Are Banked Red Blood Cell Concentrates Dangerous?

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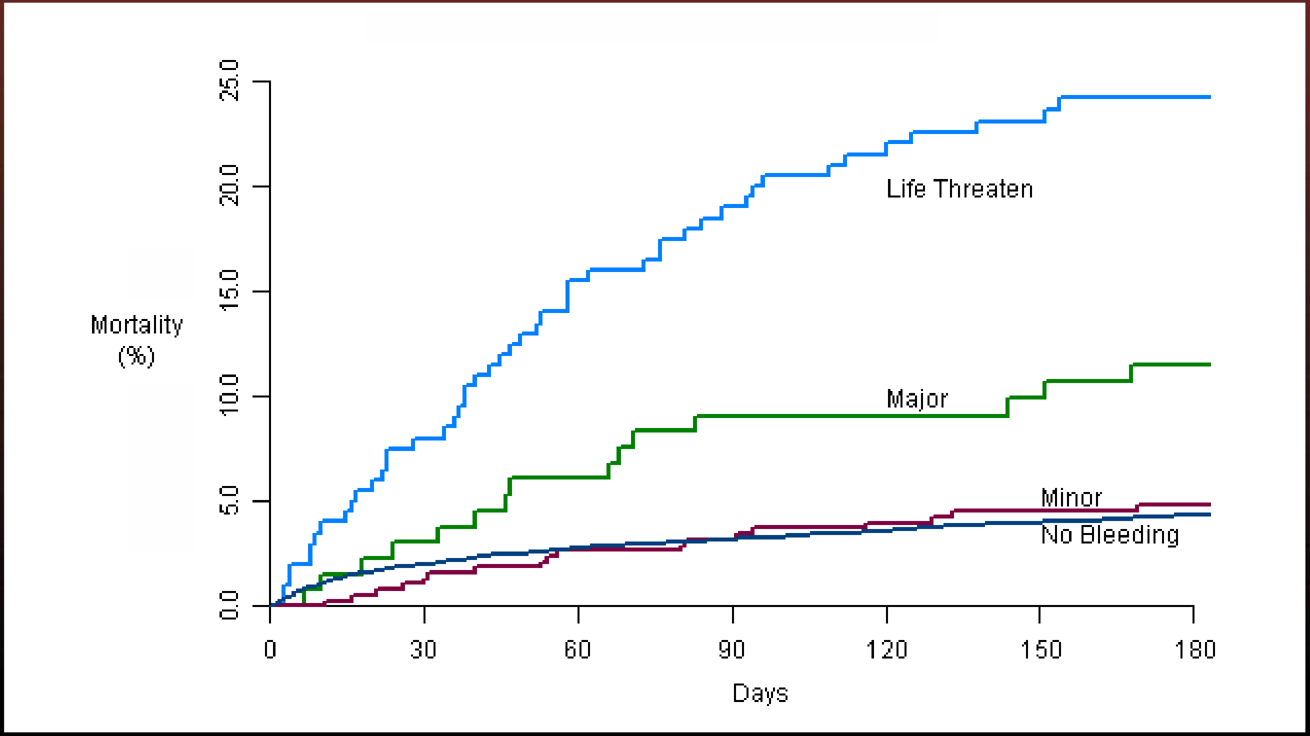
EHS Frankfurt 2008

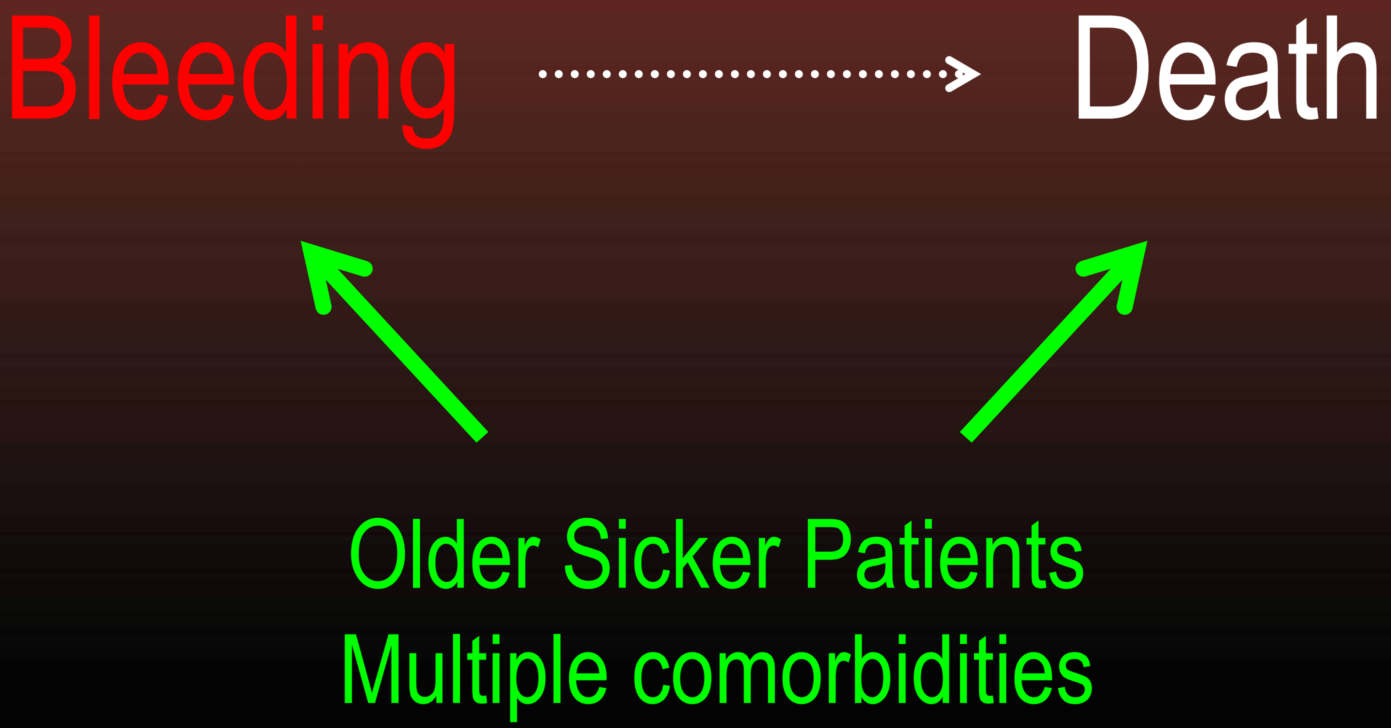
OASIS-2 and 4, OASIS Registries Severity of Bleeding and Death

N = 34,126



Eikelboom JW, et al. Circulation 2006; 114: 774-82

Confounded?



provided by JW Eikelboom

Directly Causal?

Bleeding ⟶ Death

Hypovolemia/Shock

Altered O_2 Supply/Demand

provided by JW Eikelboom

Indirectly Causal?

Bleeding → Death

Withdrawal of antithrombotic treatments

Hemostatic treatments (e.g., aprotinin)

Red Blood Cell Transfusion

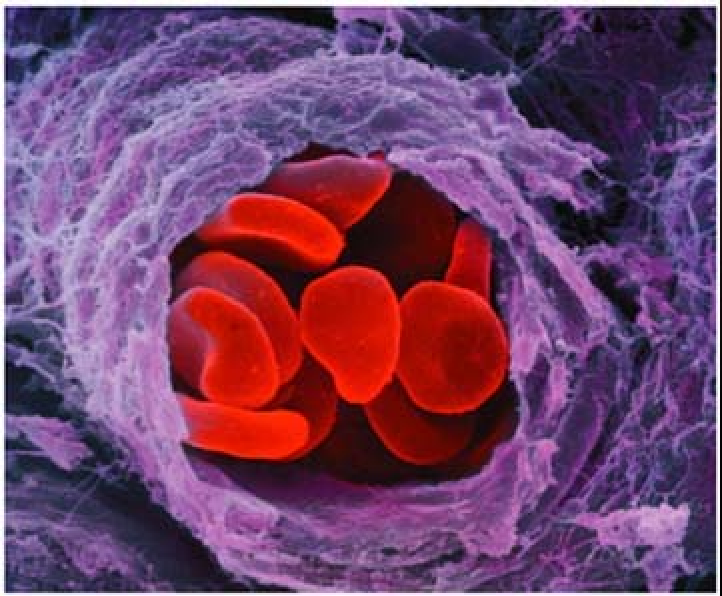
provided by JW Eikelboom

The Goal of Blood Transfusion:

Improve Tissue Oxygen Delivery
Avoid Critical Tissue Hypoxia

Determinants of Oxygen Delivery (DO_2) to Tissues

- <u>In Health:</u>
  - DO_2 2 to 4-fold greater than requirements



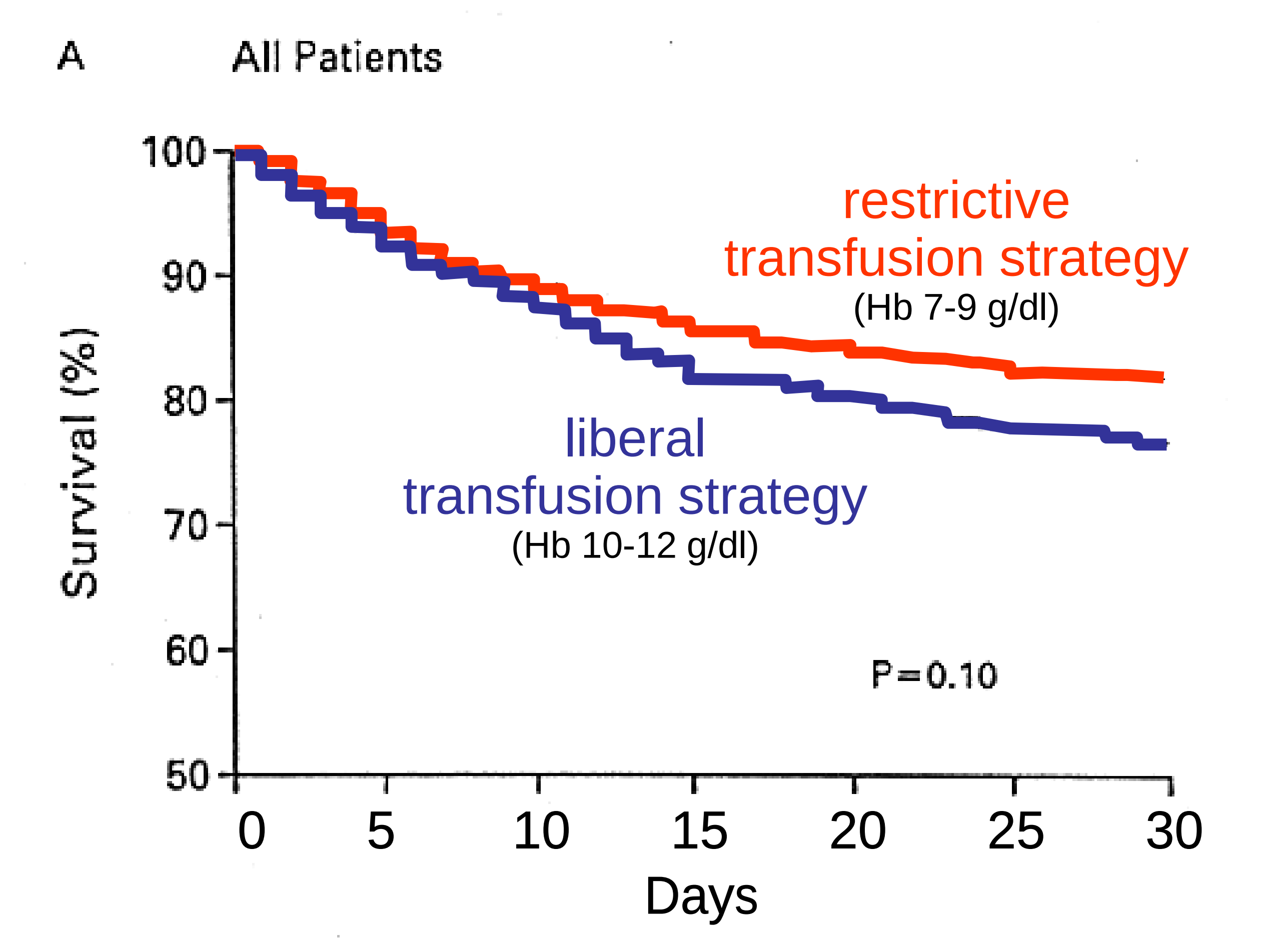
- <u>Determinants of DO_2:</u>
  - Hb level
  - Oxygen saturation
  - Cardiac output
  - Microcirculation
  - Hb O_2 release

black box in clinical practise

Hebert PC, CMAJ 1997; Tinmouth et al, Transfusion 2006

The TRICC Trial

A MULTICENTER, RANDOMIZED, CONTROLLED CLINICAL TRIAL OF TRANSFUSION REQUIREMENTS IN CRITICAL CARE



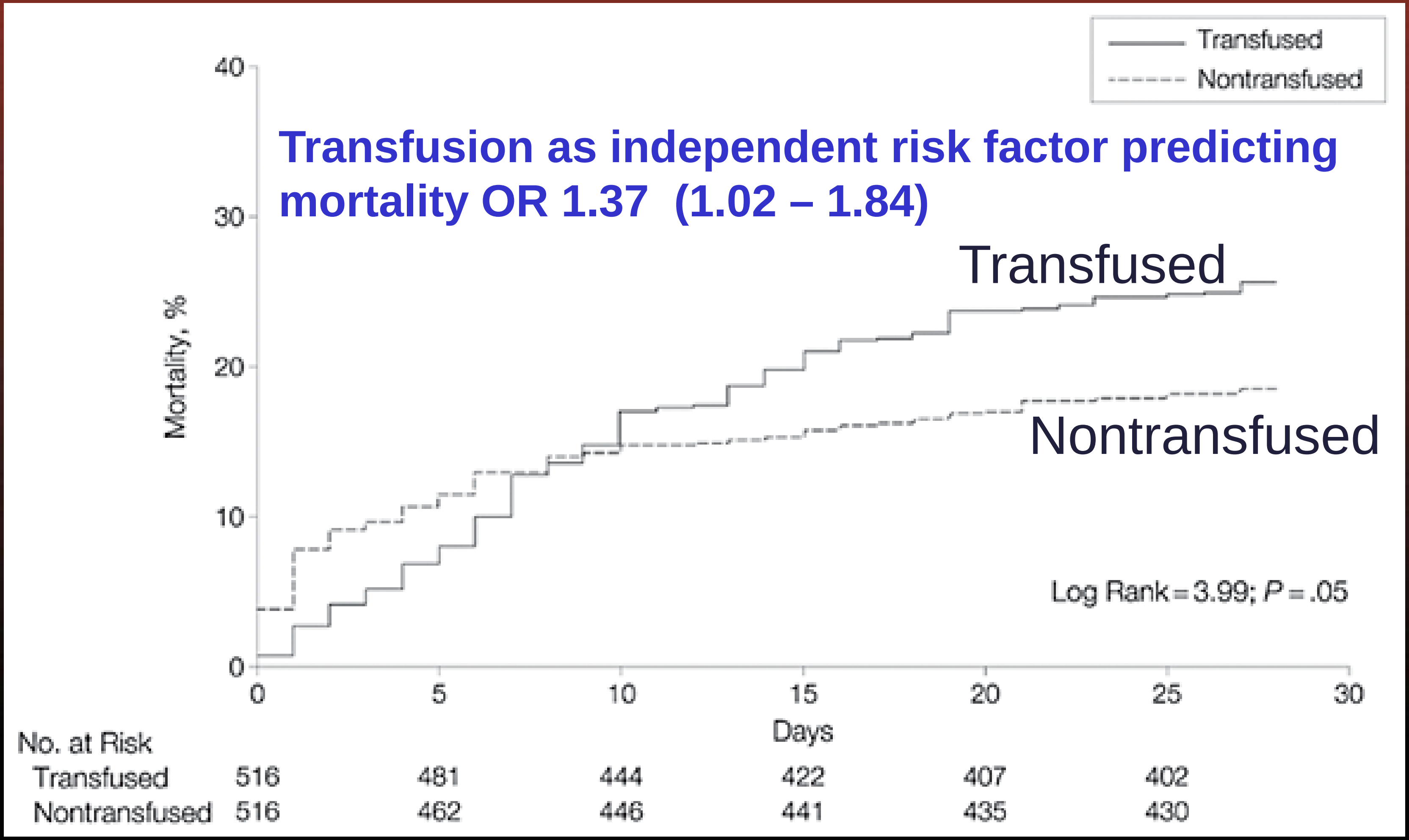
RBC 2.6 ± 4.1

RBC 5.6 ± 5.3

Hébert et al., N Engl J Med 1999;340:409-417

ABC Study: European ICU observational study

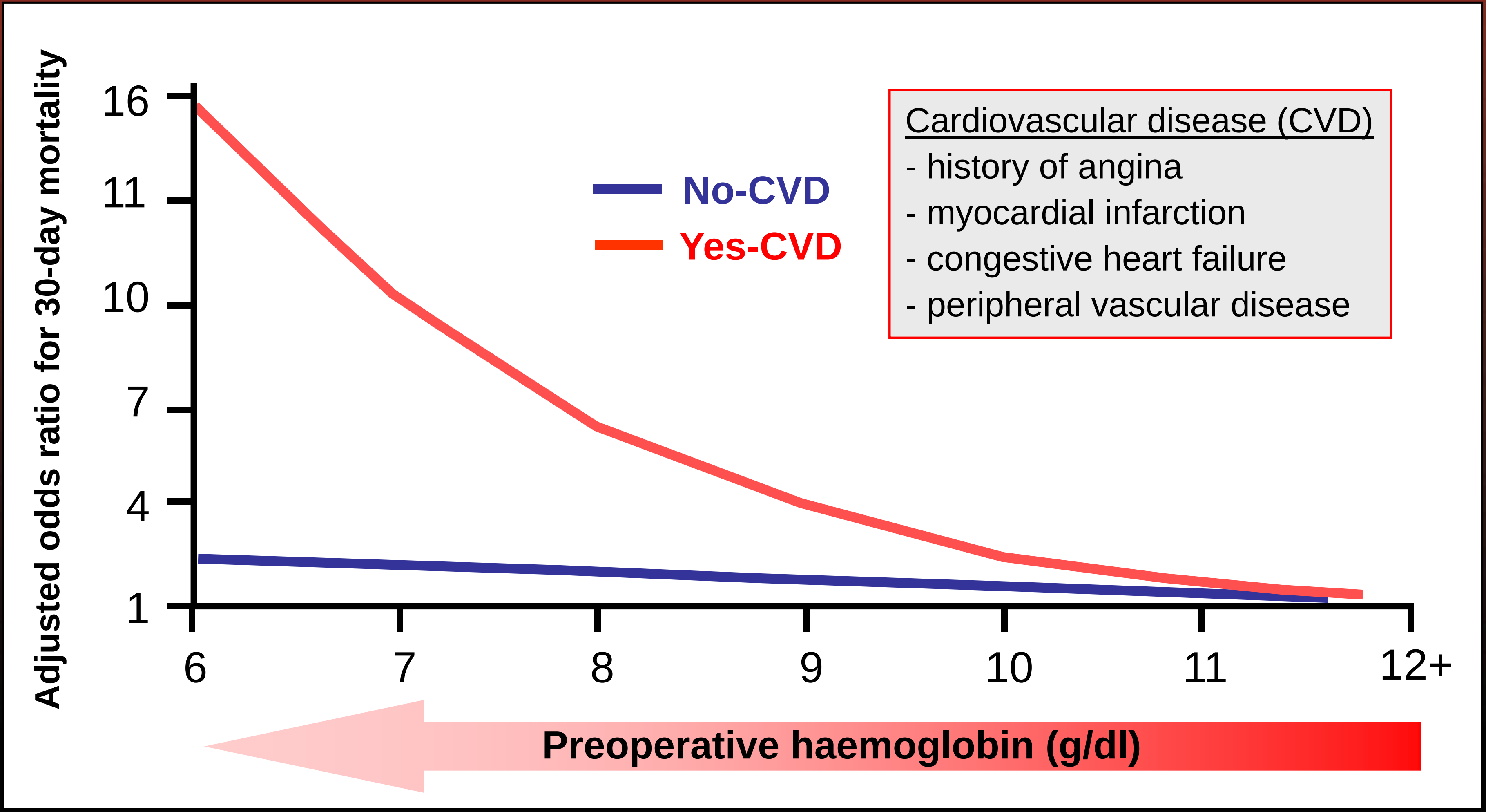
Survival Analysis by Transfusion Status among Propensity-Matched Patients



Vincent et al. JAMA 2002

Risk Factor Anemia – Cardiovascular Patients

retrospective cohorte study, 1958 Jehova's witnesses, 12 hospitals, 14 years



Carson et al., Lancet 248:1055-1060,1996

Transfusion in Cardiac Disease

Indicator for Adverse Consequences

Myocardium has a high extraction ratio of O_2 (60-75%) at normal Hb levels

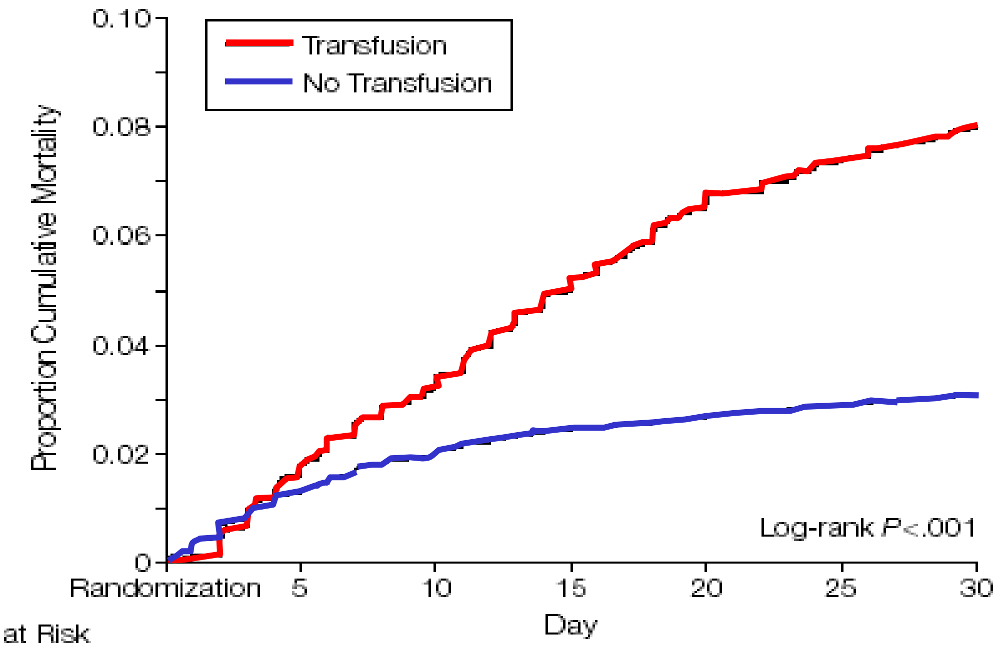
- increased coronary blood flow compensates for decrease in O_2 availability
- impaired in cardiac disease

Transfusion in Patients with ACS

Secondary analysis, ACS (GUSTO IIb, PURSUIT, PARAGON B)

N = 24,112, 10% transfused

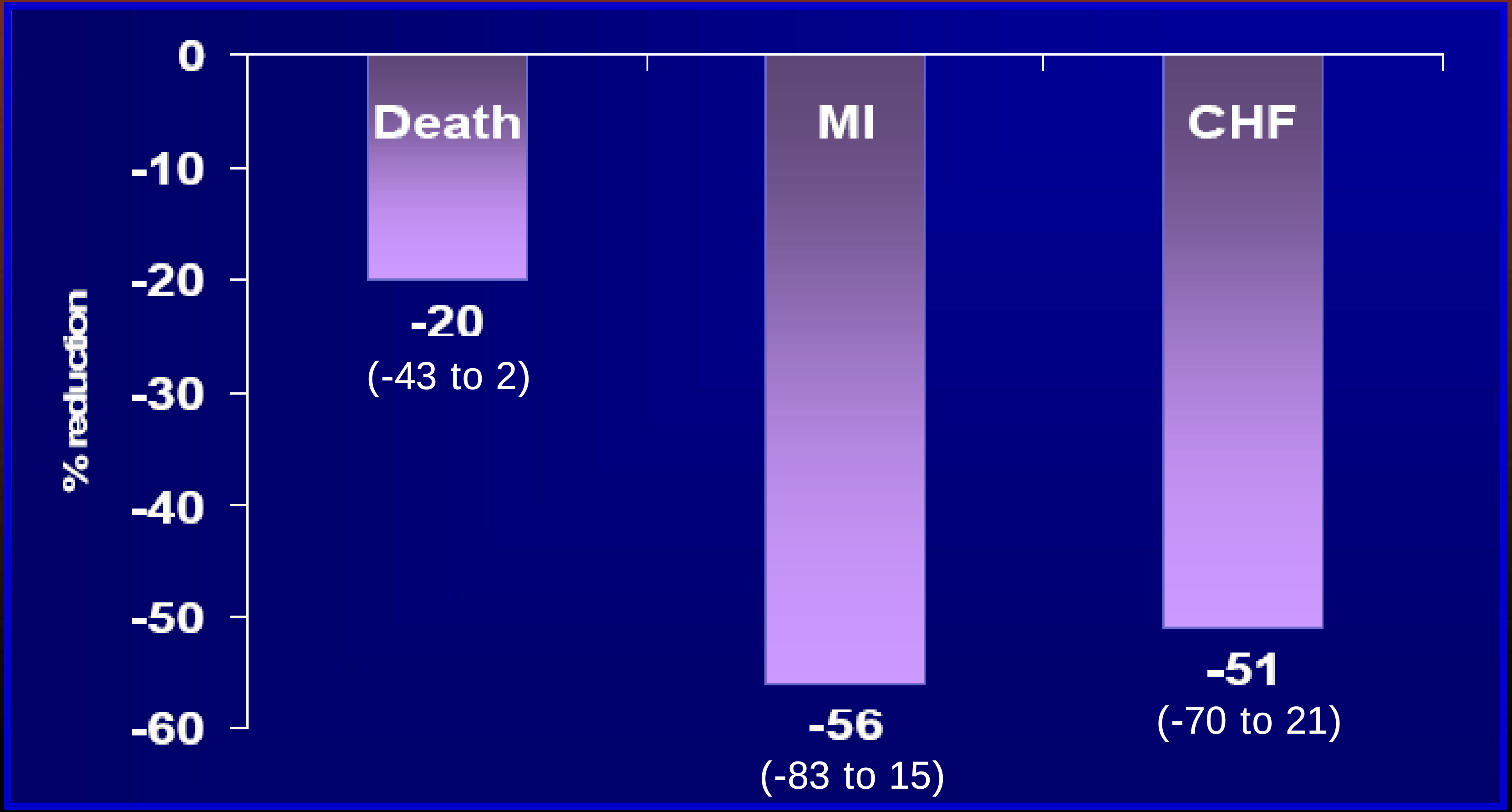
Figure 1. Kaplan-Meier Estimates of 30-Day Mortality Among Patients Who Did and Did Not Receive Blood Transfusion



No. at Risk							
Transfusion	2398	2356	2317	2274	2237	2221	2189
No Transfusion	21 684	21 408	21 248	21 162	21 102	21 062	20 884

Rao et al., JAMA; 2004; 292: 1555-1562

Strong Trend for Benefit of Restrictive Transfusion Strategy



N = 1,760

Hill et al. Cochrane 2000

Transfusion in Patients with ACS

Secondary analysis ACS (GUSTO IIb, PURSUIT, PARAGON B)

N = 24,112, 10% transfused

Table 4. Adjusted Predicted Probabilities of 30-Day Death With and Without Transfusion by Nadir Hematocrit Value

	Nadir Hematocrit, %*			
	20	25	30	35
Adjusted odds ratio (95% CI)†	1.59 (0.95-2.66)	1.13 (0.70-1.82)	168.64 (7.49-3797.69)	291.64 (10.28-8273.85)

Transfusion **is not** a risk factor

Transfusion **is** a risk factor

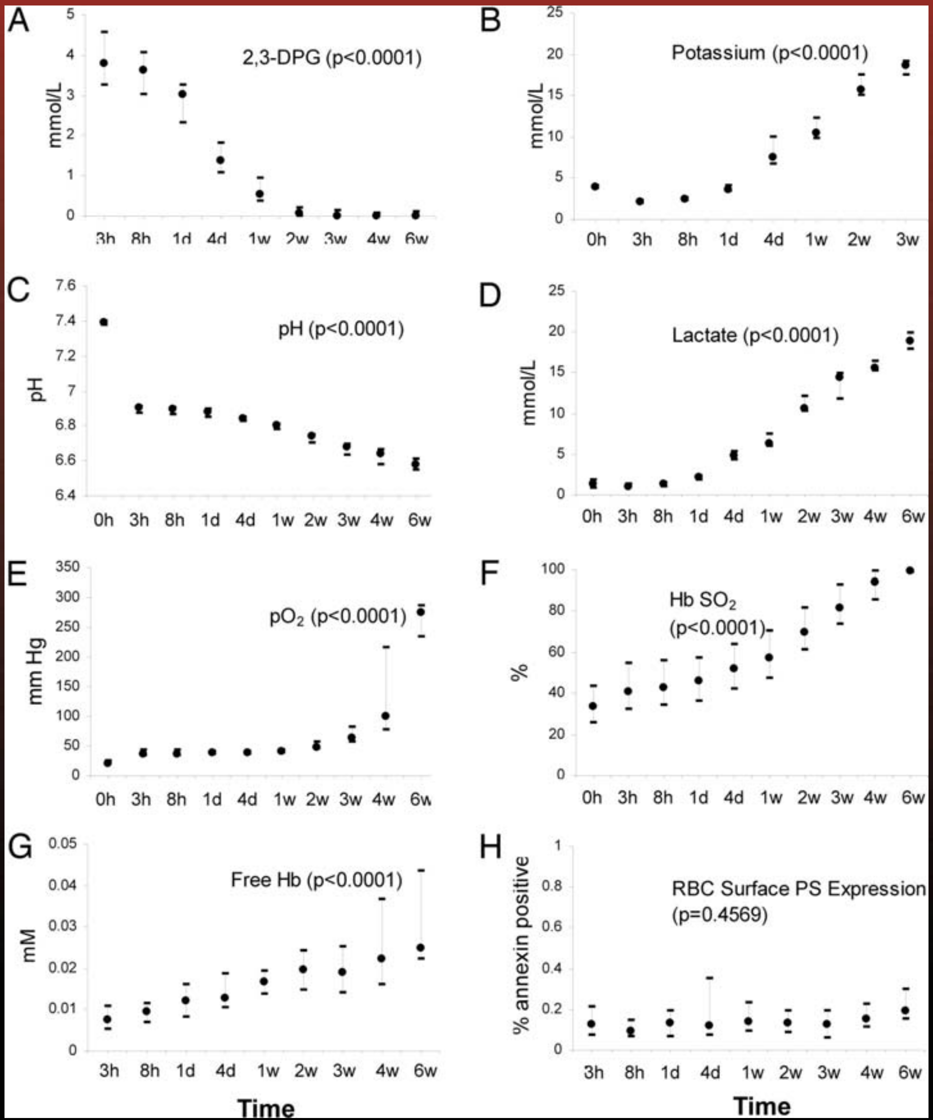
Rao et al., JAMA; 2004; 292: 1555-1562

Changes During RBC Storage

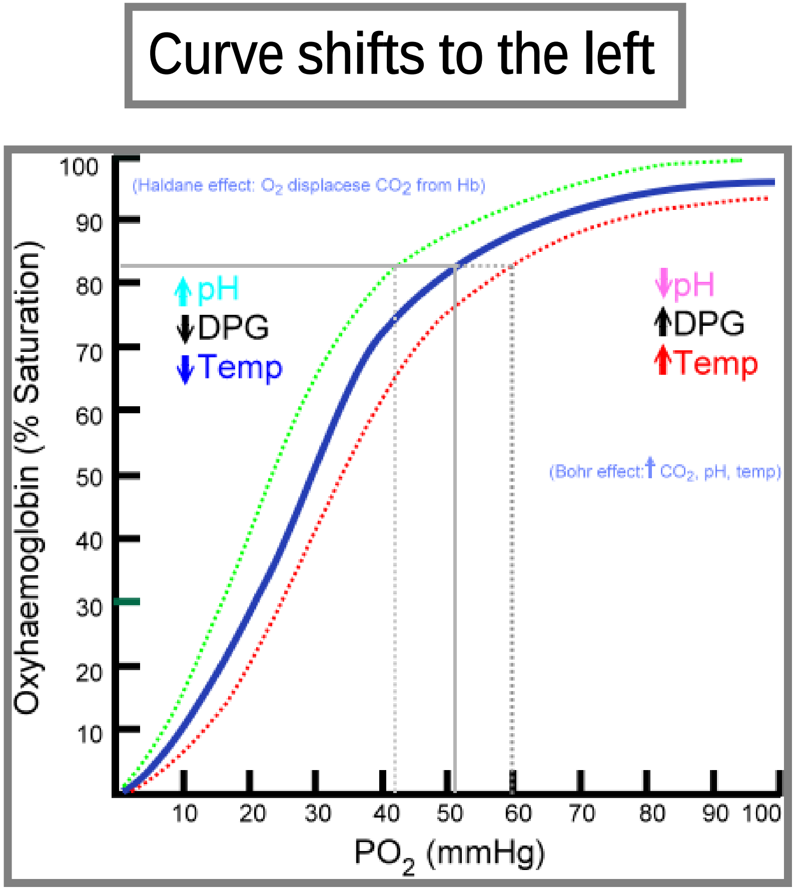
- Structural
  - Clumping of RBCs
  - Lose membrane phospholipid
  - Biconcave → spherocyte / shistocyte
- Biochemical
  - ATP depletion
  - 2,3-DPG depletion
  - NO depletion
- Inflammatory
  - ↑Inflammatory mediators

Review: Tinmouth A. et al. Transfusion 2006;46:2014-2027

Changes During RBC Storage



Bennett-Guerrero E. et al. PNAS, 2007;104:17063-17068

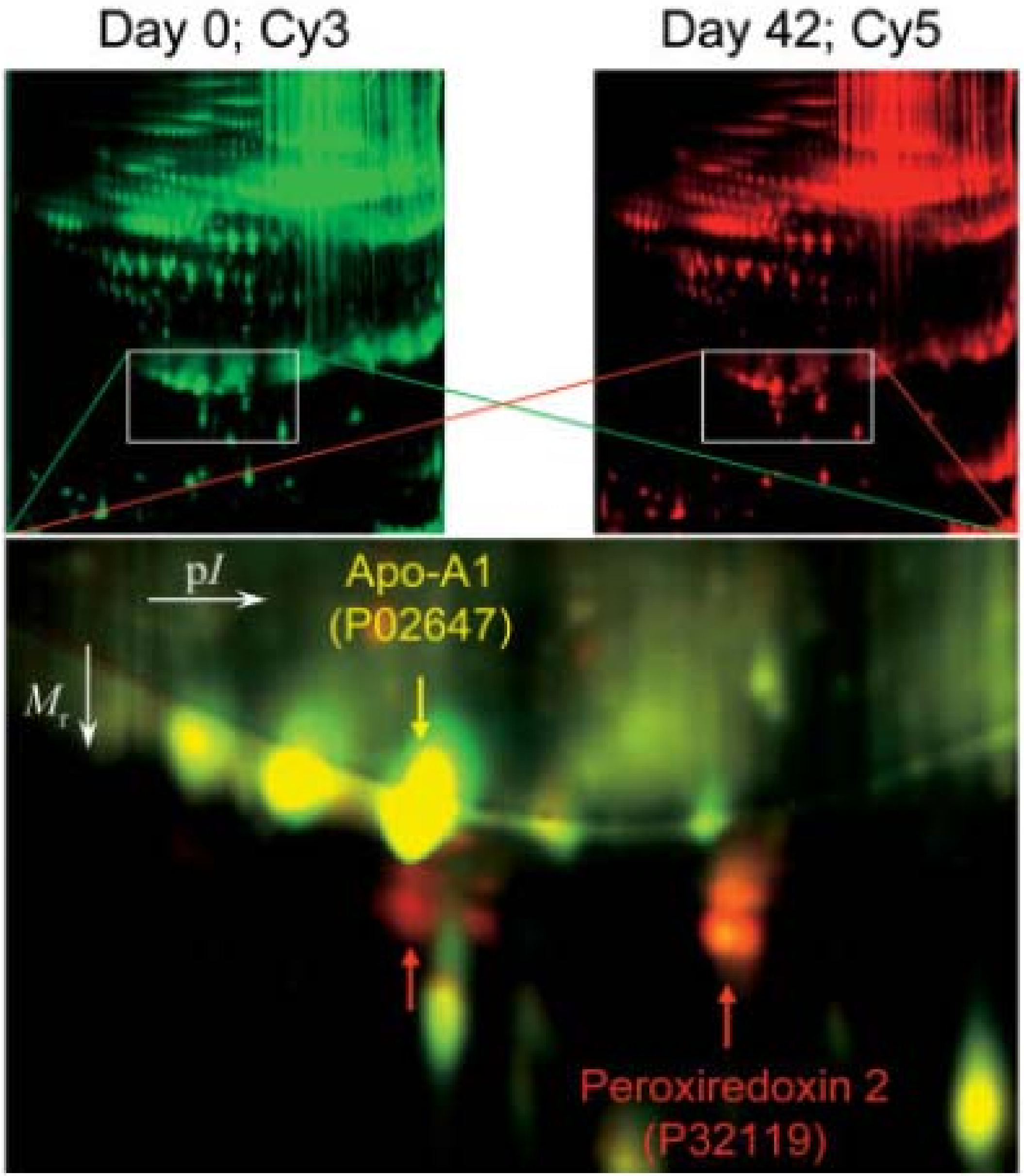


http://en.wikipedia.org/wiki/
Oxygen-haemoglobin_dissociation_curve

Impact of Altered Red Cell Rheology on Oxygen Delivery

- Rigid red blood cells less able to pass through microcirculation
- Decreased microvascular blood flow
- Reduced DO_2
- Local tissue hypoxia

Berezina et al J Surg Res 2002



Review

Proteomics and transfusion medicine: Future perspectives

Pierre-Alain Queloz[1]*, Lynne Thadikkaran[1]*, David Crettaz[1], Joel S. Rossier[2], Stefano Barelli[1] and Jean-Daniel Tissot[1]

[1] Service régional vaudois de transfusion sanguine, Lausanne, Switzerland
[2] DiagnoSwiss SA, Monthey, Switzerland

Proteom pattern of supernatants of stored RBCs

Cytokines
Histamine, Bradykinin
Complement
Cell membrane fragments
Free Hemoglobin
…

P.-A. Queloz *et al. Proteomics* 2006, *6*, 5605–5614

Impact of Impaired O_2 Delivery

- Transfusion improved mixed venous oxygen tension

  BUT
- No definite improvement / deterioration in tissue DO_2
- Anaerobic glycolysis occurs at higher Hb level (critical tissue hypoxia at higher Hb)
- Shock develops at higher Hb threshold in transfused patients

Spiess BD. Hem Onc Clin North Am 2007

Age of Transfused Blood

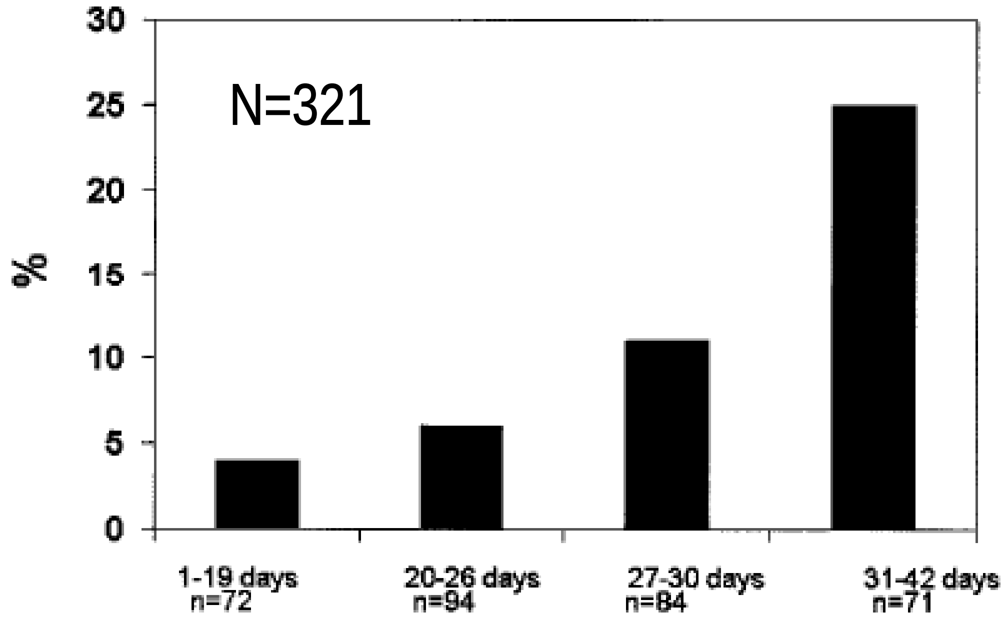


Figure 1. The rate of in-hospital mortality presented by quartiles of maximum duration of storage of transfused red blood cells (RBCs).

Basran et al. Anesthesia and Analgesia 2006

Consequence of RBC Storage

TABLE 3. Association of RBC storage with clinical outcomes: observational studies

Study: first author, year	Population	Design	Number	Outcomes
Basran, 2006[115]	Cardiac surgery	Retrospective cohort	321	Increased mortality associated with mean age of RBC units and age of oldest RBC unit
Leal-Noval, 2003[104]	Cardiac surgery	Prospective cohort	897	Increased pneumonia associated with oldest unit
Keller, 2002[117]	Trauma	Retrospective cohort	86	Increased LOS with number of RBC units >14 days
Offner, 2002[105]	Trauma	Prospective cohort	61	Increased infections with number of units >14 and 21 days
Vamvakas, 2000[118]	Cardiac surgery	Retrospective cohort	268	No change in LOS or mechanical ventilation associated with age of RBC units
Vamvakas, 1999[103]	Cardiac surgery	Retrospective cohort	416	Increased risk of pneumonia with median age of transfused RBC units
Zallen, 1999[116]	Trauma	Prospective cohort	63	Increased multiorgan failure with number of units >14 and 21 days
Purdy, 1997[114]	Septic ICU	Retrospective cohort	31	Increased mortality associated with older median age of RBC units
Martin 1993[99]	ICU	Retrospective cohort	698	Increased LOS with number of units >14 days

n=2841

Tinmouth et al. Transfusion 2006

Protein *S*-nitrosylation: purview and parameters

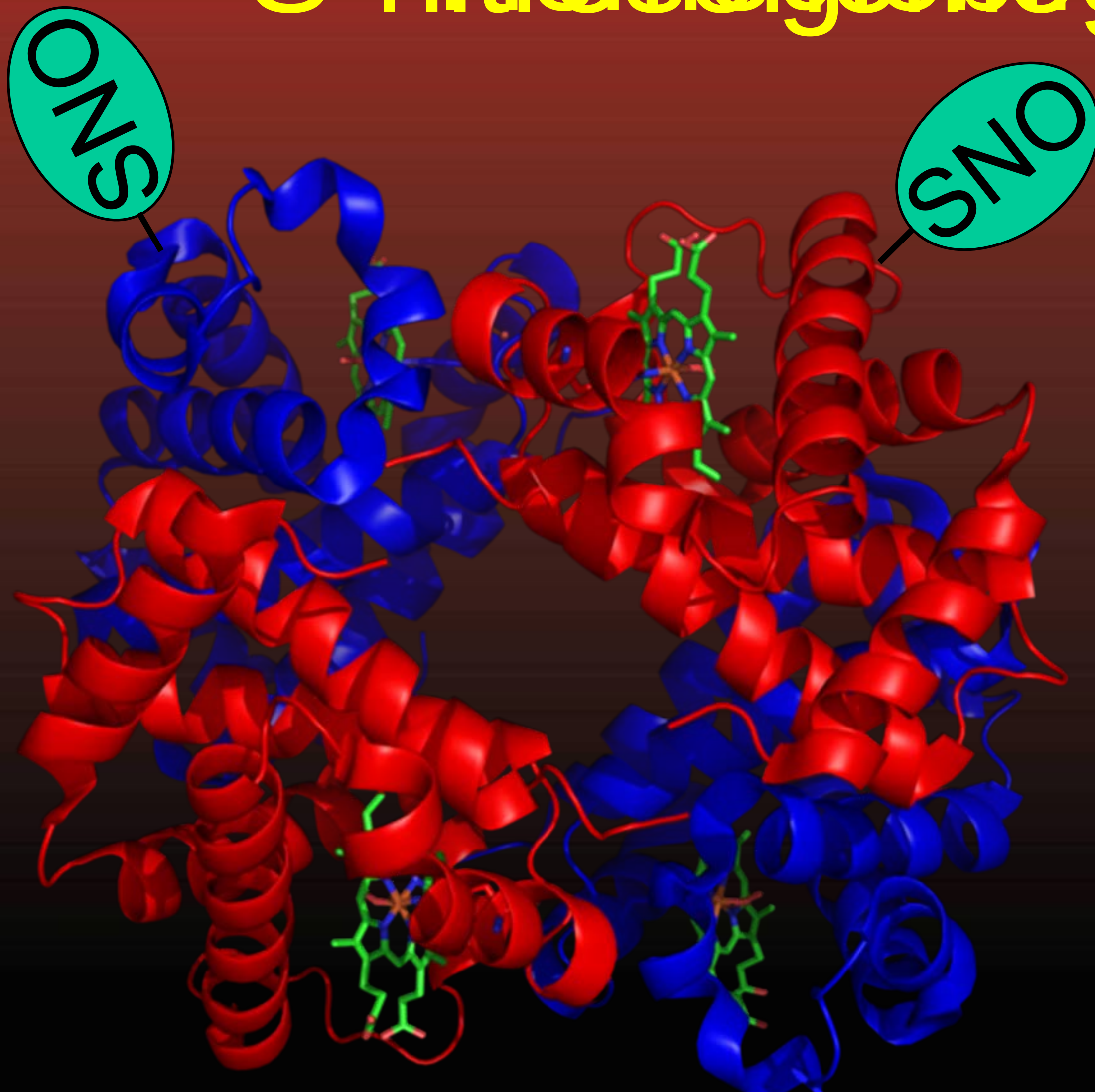
Hess D.T. et al

Nature Reviews Molecular Cell Biology

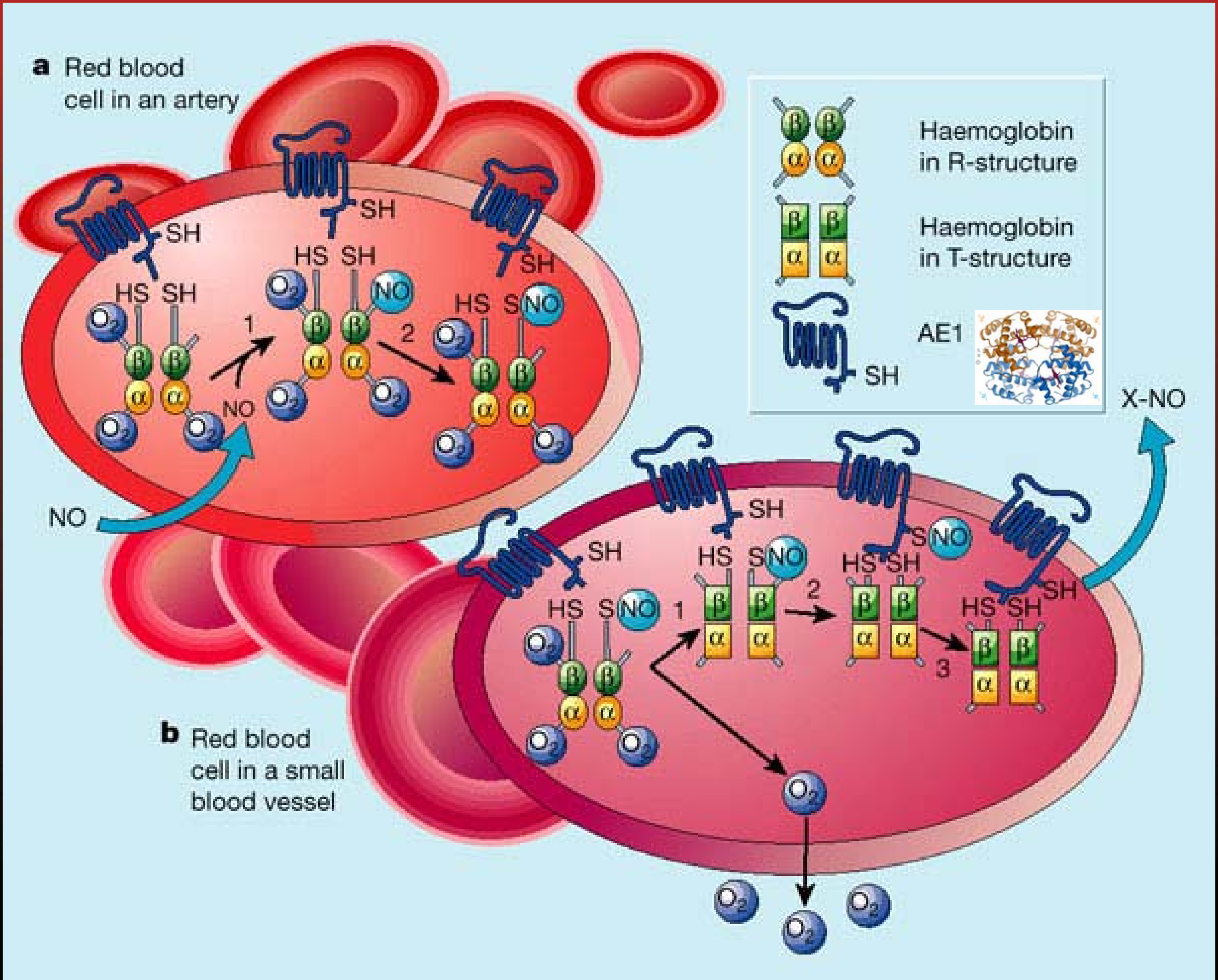
2005;6:150-166

Hemoglobin

S-nitrosohemoglobin

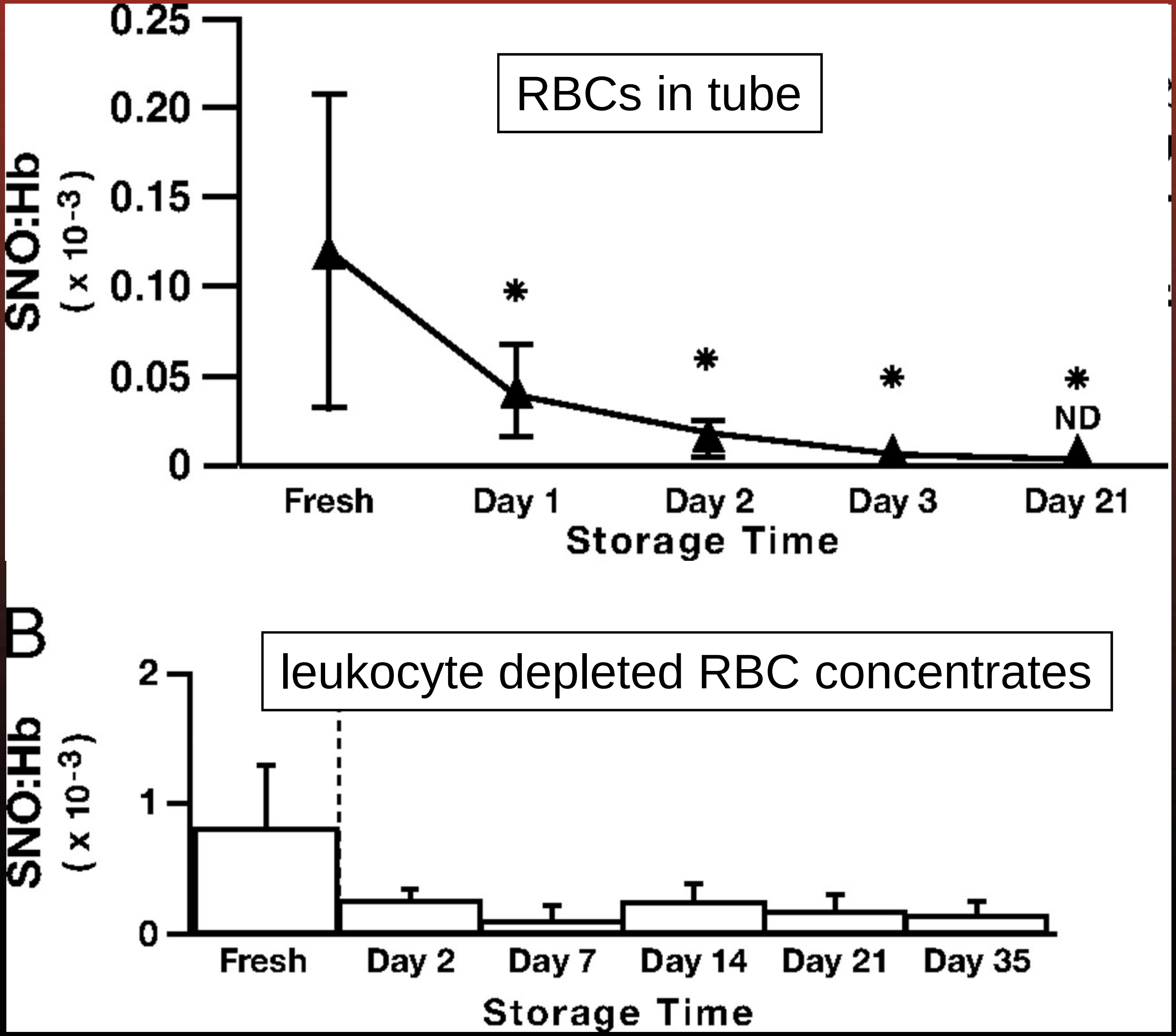


- 4 subunits
- Heme
  - Porphyrin ring
  - Fe^{2+}
- Hemoglobin is the O_2 sensor
- Cooperative O_2 binding
- SNO is the signal transducer
- CO binding
- Binds NO at ß-Cys 93



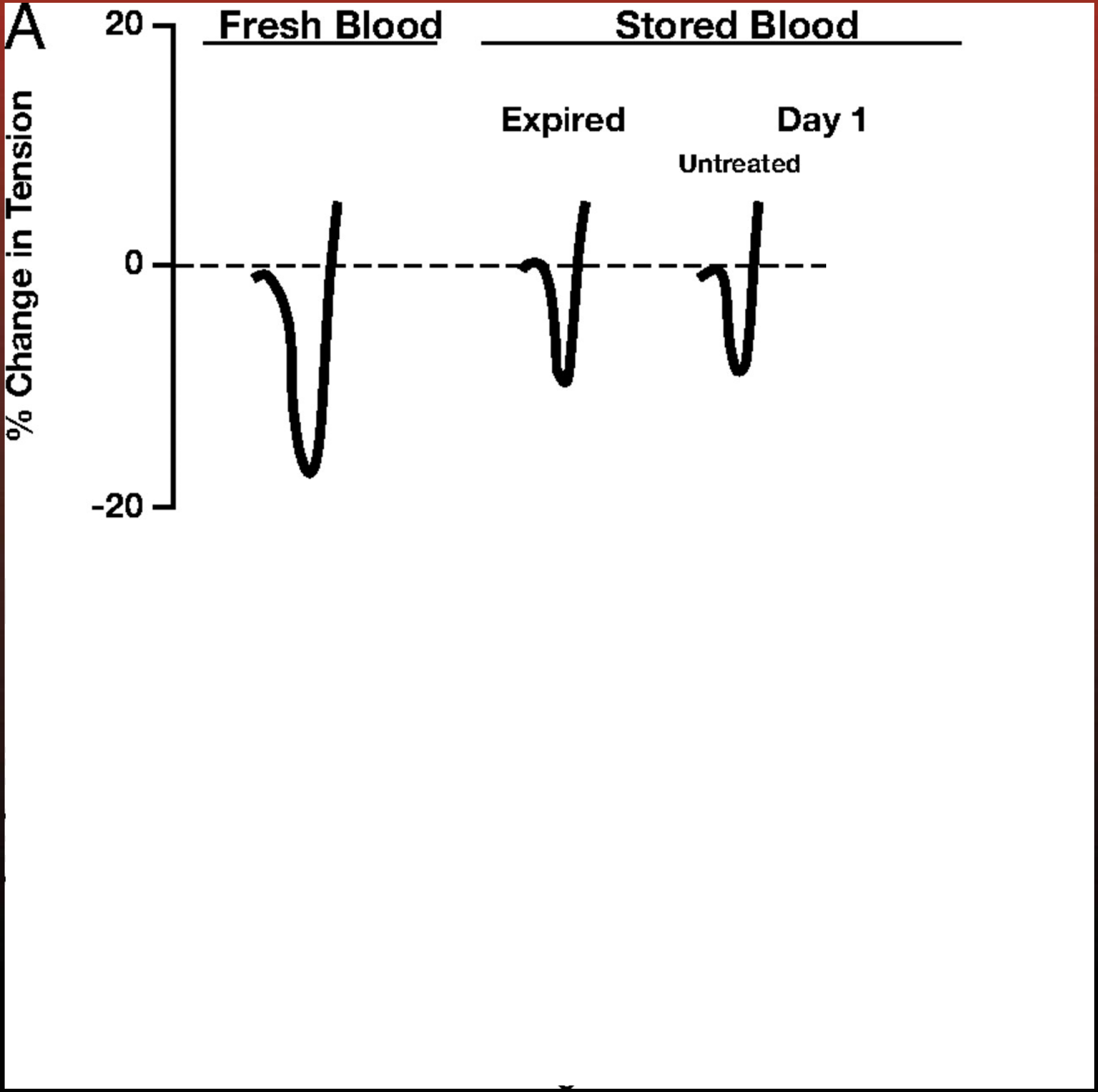
Gross SS, Nature 2001;409:622-6

SNO-Hb in Stored Blood



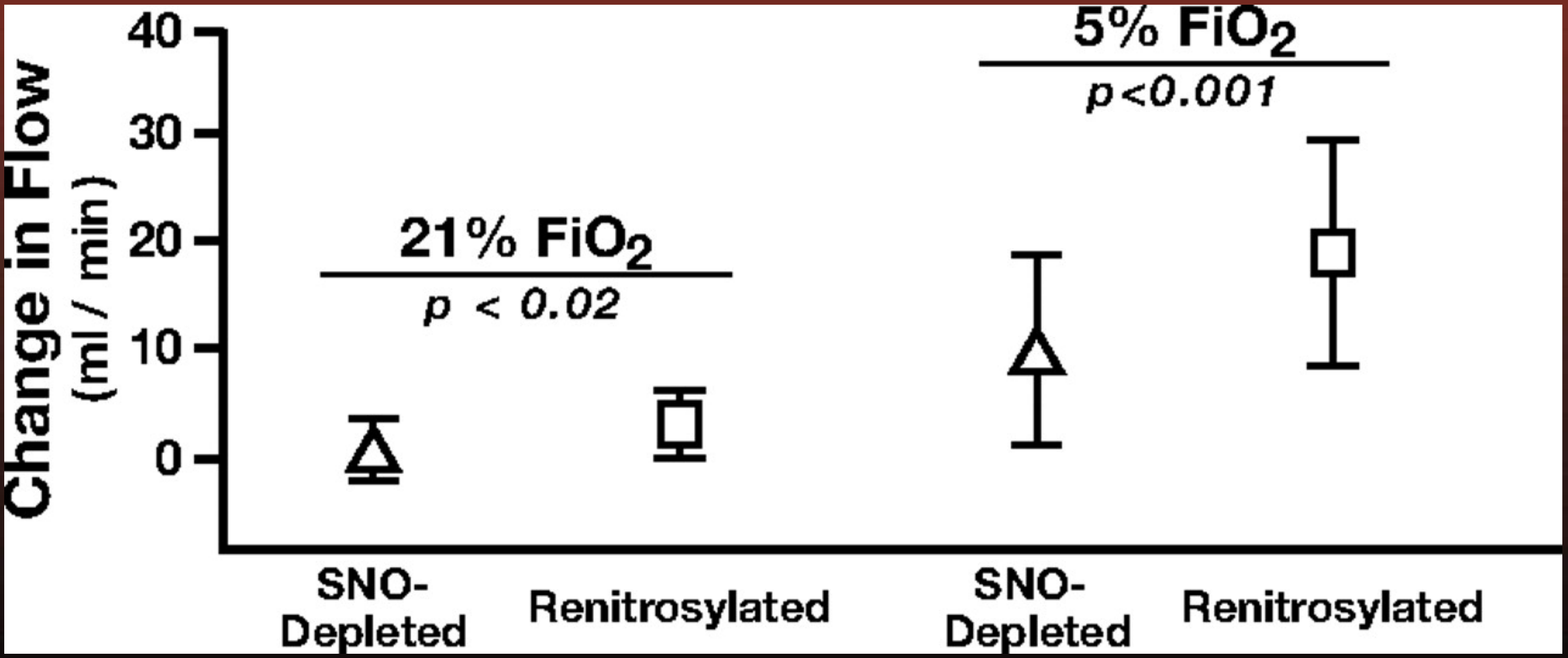
Reynolds JD et al. 2007. PNAS;104:17058-62

SNO-Hb content and vasodilatory activity of renitrosylated RBCs

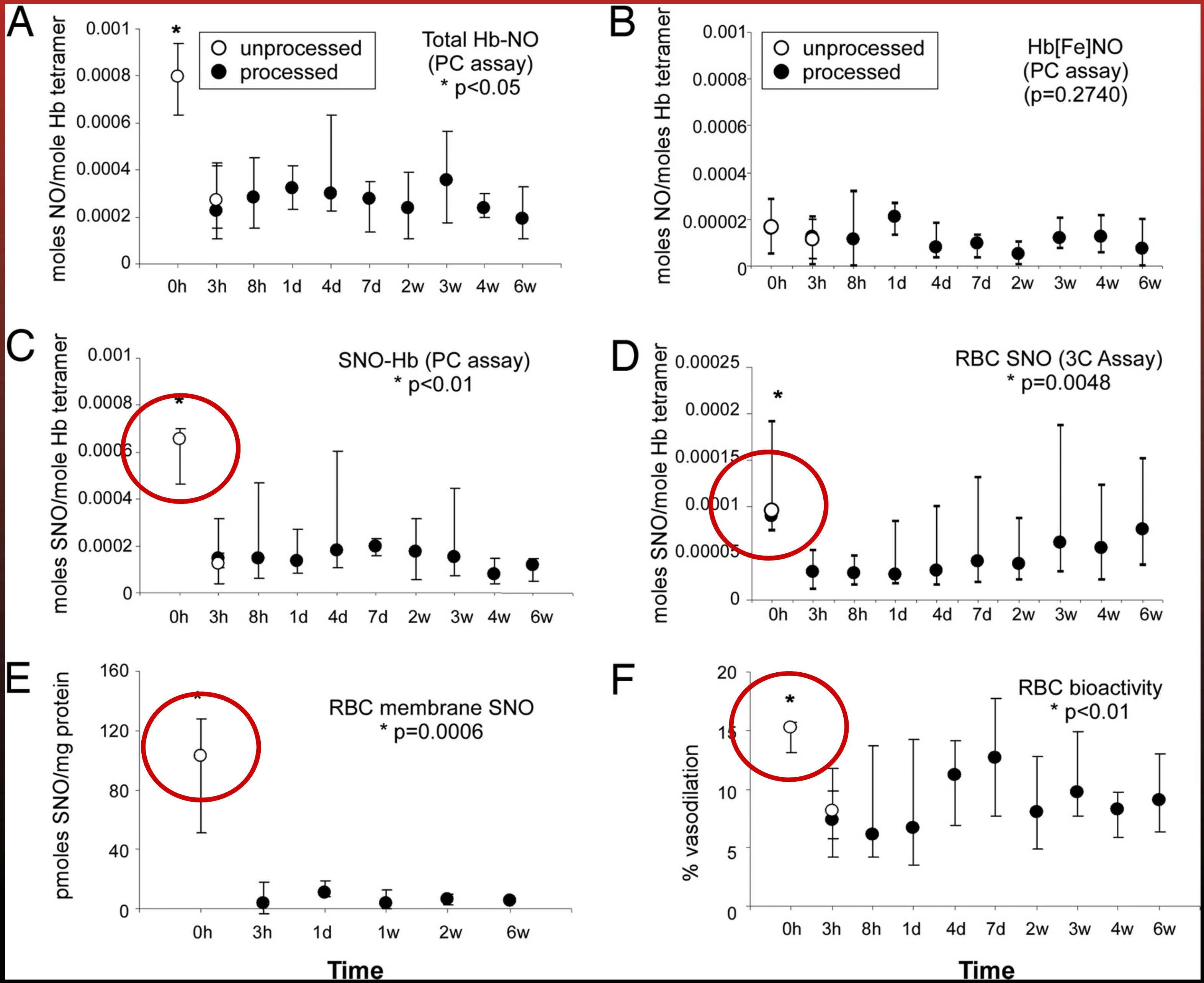


Reynolds JD et al. 2007. PNAS;104:17058-62

Hypoxic vasodilation by stored and renitrosylated RBCs in vivo



Reynolds JD et al. 2007. PNAS;104:17058-62



Bennett-Guerrero E. et al. 2007. PNAS;104:17063-8

letters to nature

S-Nitrosothiols signal the ventilatory response to hypoxia

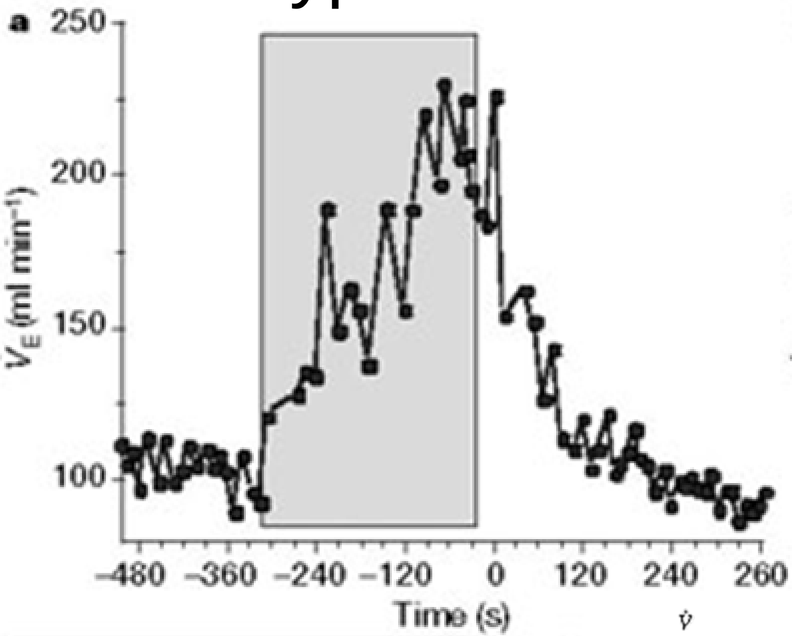
Andrew J. Lipton*, Michael A. Johnson†, Timothy Macdonald†, Michael W. Lieberman‡, David Gozal*§ & Benjamin Gaston§‖

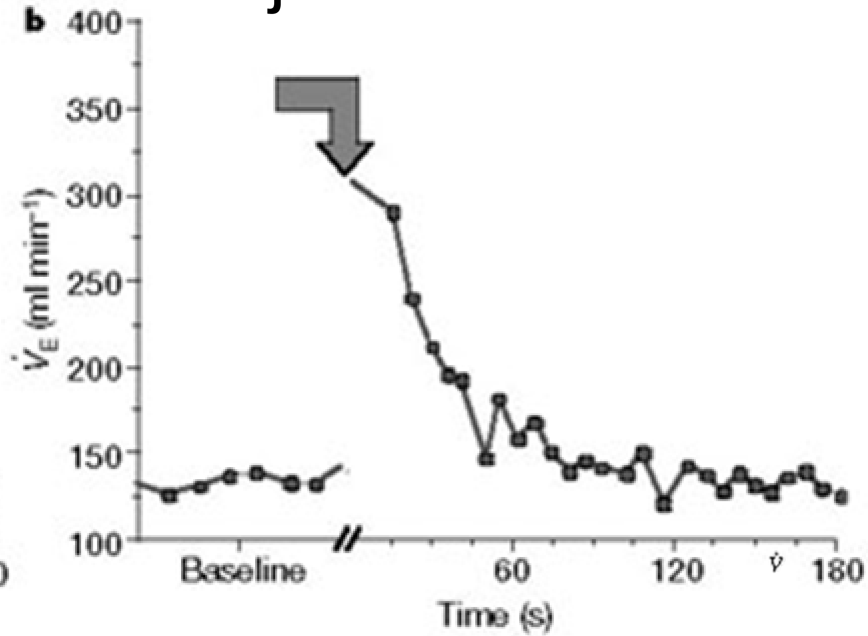
* *Kosair Children's Hospital Research Institute, Departments of Pediatrics, Pharmacology and Toxicology, University of Louisville, Louisville, Kentucky 40202, USA*

AJ Lipton et al. *Nature,2001;***413**: 171-174

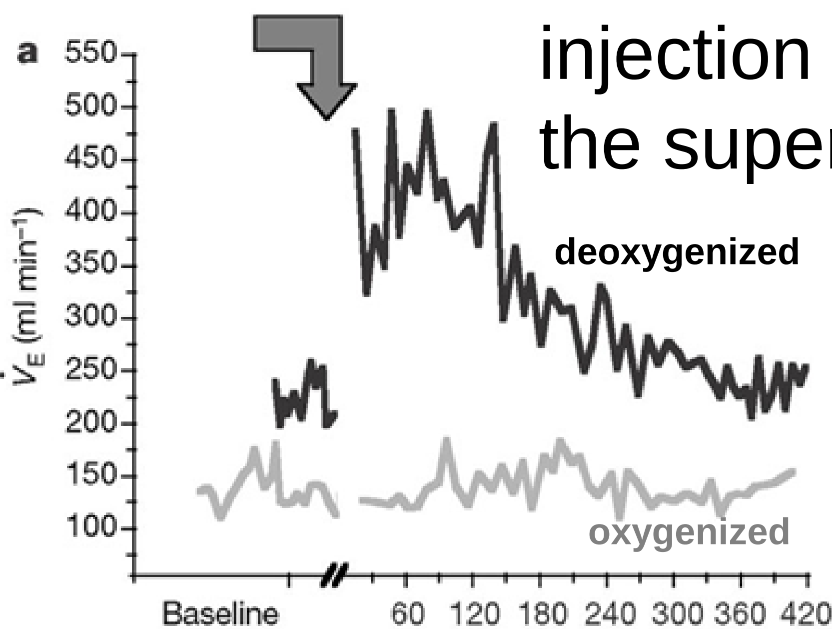
hypoxia

injection of CGSNO

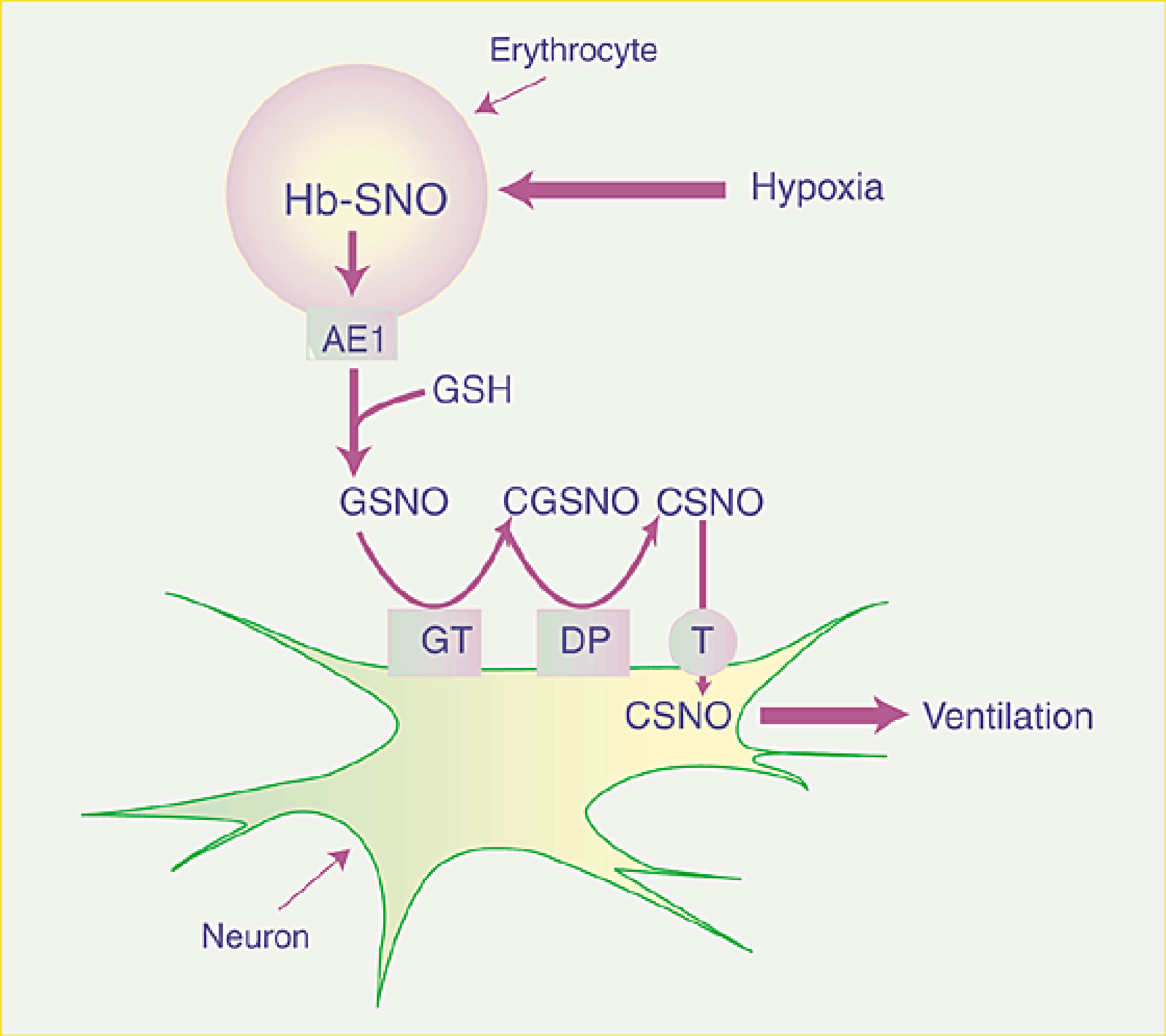




injection of <10kDa fraction of the supernatant of erythrocytes



AJ Lipton et al. *Nature,2001;***413**: 171-174



Mato JM et al. *Nature Medicine* 2001;7:1107 - 1108

Implications for Research

- Epidemiological
  - Confirming association of transfusion and adverse outcomes
  - Mechanisms (age of transfusion)
- Randomised
  - lower transfusion thresholds
  - fresh blood vs older blood

Implications

	Population	RBCs transfused/year
Germany	82 mio Ratio: 2.6	4.3 mio Ratio: 4.8
Canada	32 mio	0.9 mio

Conclusion 1

- Clinical observations and secondary analyses indicate that transfusion of stored blood might be associated with adverse outcomes
- Patients with impaired cardiac function are a high risk group for storage related adverse events of transfusion and an indicator for HV
- Prospective randomized trials are urgently needed comparing outcomes related to storage time of transfused RBCs

Conclusion 2

- NO in erythrocytes is a key player in hypoxia
- O_2 release is increased via SNO-Hb by
  - vasodilation
  - central stimulation of ventilation
- SNO-Hb decreases in stored blood < 1day
- Currently all RBCs are SNO-Hb depleted
- Do we suffocate patients by RBC transfusion (microcirculation and central)?
- Renitrosilation of stored blood might be a perspective

Dr Kathryn Webert

Dr John W. Eikelboom

McMaster University, Hamilton Canada

kindly provided me with slides of their educational talks on RBC transfusion and adverse effects

Reynolds et al PNAS 2007

- RBCs depleted in SNO-Hb were renitrosylated with purified NO solutions (1). Aliquots of fresh or packed RBCs at storage days 1, 7, 14, and 21 along with samples of expired RBCs were mixed with PBS (pH 7.4) in gas-tight vials and subsequently degassed with argon. (The expired RBC samples required additional pH buffering by washing in PBS.) NO was slowly added to the vials to obtain a final NO:heme ratio of either 1:250 or 1:500. The vials were then opened to room air and gently vortexed to achieve rapid oxygenation of the RBCs [as previously determined, the vasodilatory activity generated at 1:500 and 1:250 was indistinguishable, indicating saturation in the amount of SNO-Hb produced (1-3)]. After resuspending RBCs in PBS (»750 ml),150 ml was used for determining NO levels and the remainder was used in bioassays. RBCs used in the canine study were depleted of SNO-Hb by overnight storage (up to 24 h) at 4°C; these RBCs were then mixed with PBS (without NO) and gently aerated.

Bennet guerrero PNAS

- Blood Donation and Processing. Subjects were asked to consume nothing by mouth after midnight before the donation. On arrival to the donation site (DUMC Post Anesthesia Care Unit), using aseptic technique and a blood pressure cuff, »500 ml of whole blood was collected from a peripheral vein directly into a Pall Medical Leukotrap WB System containing 70 ml of CP2D anticoagulant (containing 0.0156 M citric acid, 0.1227 M sodium citrate, 0.016 M monobasic sodium phosphate, and 0.257 M dextrose). After leukocyte depletion and centrifugation, packed RBCs were resuspended in 110 ml of Nutricel [additive solution-3; AS-3] preservative solution. Each unit of RBCs was stored in a monitored and alarmed refrigerator at 1-6°C. Aliquots of RBCs were withdrawn by using a sterile docking device (Sterile Tubing Welder TSCD model SC-201A; Terumo Medical Corp, Somerset, NJ) and transferred on ice to the appropriate laboratories for analysis at: 3, 8, 24, and 96 h, and 1, 2, 3, 4, and 6 weeks.