The red blood cell (RBC) storage lesion:

Is it clinically significant?

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Red Blood Cell (RBC) Transfusion is a Common Medical Practice

- ~ 14 Million RBC units are collected/year in US
  - Almost all are transfused (wastage is minimal)
- Complex logistics of procurement and supply
  - Goal is optimal utilization of RBC resources
- RBC units can be stored up to 42 days at 1-6^{o}C prior to transfusion
  - FDA licensure of transfusion technologies (bags, collection solutions, etc) is based on survival of RBCs (not function)
  - *Assumption*: if RBCs survive, they are working
  - However……

Many biochemical and functional changes occur during RBC storage…

- ↑ Inflammatory mediators
- ↑ RBC adhesion
- ↑ Immunomodulation
- ↑ Hemolysis
- ↓ RBC deformability
- ↓ ATP
- ↓ 2,3-DPG
- ↓ NO

…The critical question is: *do these changes sufficiently alter RBC function to produce different outcomes in recipients?*

The results will have significant implications for blood supply logistics

20+ major clinical studies addressing this issue

- Decidedly mixed results
  - Some show effects of storage on recipient outcomes
  - Others do not
- Factors complicating analysis
  - Single vs. multiple center
  - Study size ranges from 15 – 400,000 patients
  - Variable RBC processing & storage methods
- How generalizable are results of one study to another patient group?

Koch *et al*: storage impairs outcome

The NEW ENGLAND JOURNAL of MEDICINE

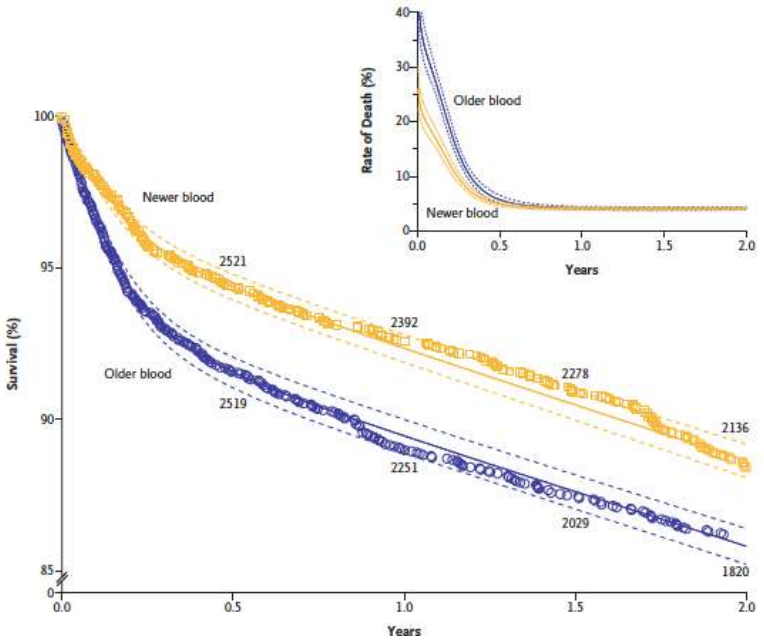
ORIGINAL ARTICLE

Duration of Red-Cell Storage and Complications after Cardiac Surgery

Colleen Gorman Koch, M.D., Liang Li, Ph.D., Daniel I. Sessler, M.D., Priscilla Figueroa, M.D., Gerald A. Hoeltge, M.D., Tomislav Mihaljevic, M.D., and Eugene H. Blackstone, M.D.

Retrospective review of cardiac surgery pts

- 8802 units stored median 11d given to 2872 pts
- 10,782 units stored median 19d given to 3130 pts



	MORTALITY	VENT > 72 H	COMPOSITE
RBC: mean 11d	1.7%	5.6%	22.4%
RBC: mean 20d	2.8%	9.7%	25.9%
P-value	0.004	< 0.001	0.001

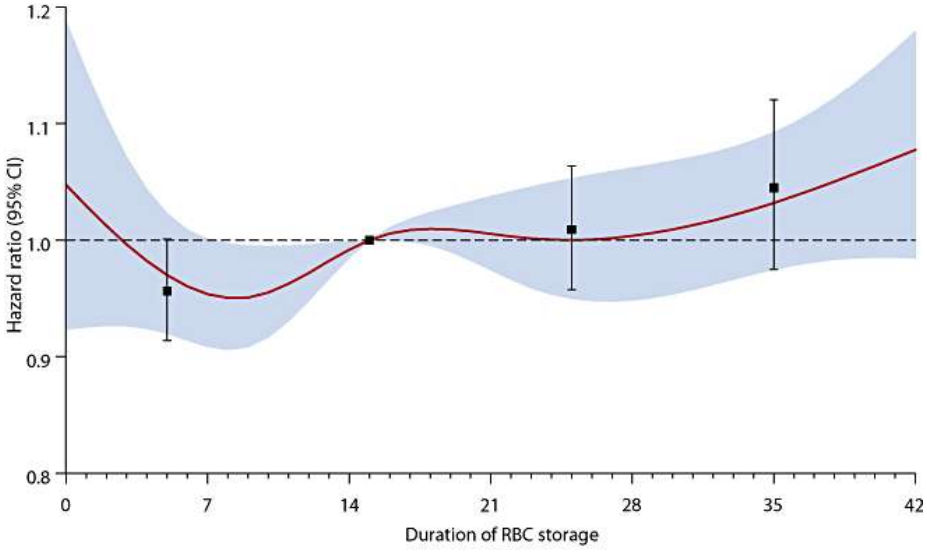
Edgren *et al*: No effect of storage time

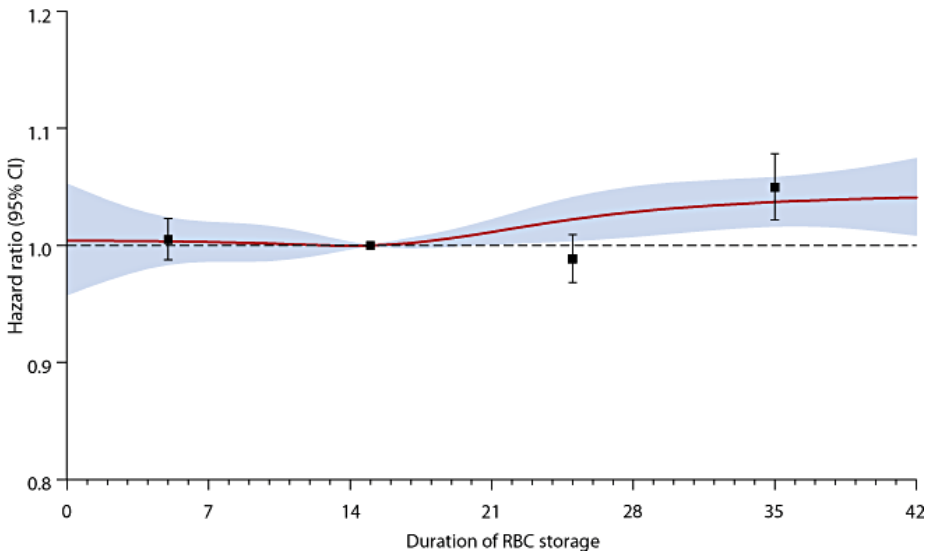
CME

Duration of red blood cell storage and survival of transfused patients

Gustaf Edgren, Mads Kamper-Jørgensen, Sandra Eloranta, Klaus Rostgaard, Brian Custer, Henrik Ullum, Edward L. Murphy, Michael P. Busch, Marie Reilly, Mads Melbye, Henrik Hjalgrim, and Olof Nyrén

TRANSFUSION 2010;50:1185-1195.





405,000 transfusion episodes studied: *"Although a small excess mortality was noted in recipients of the oldest RBCs, the risk pattern was more consistent with weak confounding…"*

Retrospective studies have problems

- It is almost impossible to remove all biases from retrospective studies
- Based on research questions that were first examined by retrospective studies, and then prospective RCT:
  - Retrospective studies overstate benefits
  - Retrospective studies understate risks
  - Retrospective studies reach incorrect conclusions > 50% of the time

- "Lies, Damned Lies, and Medical Science"
  - *(Atlantic Magazine; November 2010)*

Effect of Red Cell Storage Age: *Equipoise Exists*

- Results from large retrospective studies are in disagreement
- Until RECESS (which has re-started accrual) no large, prospective human RCT has evaluated the effects of transfusion of RBC units stored for different periods on:
  - Clinical outcome
  - Hemodynamic variables & end-organ function
  - Immediate O_2 delivery enhancement
  - Microvascular circulatory changes

We are taking another approach: Investigate effects at an "individual patient level"

- Focus on the possible role of alterations in peripheral NO activity following exposure to stored RBCs
- INOBA hypothesis: Insufficient NO Bio-Availability
  - A hypothesis to explain the adverse effects of aged blood?

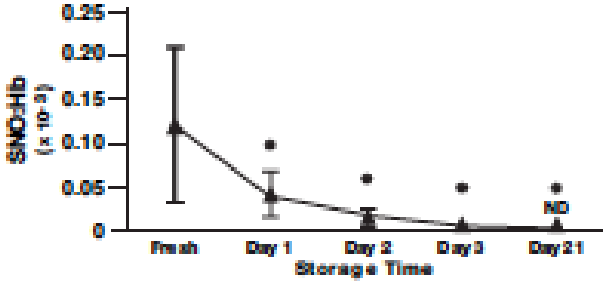
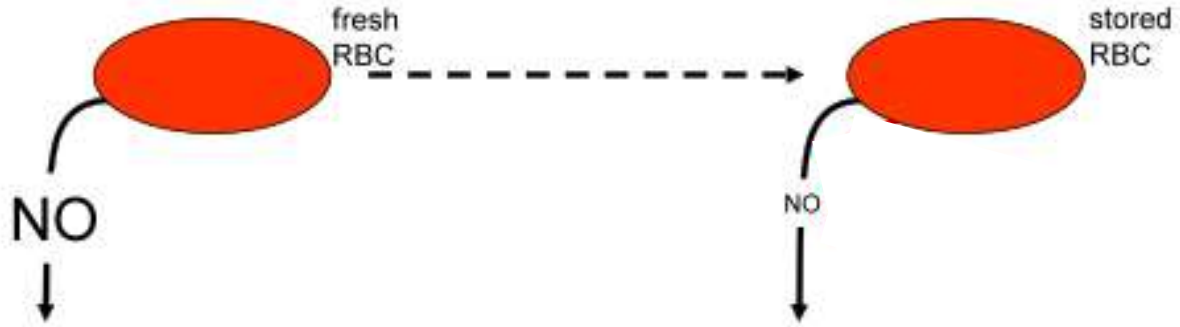


Fig. 1. SNO-Hb in stored human whole blood. Shown is the change in levels over time in human RBCs ($n = 4$) stored in citrate phosphate dextrose/additive solution 1. Packed cells were kept in vacutainers at 4°C and serially sampled by using an aseptic technique at the times indicated. *, $P < 0.05$ (significant difference from fresh blood). At day 21, the level of SNO-Hb was below the sensitivity of the assay (ND, not detected).

Reynolds, et al, PNAS 104: 17058 (2007)

Nitric Oxide

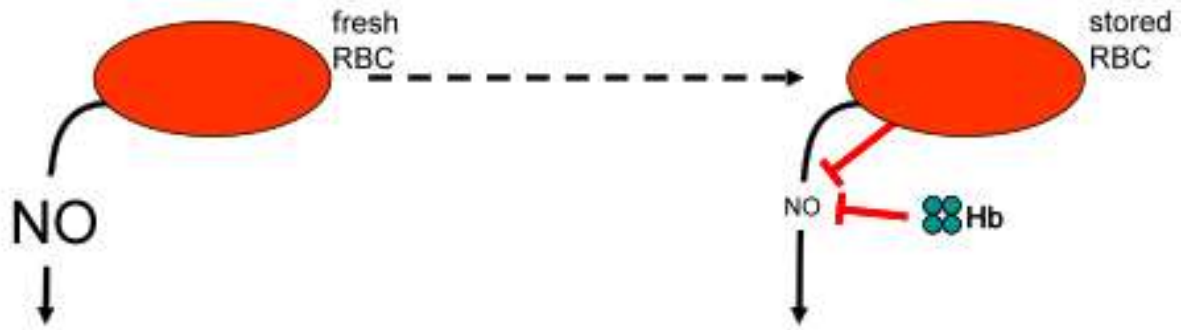
- In addition to carrying O_2 and CO_2, RBCs/Hb can also control local concentrations of NO
- NO regulation by RBCs is believed to be important for the process of "hypoxic vasodilation"
  - NO concentrations in the periphery help match blood flow (and O_2 supply) with O_2 need
  - Under low pO_2 tissue conditions, more NO is "released" from RBCs ➔ increased blood flow
- <u>Simplest form of Hypothesis</u>: Stored RBCs carry/catalyze less NO for hypoxic vasodilation; transfusion of these cells impairs blood flow.



Nitric Oxide

- INOBA hypothesis: Insufficient NO Bio-Availability
  - A hypothesis to explain the adverse effects of aged blood?

- *Potential problem with the simple hypothesis*:
  - How can a few units of older RBCs (assuming depleted NO) adversely affect outcomes in the presence of other normal RBCs?
  - Alternatively, NO scavenging/buffering may be important…



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- Aim 1: determine effects of common RBC storage and processing methods on NO-dependent vasoreactivity using "high throughput" aortic ring models
- Aim 2: select most interesting conditions and investigate their effects on NO-dependent vasodilatory function in healthy volunteer transfusion recipients
- Aim 3: select 1-3 specific conditions (eg, <10 days vs. >21 days) and investigate in hospitalized patients, correlating with endothelial dysfunction

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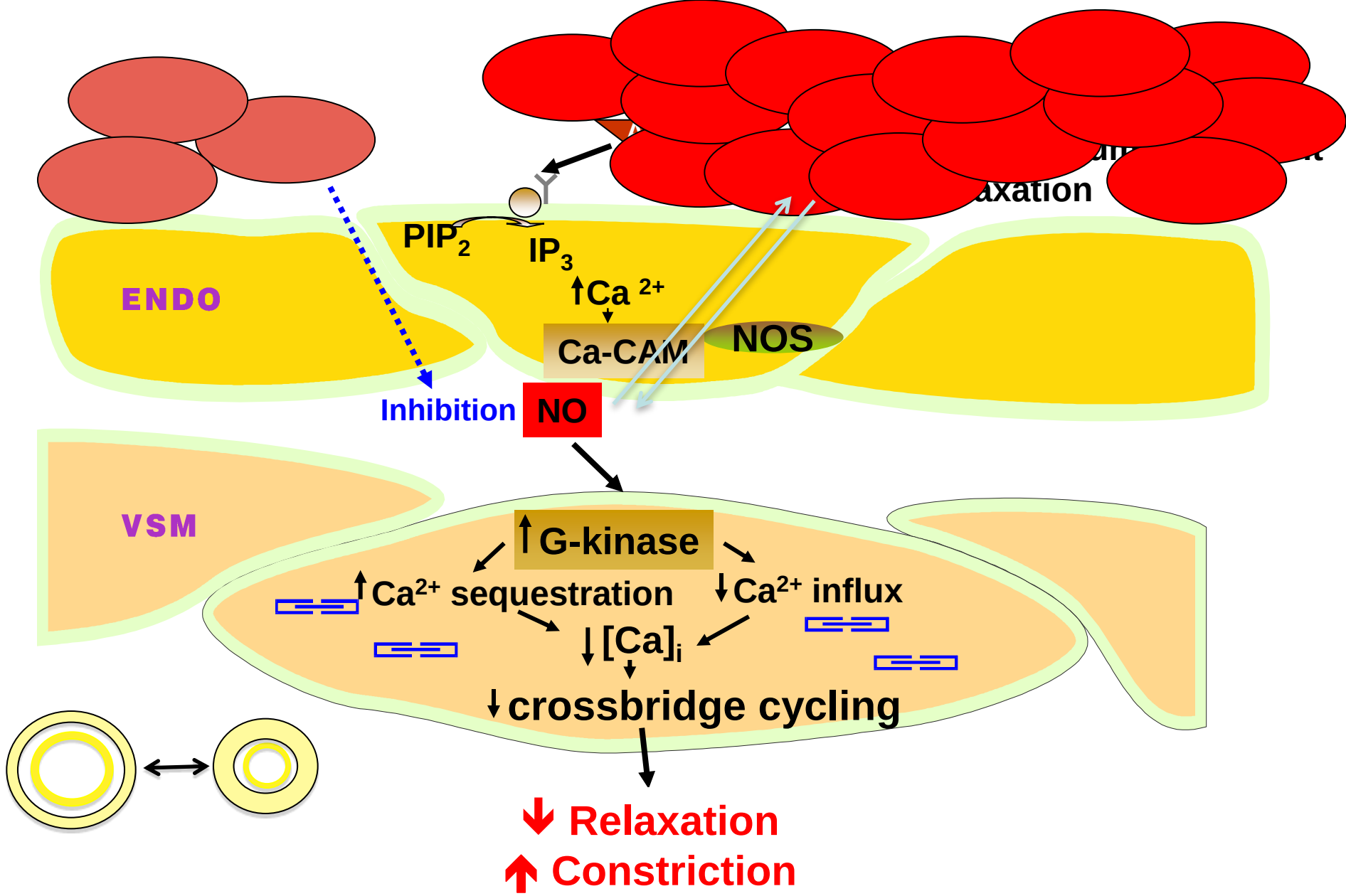
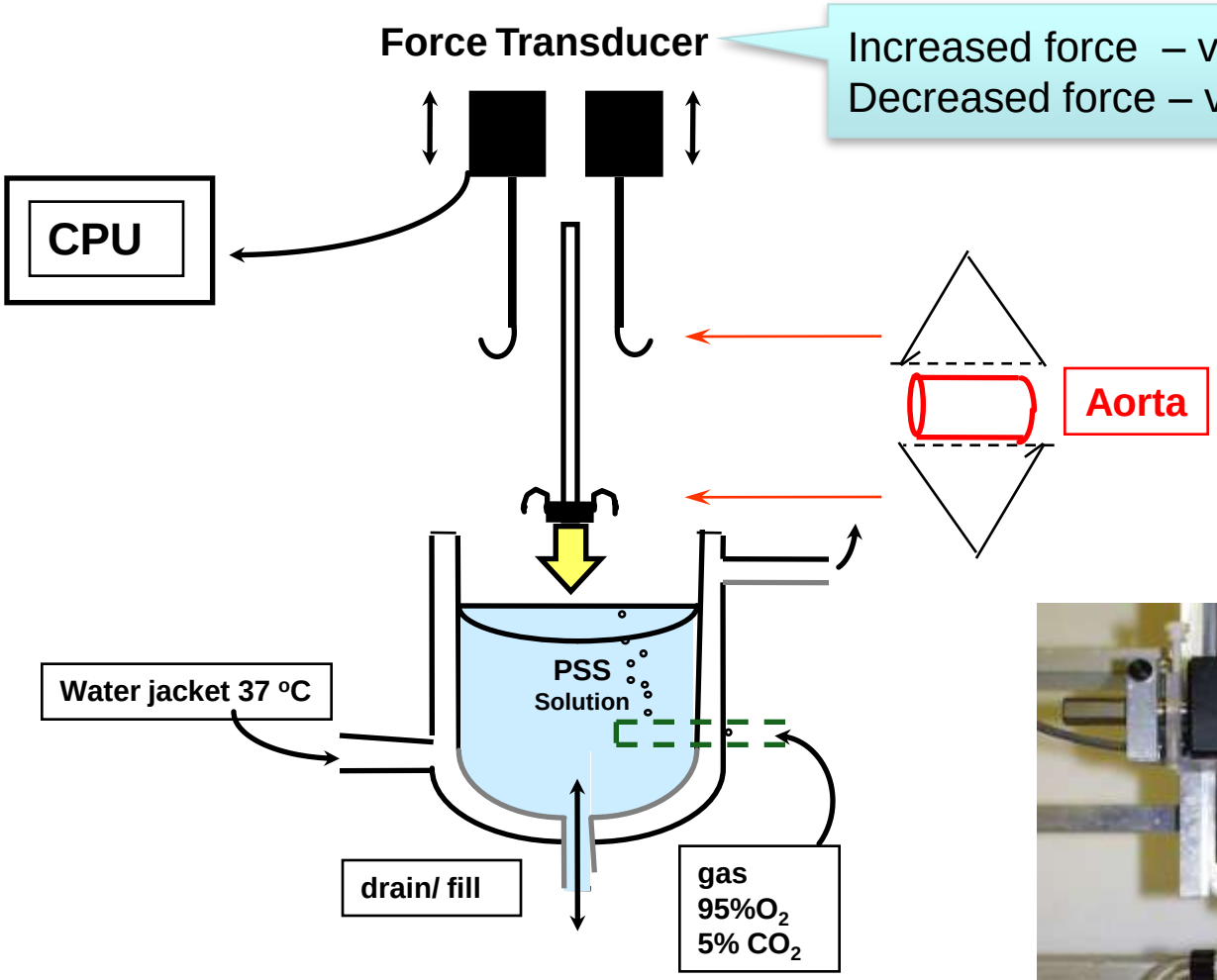
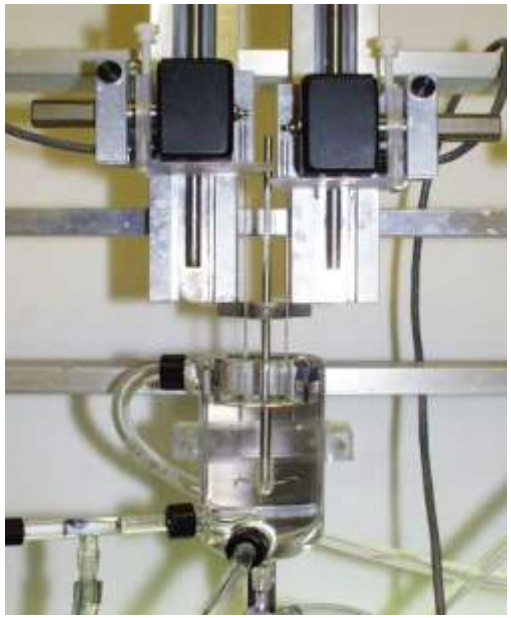


If stored RBCs express potent inhibitory activity…..relatively small numbers of these cells could exert global inhibition of vascular NO signaling

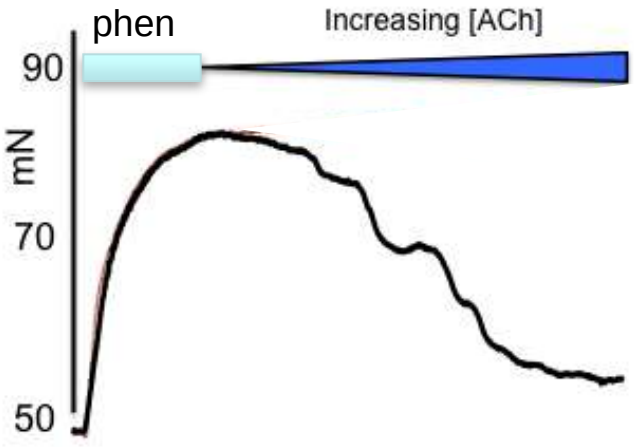
Contractility measurements

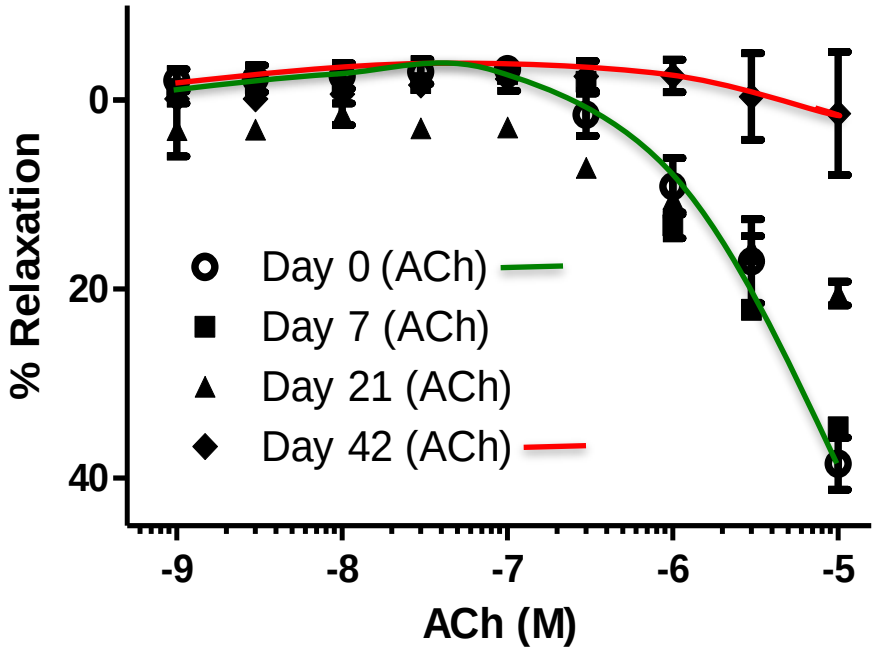




Determine effects of leukoreduced AS-3 RBC storage time on NO-dependent vasoreactivity

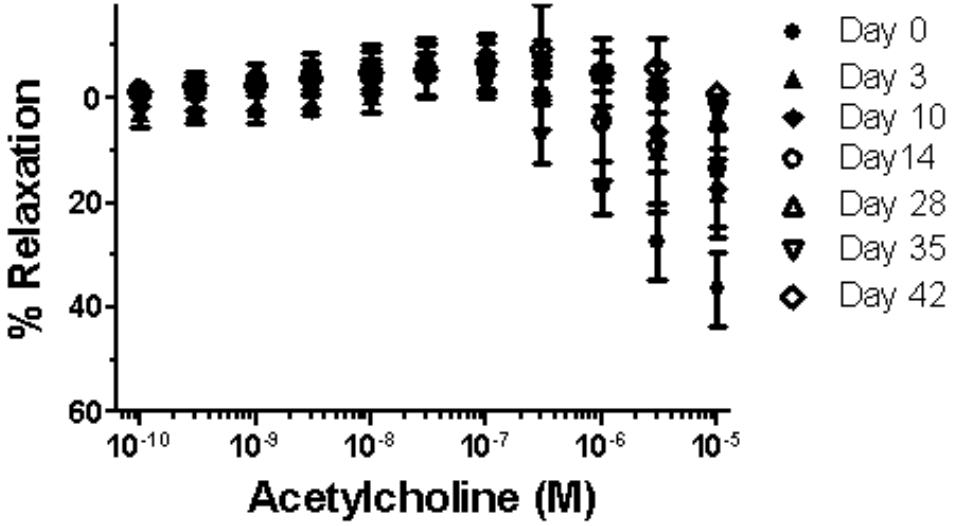
ACh Dose Response

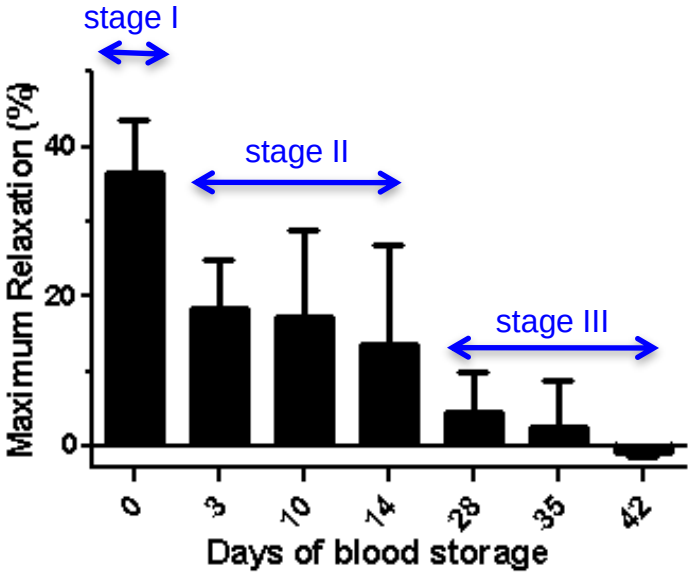




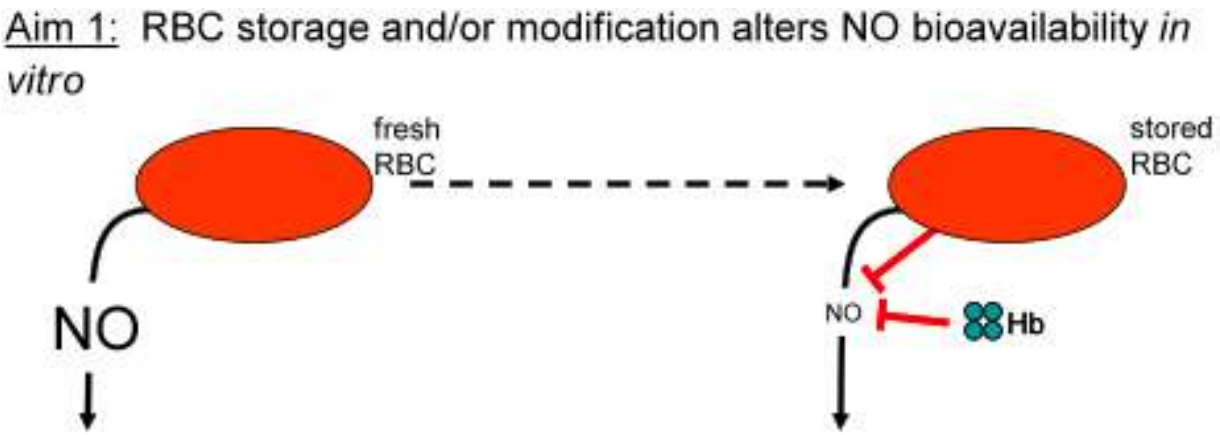
- *When RBCs are added before ACh:*
  - Interfere with ACh-stimulated, NO-mediated vasodilation
  - Inhibitory effect significantly greater with old vs. fresh RBC units

normal protocol





spun (volume reduction) protocol



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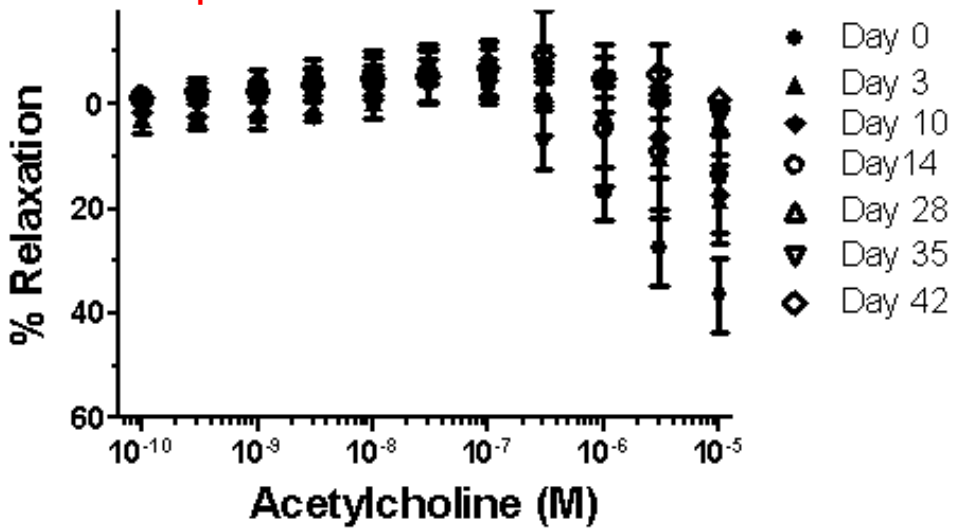

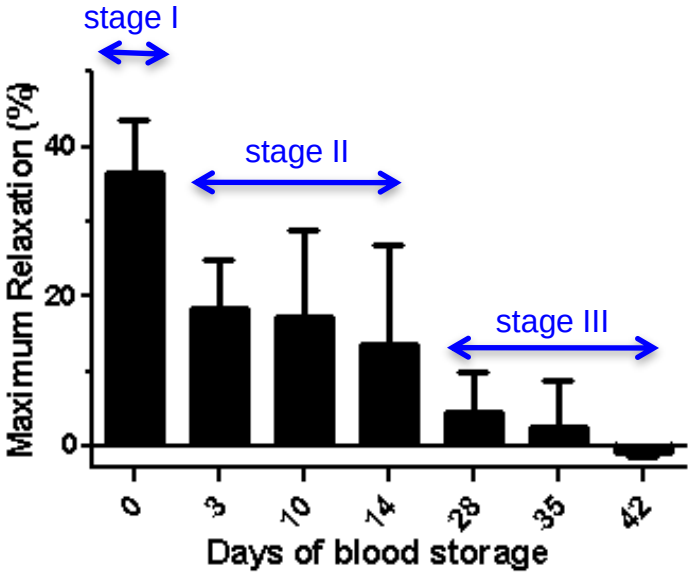

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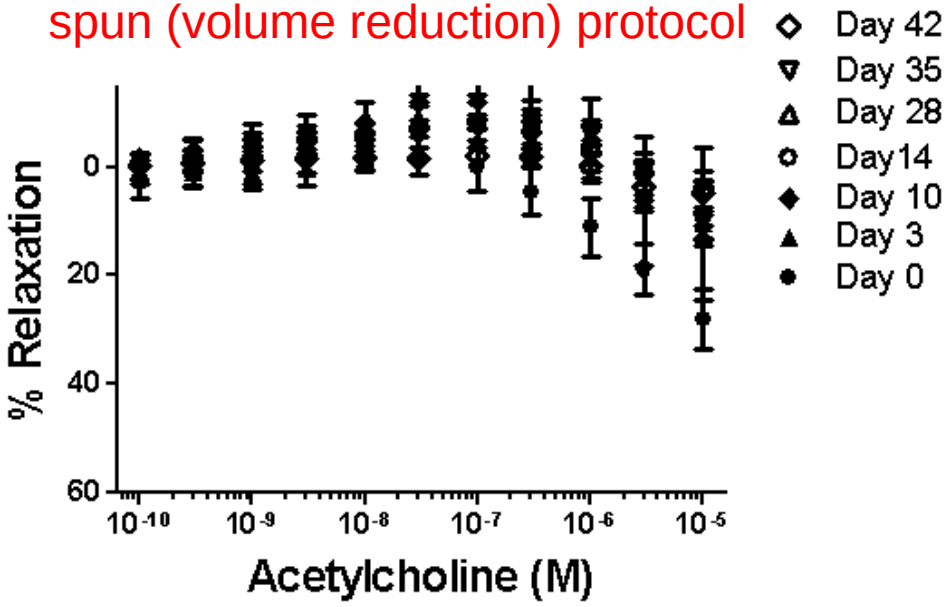
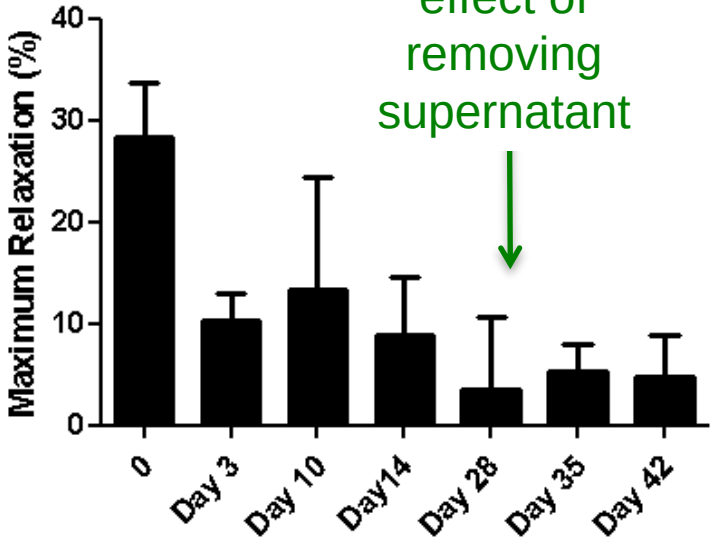
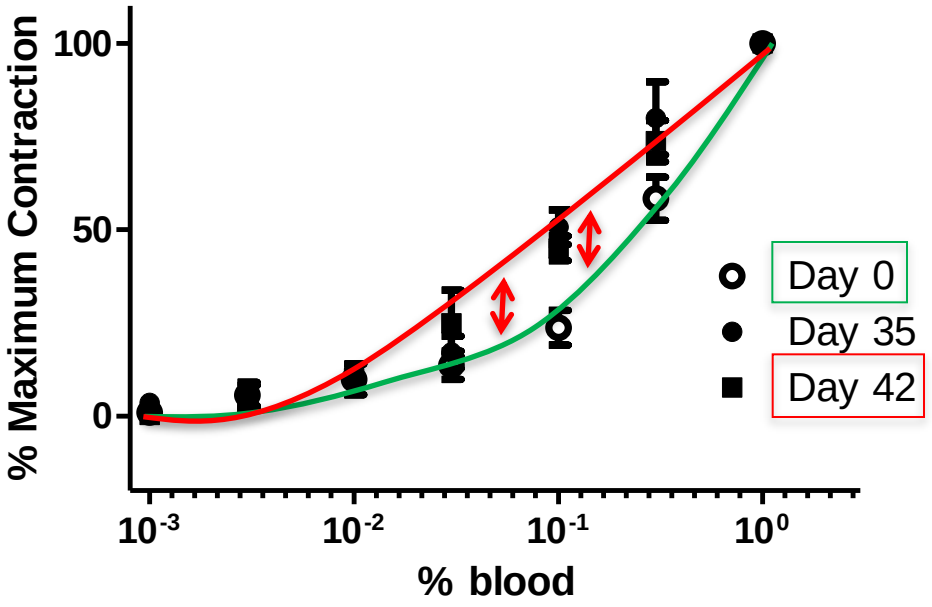


no / minimal effect of removing supernatant



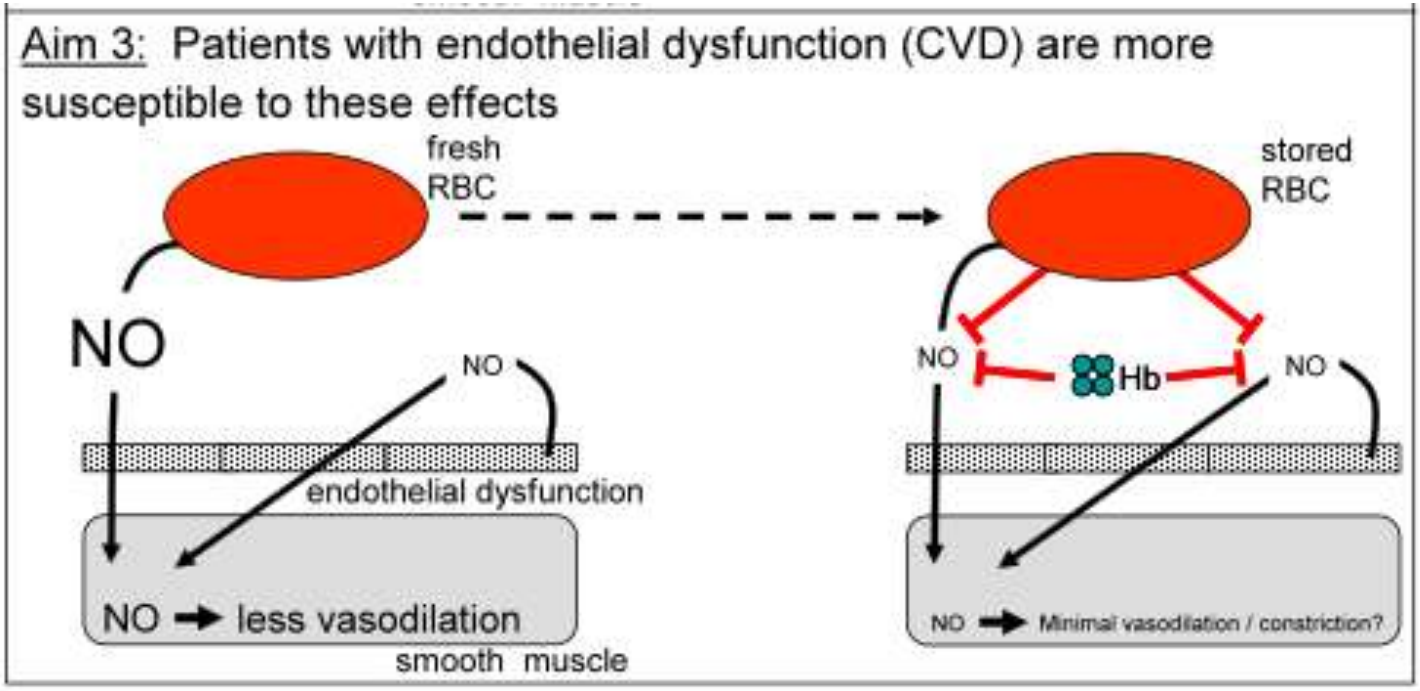
ACh Dose for Maximal Relaxation → Blood Dose Response to Elicit Contraction (Reverse Relaxation)

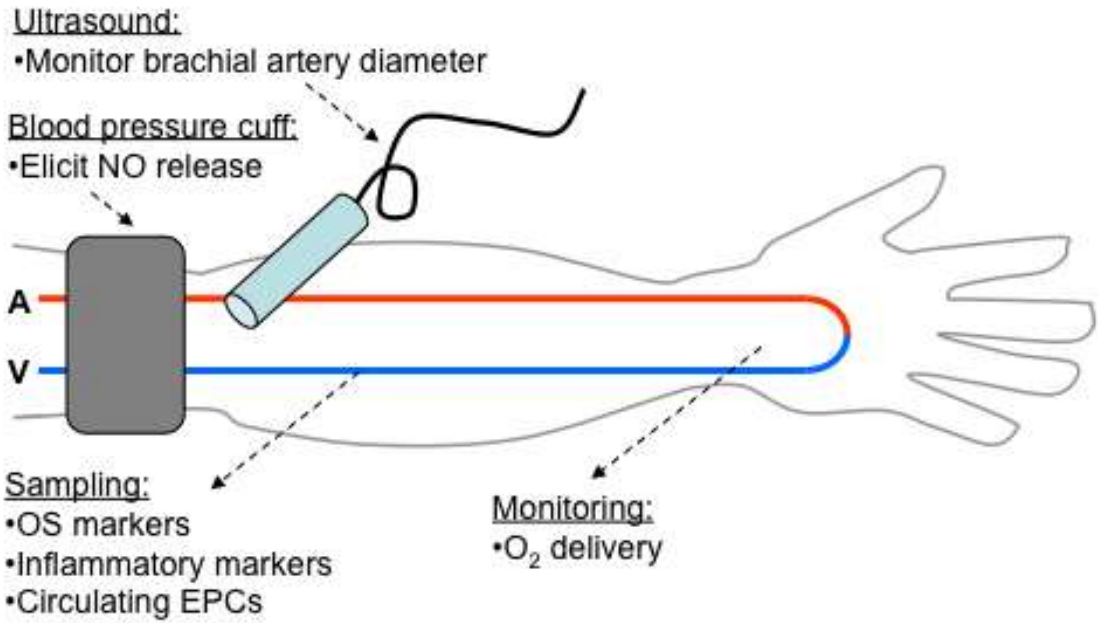


- *When RBCs are after ACh:*
  - Reverse ACh-stimulated, NO-mediated vasodilation
  - Inhibitory effect significantly greater with old vs. fresh RBC units

Aim 1 - summary

- ACh-stimulated, NO-mediated vasodilation is impaired by RBCs, with inhibition increasing with storage time
  - distinct stages of storage?
- Supernatant (free Hb) removal by centrifugation is ineffective at reversing changes
  - Is washing more effective?
- Maximal ACh-stimulated relaxation can be reversed by addition of RBCs; stored RBCs are more inhibitory than fresh
- Will repeat under hypoxic conditions

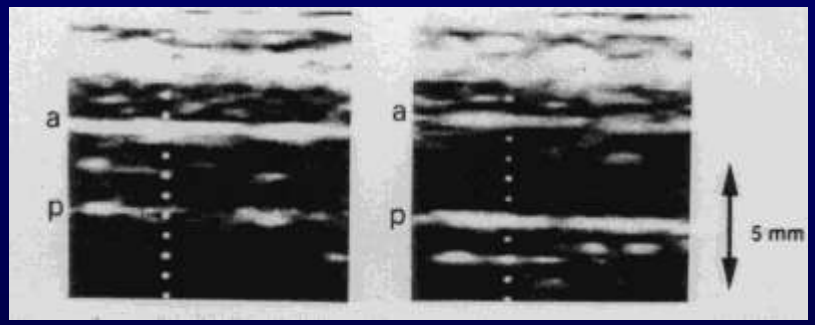

Aim 3: Patients with endothelial dysfunction (CVD) are more susceptible to these effects
fresh RBC
stored RBC
NO
NO
NO
Hb
NO
endothelial dysfunction
NO → less vasodilation
smooth muscle
NO → Minimal vasodilation / constriction?

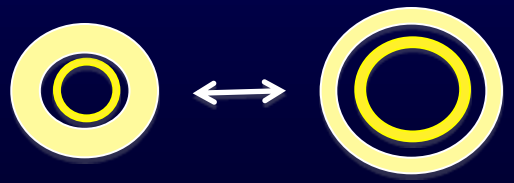

Ultrasound:
•Monitor brachial artery diameter
Blood pressure cuff:
•Elicit NO release
A
V
Sampling:
•OS markers
•Inflammatory markers
•Circulating EPCs
Monitoring:
•O_2 delivery

Endothelium-dependent and -independent function

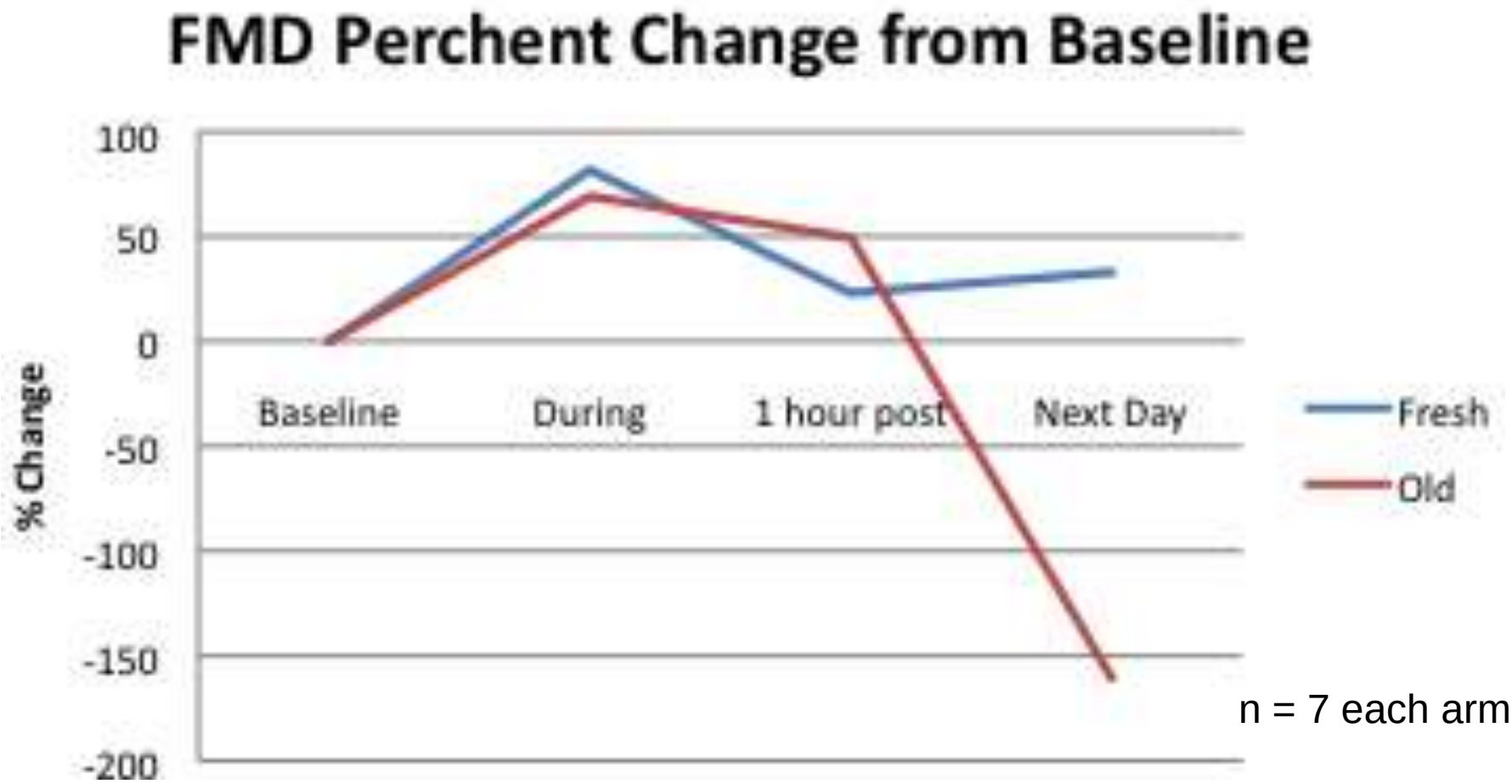
- Flow-mediated vasodilation (FMD)
  - Endothelium-dependent
    - Baseline measurements
    - Reactive Hyperemia

Rest | **Reactive Hyperemia**





Transfusion of aged RBCs leads to delayed decreased responses to NO as compared to fresh RBCs

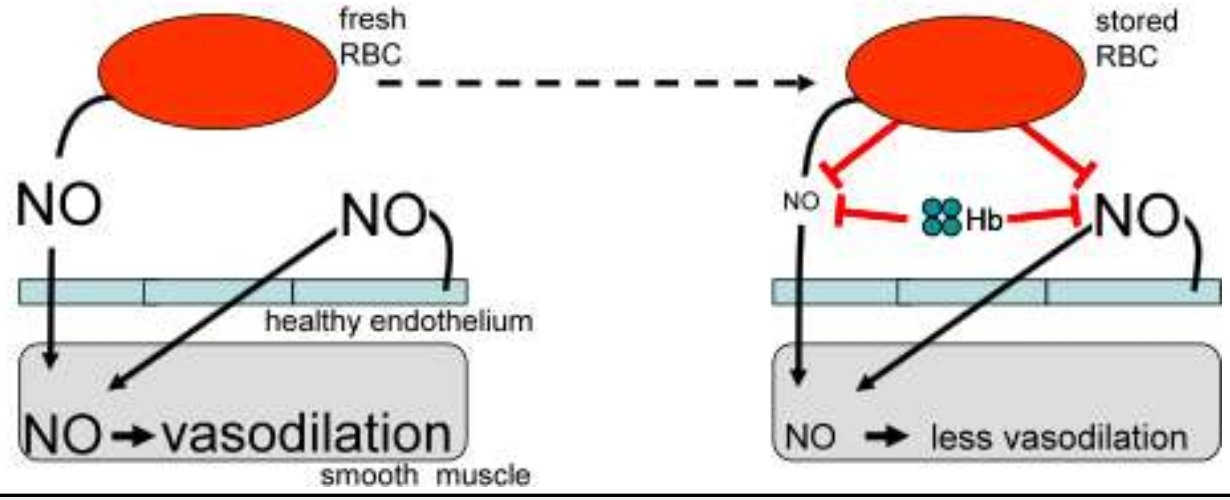


n = 7 each arm

Aim 3 - summary

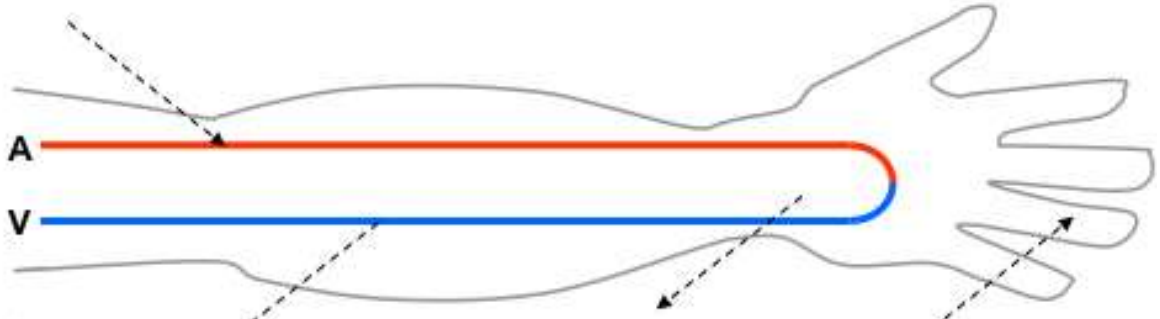
- NO-responsive large arterial vasodilation can be measured in transfused patients using ultrasound FMD measurements
- There are significant changes in FMD that occur following RBC transfusion of anemic patients
  - Acute changes are greater than are seen in most drug studies
- During transfusion, and within 1 hour of completion, fresh (< 7d) and aged (35-42d) RBC transfusion exert similar effects on FMD
- Measured on the day after transfusion, there appears to be a significant arterial constrictive effect of aged vs. fresh RBCs

Aim 2: Transfusion of stored/modified RBCs to healthy recipients inhibits NO-mediated increases in blood flow and O_2 delivery



Infusions:
- L-NMMA (NOS inhibitor)
- TEA (EDHF inhibitor)

Contralateral arm:
- RBC transfusion



Sampling:
- [Nitrite]
- pH
- pO2
- $HbFe^{2+}$•NO
- Hb-SNO
- $HbFe^{3+}$

Monitoring:
- Blood flow
- O_2 delivery

Grip exercises

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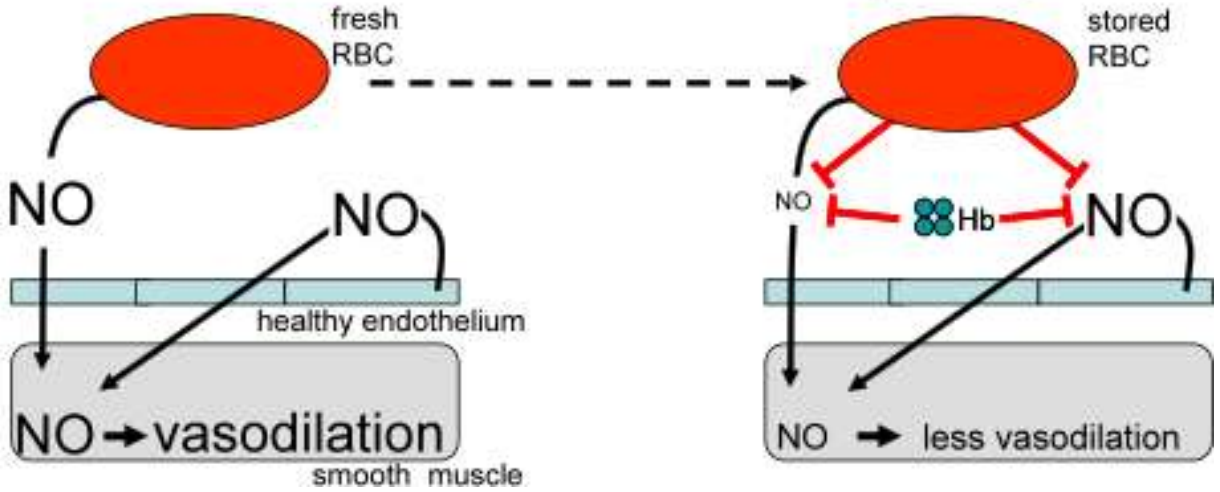

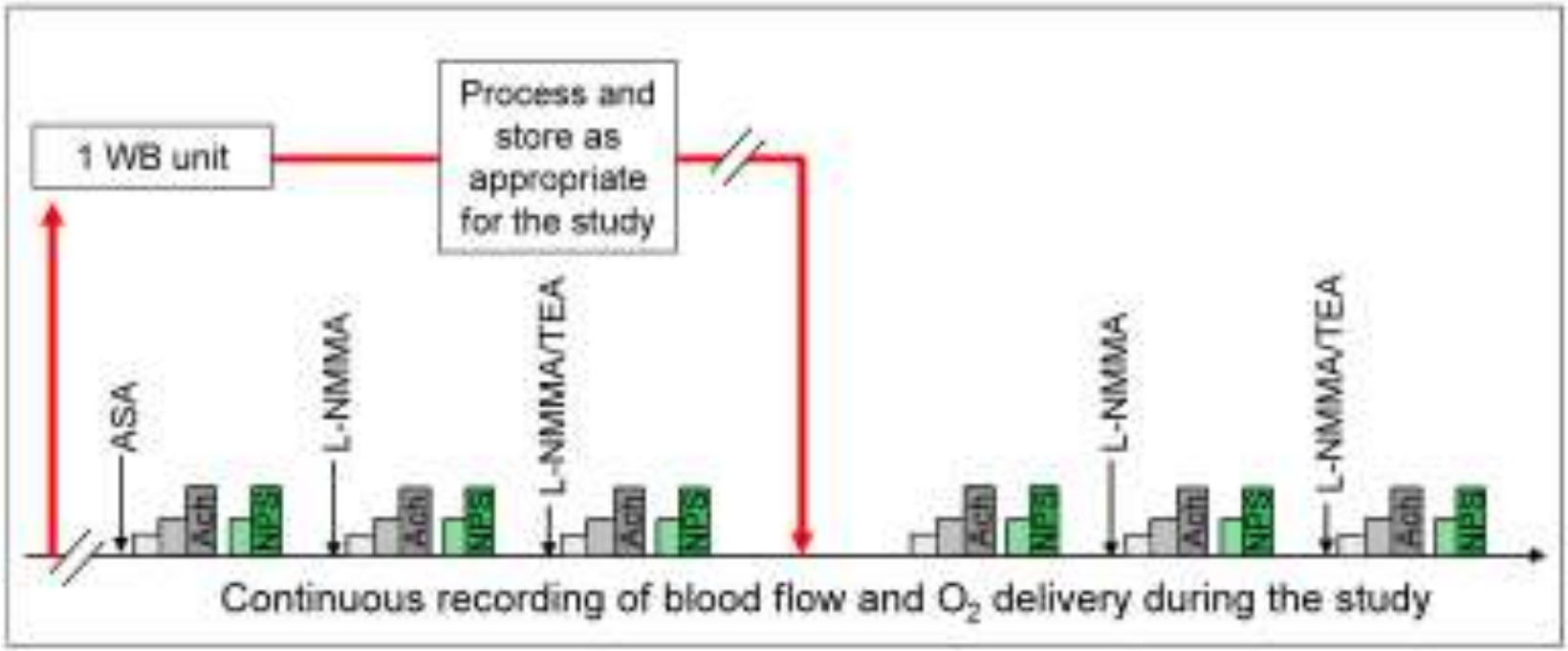

Summary

- INOBA (Insufficient NO bioavailability due to reduced delivery and/or increased scavenging by aged RBCs) may underlying adverse effects ascribed to stored RBCs
- In vitro studies show that RBCs interfere with Ach-stimulated NO-mediated vasodilation (aged > fresh
  - Furthermore, maximal ACh-stimulated relaxation can be reversed by addition of RBCs (again, aged > fresh)
- Preliminary FMD studies in transfused patients also suggest that aged RBCs have a delayed vasoconstrictive effect on the brachial artery
- These results will need to be correlated with clinical outcomes as measured in RECESS and other studies
- This work may lead to improved approaches to RBC storage and/or matching of RBC units to recipients

Contributors

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