8 ± 1	8 ± 1
Coronary Venous Hemoglobin Saturation, %				
Vehicle	15.6 ± 3.3	17.3 ± 3.0	12.6 ± 3.2	10.6 ± 2.2
Purinergic Blockade	13.7 ± 1.5	10.9 ± 1.5	6.4 ± 1.1	5.8 ± 1.0
Arterial Oxygen Content, ml O_2/dl blood				
Vehicle	17.1 ± 1.1	16.5 ± 0.6	16.9 ± 0.7	17.6 ± 0.6
Purinergic Blockade	17.0 ± 0.6	17.4 ± 0.7	17.2 ± 0.8	17.3 ± 0.7
Coronary Venous Oxygen Content, ml O_2/dl blood				
Vehicle	3.2 ± 0.7	3.6 ± 0.5	2.5 ± 0.6	2.1 ± 0.4
Purinergic Blockade	3.1 ± 0.3	2.2 ± 0.3	1.1 ± 0.3	0.9 ± 0.2
Hematocrit, %				
Vehicle	39 ± 3	39 ± 2	39 ± 2	39 ± 2
Purinergic Blockade	39 ± 2	39 ± 2	39 ± 2	39 ± 2

Values are means ± SE. Results from 7 dogs treated with vehicle or purinergic blockade [L-nitroarginine (LNA) + 8-phenyltheophylline (8-PT) + 2-iodo-N^6-methyl-(*N*)-methanocarba-2′-deoxyadenosine-3′,5′-biphosphate (MRS 2500)]. All dogs were studied under both conditions.

Evidence for ATP release from red cells

- Deoxygenating red cells release ATP
  - Bergfeld & Forrester 1992 Cardiovasc Res 26: 40-7
- ATP in venular plasma increases with hypoxia
  - Jagger et al Am J Physiol Heart 2001 : 280 ; 2833-9
- ATP causes arteriolar vasodilation & blocking ATP receptors causes tissue hypoxia
  - Gorman..Feigl et al. 2010. Am J Physiol Heart 299: H1981-H1989

Impaired ATP release from red blood cells promotes their adhesion to endothelial cells: A mechanism of hypoxemia after transfusion

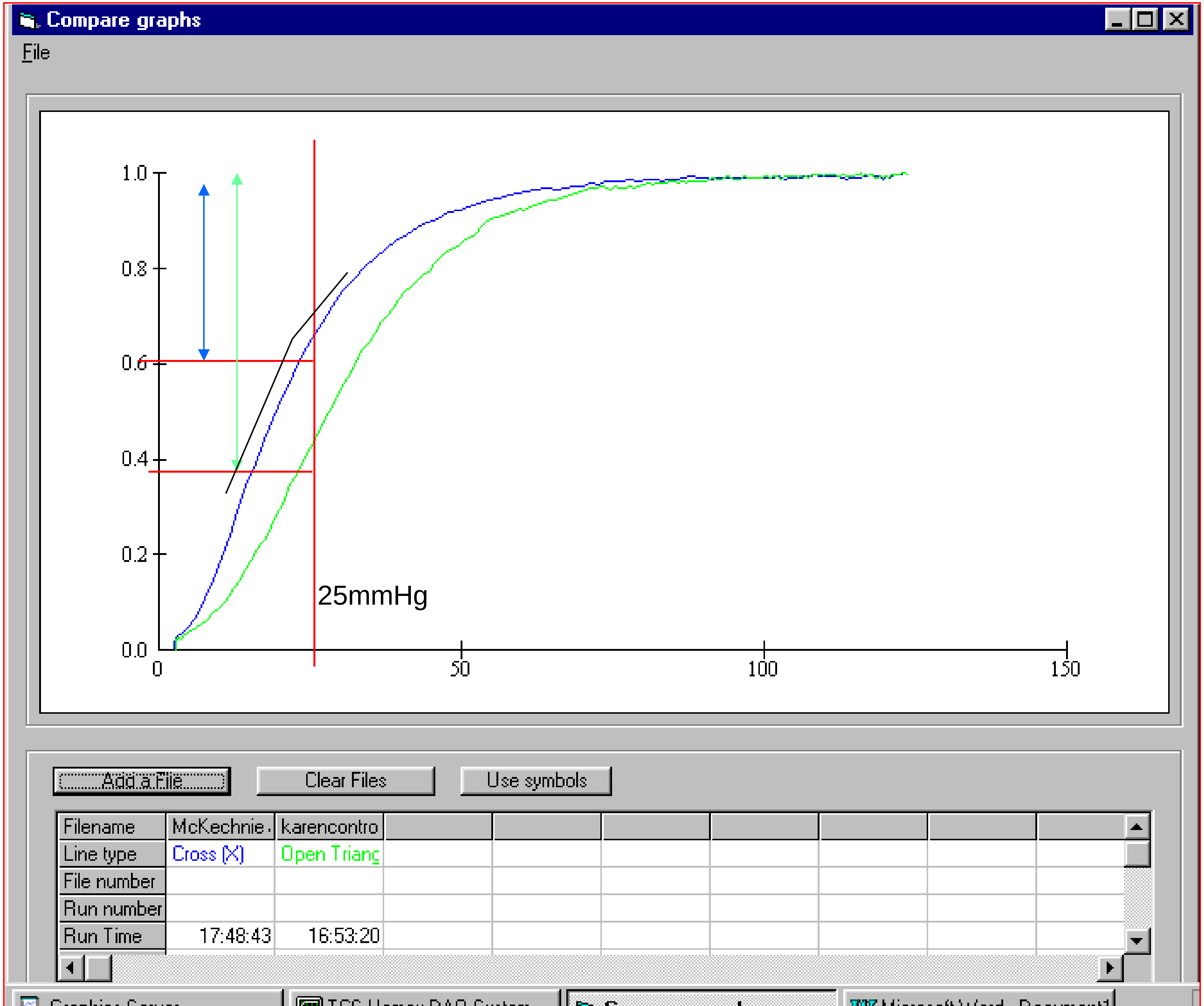
Hongmei Zhu, MS, Rahima Zennadi, PhD, Bruce X. Xu, Jerry P. Eu, MD, Jordan A. Torok, MD, Marilyn J. Telen, MD, and Timothy J. McMahon, MD PhD*

Critical Care Medicine 2011; 39: 2478

Red cell cytosol

Haemoglobin	5 mmol/L (tetrameric protein)
2,3 DPG	4.2 mmol/L
Glutathione	2.2 mmol/L
ATP	1.35 mmol/L
ADP	0.2 mmol/L

(ATP is vasoactive in low nanomolar concentration with maximal effect at 1 micromol/L)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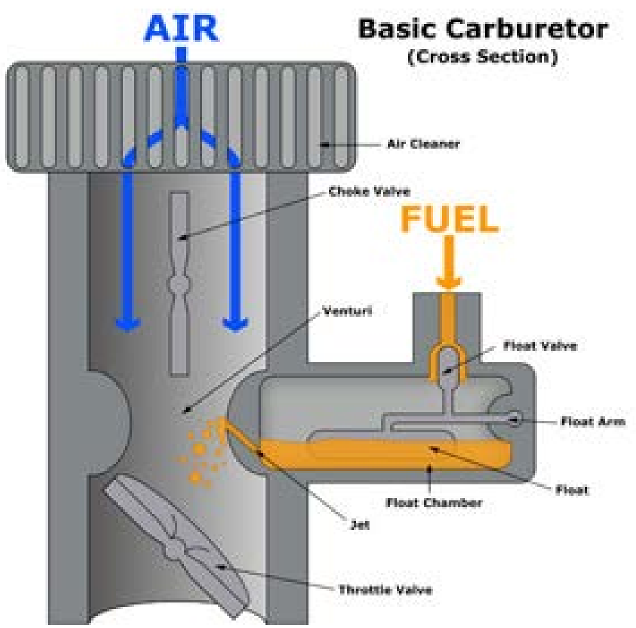
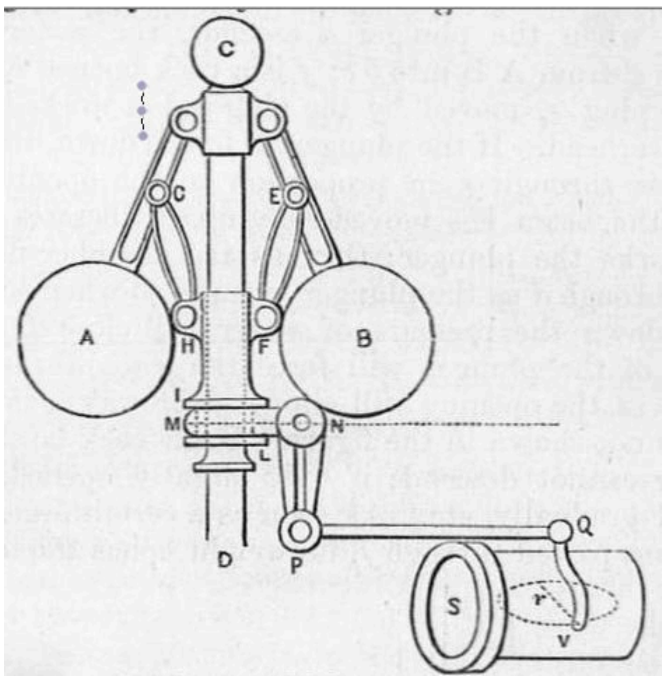
Carburetor
Feed forward control



Governor
Feed back control



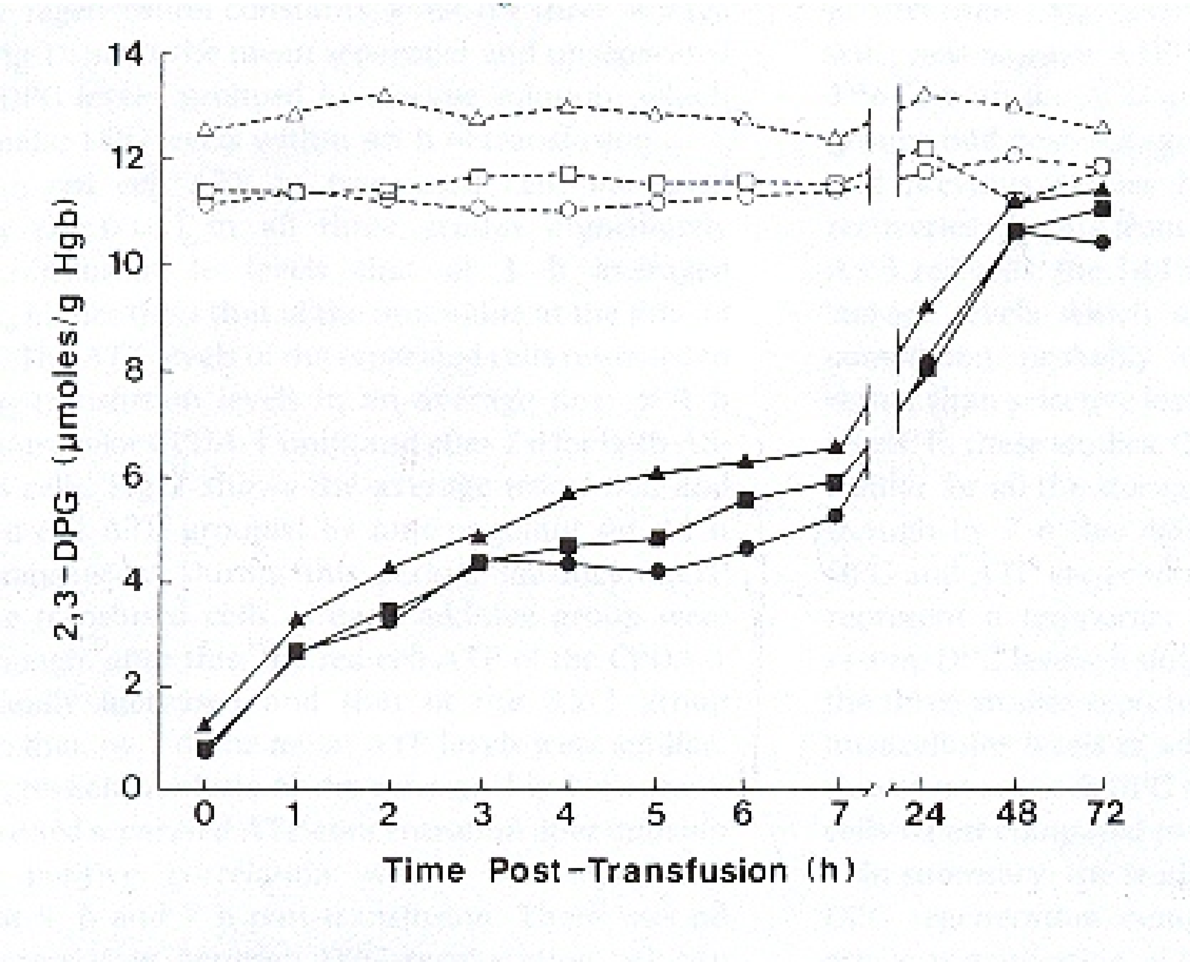
Watts governor and throttle valve (Steam and the Steam Engine - Land and Marine, 1875)" by Andy Dingley (scanner) - Scan from Evers, Henry (1875) Steam and the Steam Engine: Land and Marine, Glasgow: Williams Collins. Licensed under Public domain via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Watts_governor_and_throttle_valve_(Steam_and_the_Steam_Engine_-_Land_and_Marine,_1875).jpg#mediaviewer/File:Watts_governor_and_throttle_valve_(Steam_and_the_Steam_Engine_-_Land_and_Marine,_1875).jpg

Tissue oxygenation

- 2,3 DPG will affect Oxygen release but also ATP release
- 2,3 DPG is like the governor in a diesel engine, setting the level of feedback for microcirculatory flow control

Clinical studies: 2,3 DPG

Heaton et al 1989 BJH 71; 131-6



Summary

- Delivery of oxygen by red cells depends on oxygen carriage, HbO_2 p50 and **blood flow**
- Deoxygenation of red cells regulates local blood flow
- Failure of Hb deoxygenation will limit both flow and oxygen delivery

Why do we transfuse red cells?

1. To prevent death from exsanguination

2. To ‘top up’ haemoglobin post procedure to reduce morbidity/mortality during recovery phase

3. To improve quality of life in patients with longer term bone marrow failure

What is the optimal Hb?

O_2 extraction in resting medical students.

Mountfield's Physiology 1973

	% cardiac output	% total O_2 uptake	% Hb O_2 extracted
Skin	9	2	5.0
Renal	19	7	6.5
Other	10	5	15
Splanchnic	24	25	20
Cerebral	13	20	31
Sk' Muscle	21	30	40
Coronary	4	11	57


THINK YOURSELF THIN!
REVOLUTIONARY NEW DIET
BY
Dr J P WALLIS
TV
RADIO
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€

High Hb is not always good

Peruvians living at high altitude are prone to thrombotic events and pulmonary hypertension

Tibetans are not

Natural selection on EPAS1 (HIF2α) associated with low hemoglobin concentration in Tibetan highlanders

Beall et al 2010 PNAS 107: 11459- 64

How do we measure the benefits of transfusion?

Haemoglobin increase?

Mortality/Morbidity? TRICC/FOCUS/TRIPICU

Tissue Hypoxia? Walsh *et al* Crit Care Med

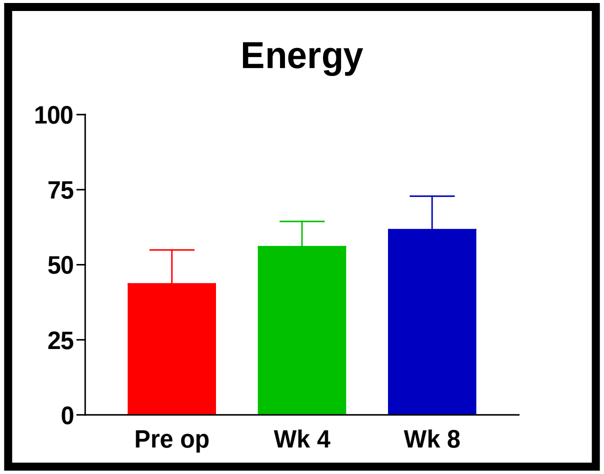
Quality of life

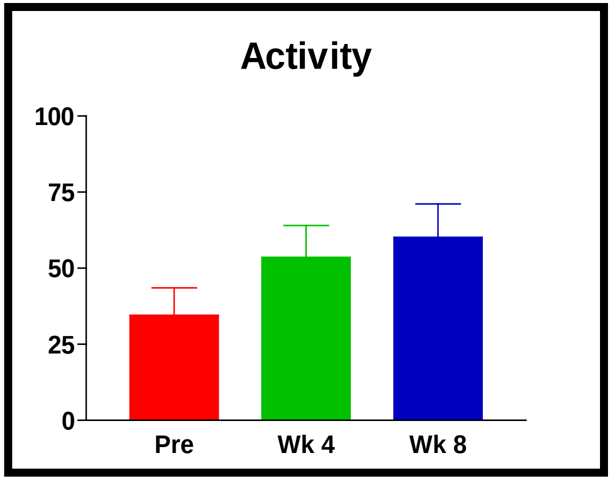
Studies of ‘top up’ transfusion

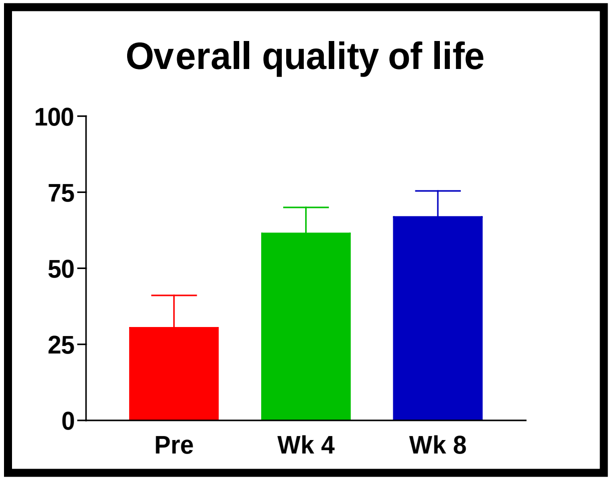
- Halm *et al*. Transfusion 2003; 43: 1358-65
- Foss *et al*. Age & Ageing 2008; 37:173-8
- Lawrence *et al*. Transfusion 2003;43: 1712-22

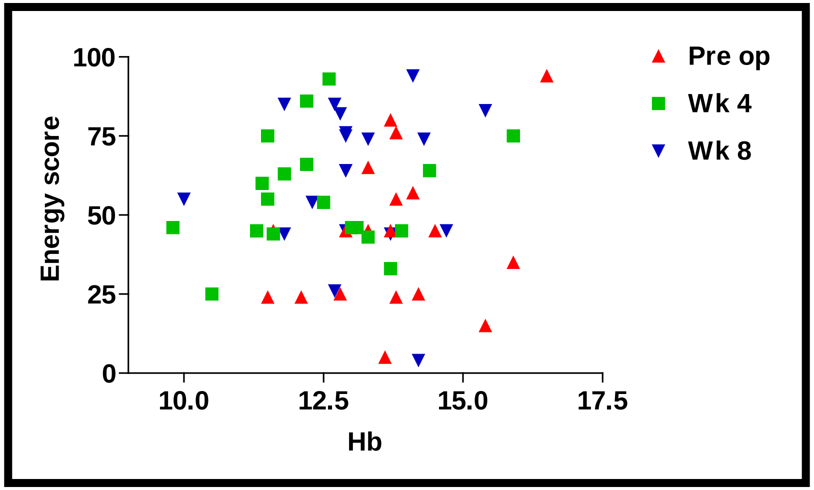
- So-Osman *et al*. Transfusion 2011; 51: 71-81
- Carson *et al* (Focus). NEJM 2011; 365: 2453-62
- Vuille-Lessard *et al*. Transfusion 2012; 52: 261-70
- Wallis *et al.* Transfusion Medicine 2005

Post operative hip surgery patients
QoL – visual analogue scale



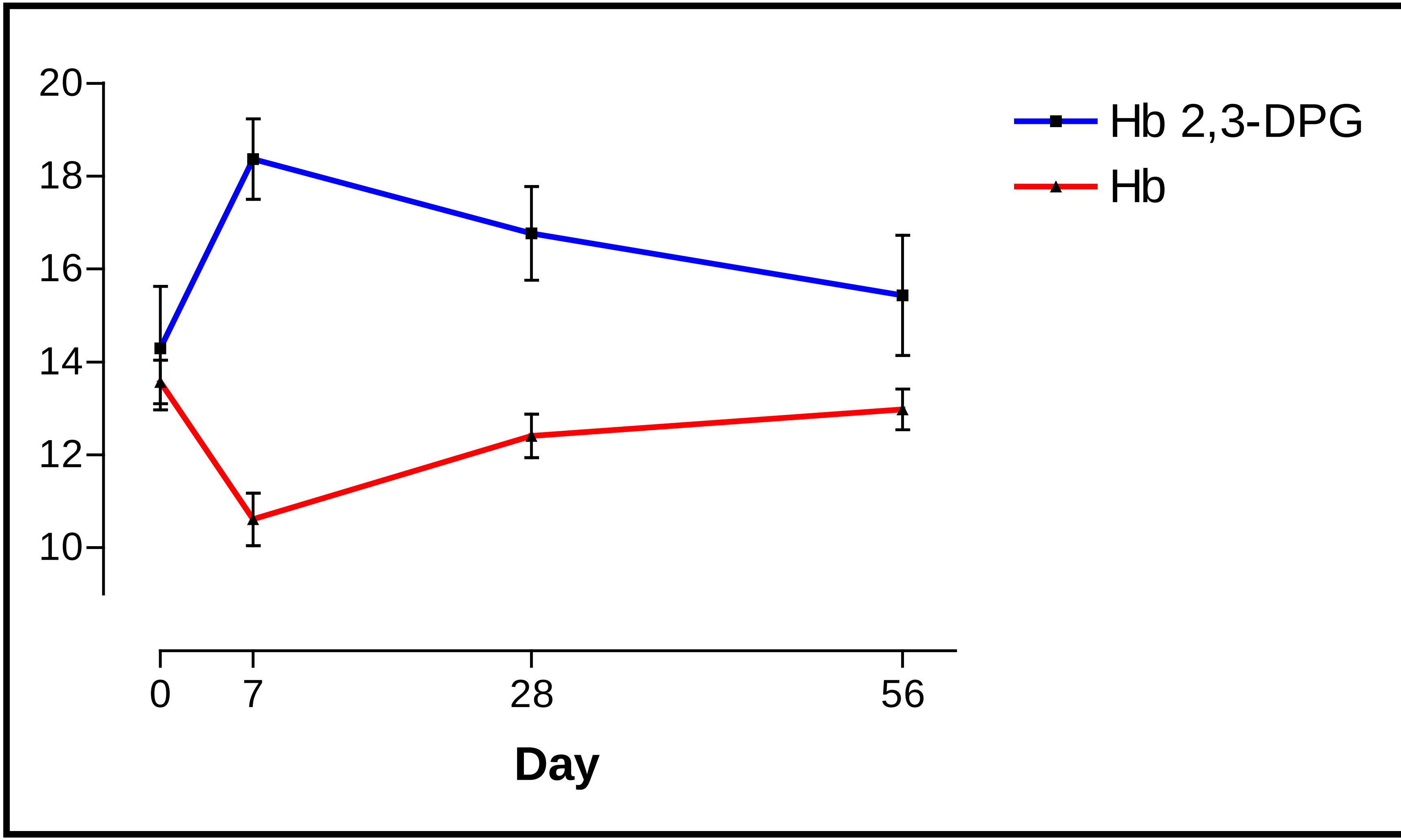


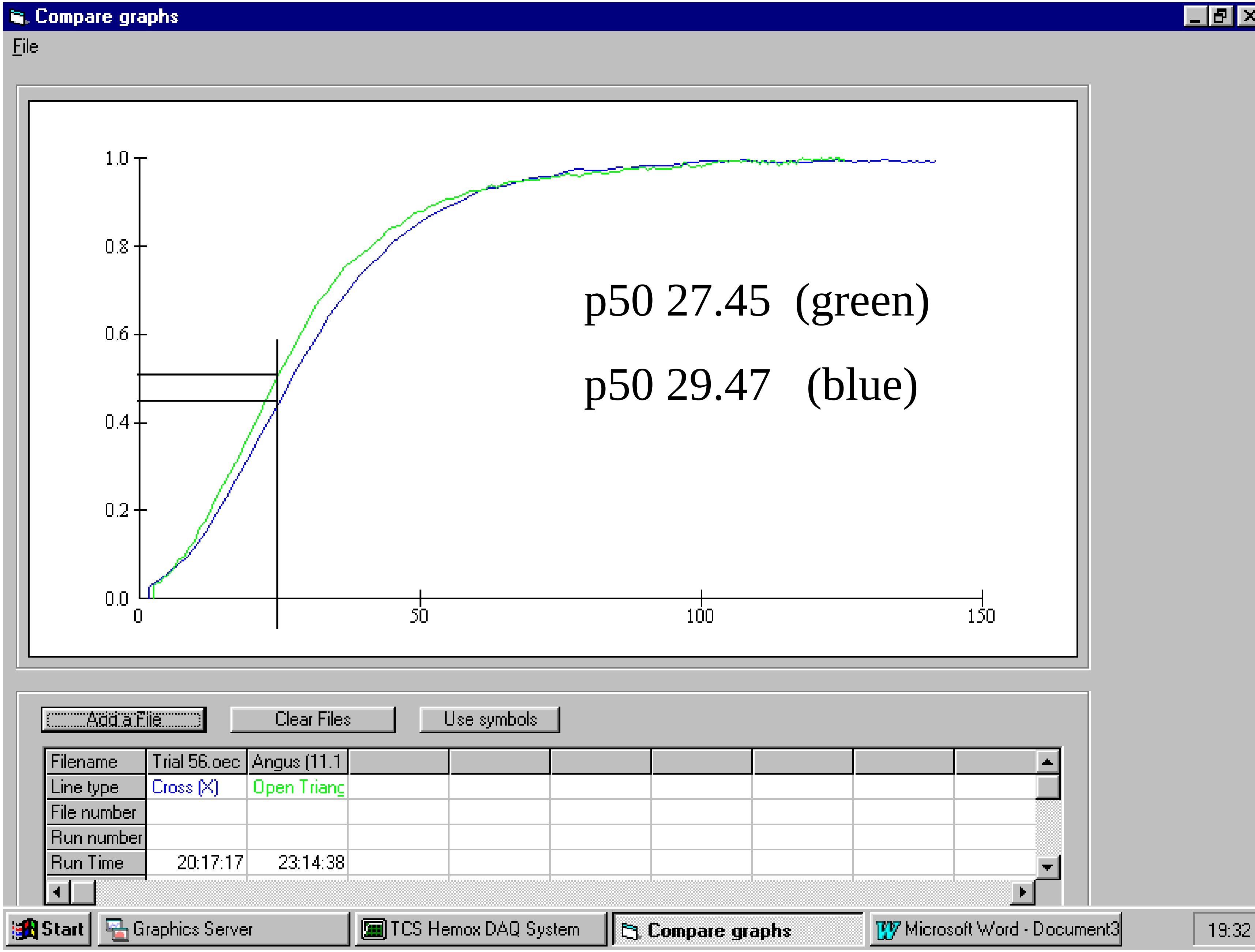




Wallis *et al.* Transfusion Medicine 2005

2,3 DPG

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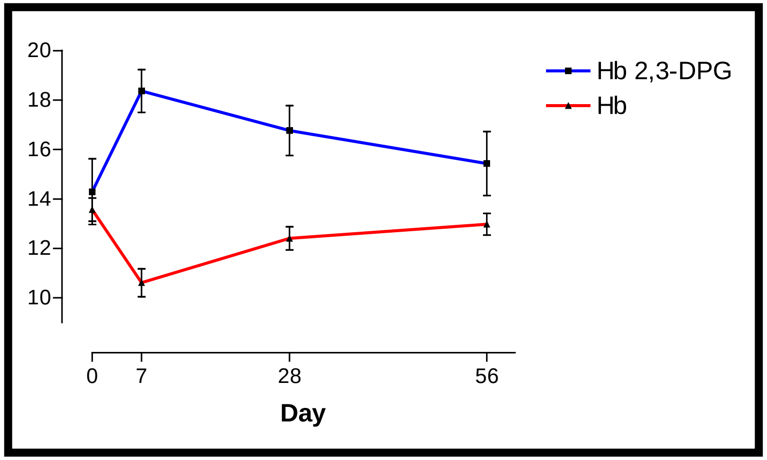
2,3 DPG

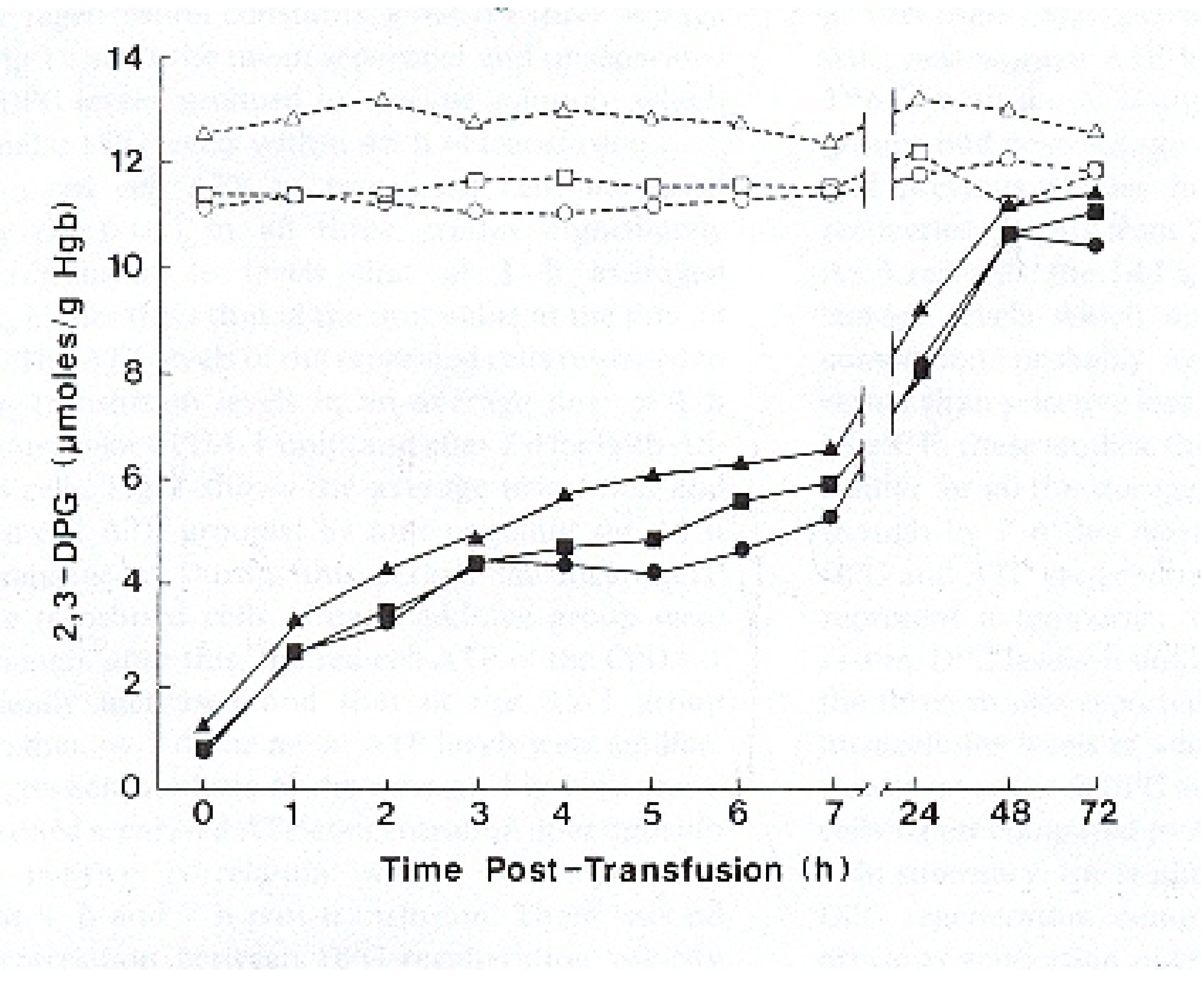


Oxygen delivery at pO_2 of 25 mm Hg

	preop	**d7**
Hb	100%	76%
D O2	100%	85%
M Circ	100%	??90%

Clinical studies: 2,3 DPG

Heaton et al 1989 BJH 71; 131-6



Skin and Visceral organs

- Blood flow to is regulated for purposes other than O2 delivery

Skeletal muscle

- Depends on physical demands

Cerebral blood flow

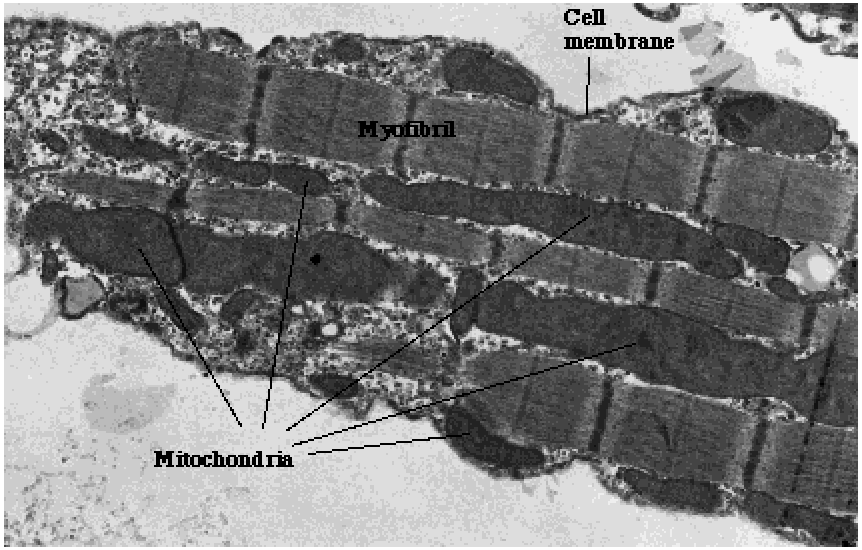
- O2 requirements fall by >30% with deep sedation
- Hb < 6g/dl associated with reduced mental function

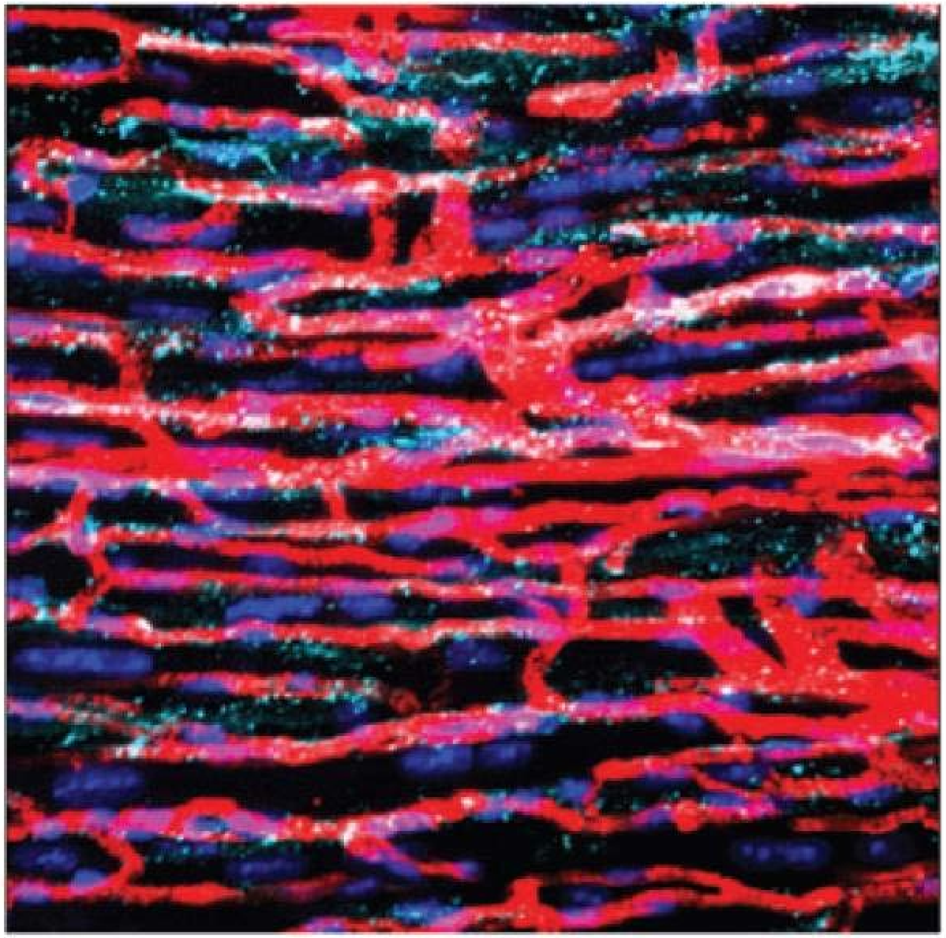
(Weiskopf RB. Anaesthesiology 2006; 104:911-20
& Clin Neurophysiol 2005; 116:1028-32)

Coronary blood flow

- Increases linearly 1:1 with cardiac work
- Little reserve available

Cardiac myocytes

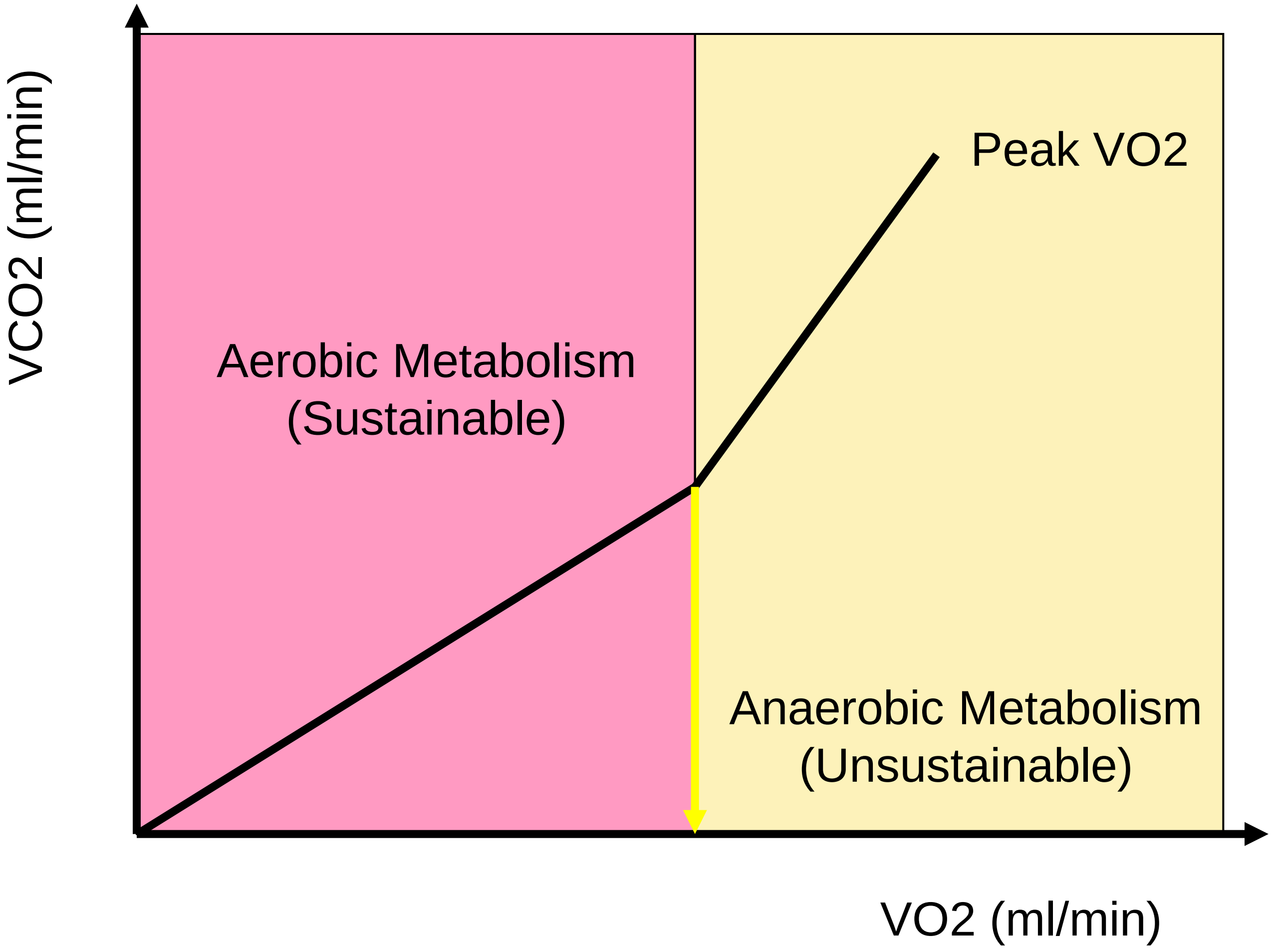




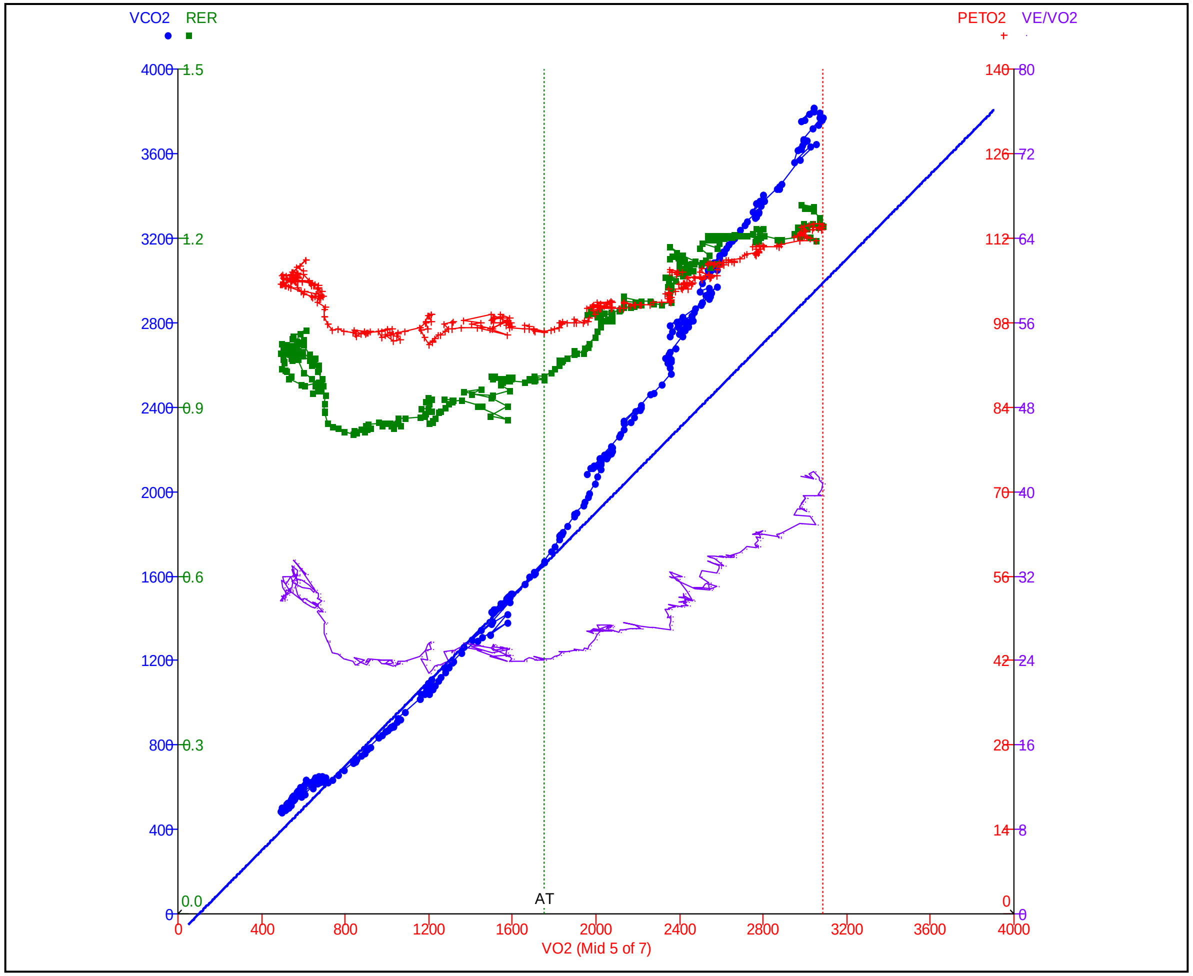
CPX testing

- Use;
  - Sports medicine
  - CHF
  - Preoperative
- Cycle
- Graded Work rate increase
- Breath VCO_2 and VO_2
- 12 lead ECG
- 6 - 10 min test
- Symptom limited
- Safe

Anaerobic Threshold
Objective, non-volitional, repeatable measurement



Submaximal derivatives



Pearce, Wright, Snowdon & Wallis

Freeman Hospital , Newcastle upon Tyne

B J Anaesth 2014.

Subjects

20 Transfusion dependant patients with bone marrow failure

No known exercise limiting coronary vascular disease

CPEX

</= 3 days pre-transfusion

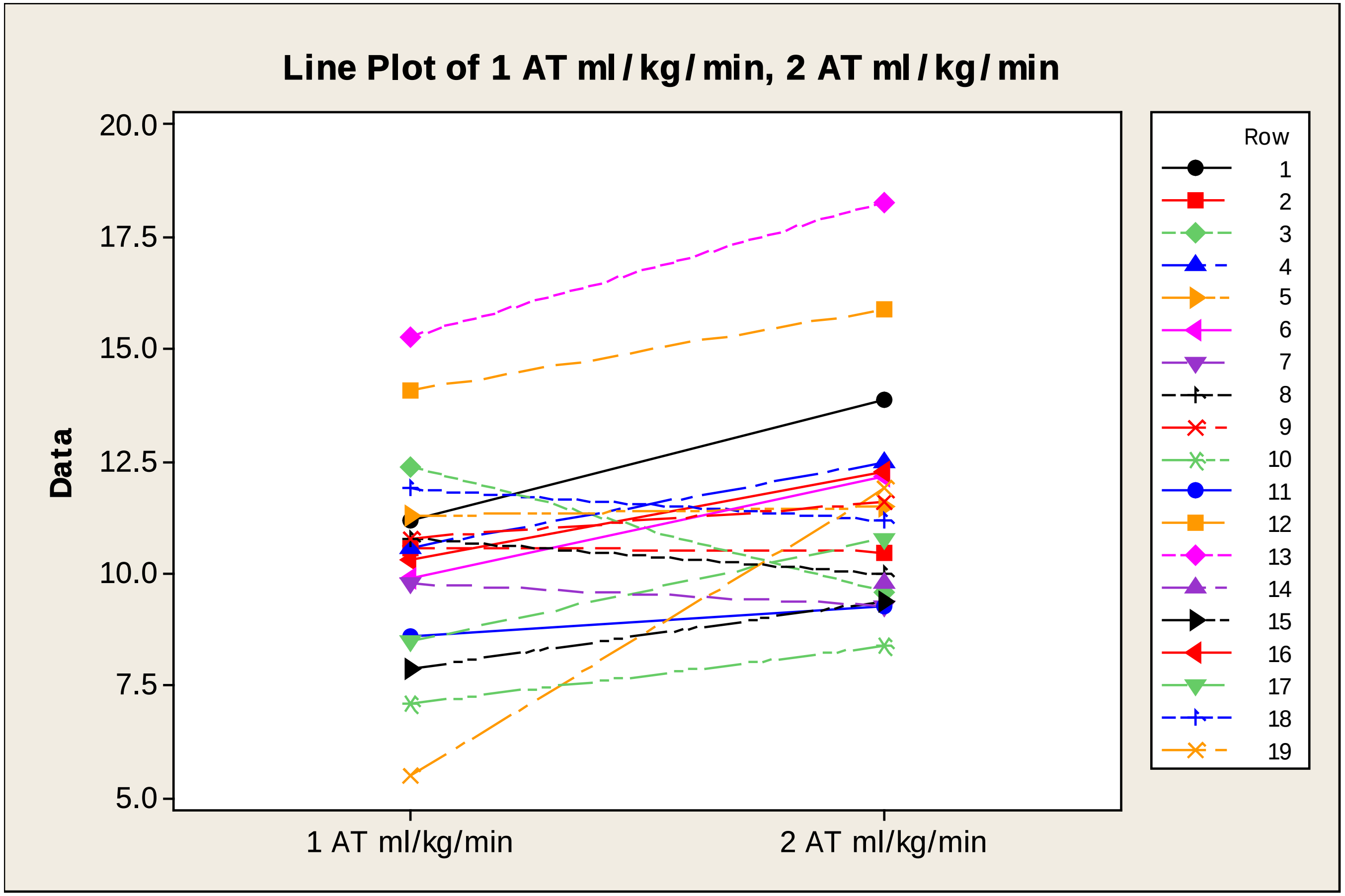
3-5 days post transfusion

Outcome : change in AT per gram Hb increase

Pearce, Wright, Snowdon & Wallis

Freeman Hospital , Newcastle upon Tyne

Manuscript in preparation



Transfusion and CPEX AT testing

- Haemoglobin increased by 2.9 g/dL
- Change in VO2 at anaerobic threshold

= 0.43 ml/min/Kg per g/dL Hb

AT testing in patients with Cardiac failure + Erythropoietin

- – Mancini et al Circulation 2003; 107: 294-9

- Hb increased by 3.3g/dL.
- Change in VO2 at anaerobic threshold

= 0.36 mL/min/Kg per g/dL Hb

- CPX testing is a reproducible measure of cardiovascular capacity
- Can be performed on older patients without morbidity
- Shows a clear relationship between Hb and AT
- Method for assessing the function of transfused red cell?

Conclusion

1. Life with Oxygen is playing with fire. It is both necessary and dangerous
2. Our microcirculation is tuned to provide adequate but not excessive and damaging levels of oxygen
3. Red cells may contribute to microcirculatory control through ATP release
4. 2,3dpg will set the thermostat on hypoxic vasodilation and O2 delivery
5. Stored blood with low 2,3DPG may limit O2 delivery
6. Anaerobic threshold (AT) testing is a safe and accurate method for assessing cardiac response to anaemia/transfusion

Thank you for listening