# *Role of Reactive Oxygen Species in Septic Shock*

Richard E. Klabunde, Ph.D.
Associate Professor of Physiology
OUCOM
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# *Outline*

- General introduction and definitions
- Pathophysiology of shock
- Pathogenesis of shock
- Current research and potential new therapies
  - Nitric oxide
  - Reactive oxygen species
  - Peroxynitrite

# *Sepsis*

- Systemic inflammatory response to a confirmed infectious process (most commonly caused by bacterial products (e.g., endotoxin)

# *Severe Sepsis*

- Sepsis with either hypotension or systemic manifestations of hypoperfusion
  - Lactic acidosis, oliguria, altered mental status

# *Septic Shock*

- Cardiovascular dysfunction associated with sepsis resulting in hypotension and organ hypoperfusion despite adequate fluid resuscitation

# *Endotoxemia & Endotoxic Shock*

- Endotoxemia
  - Elevated levels of bacterial endotoxins (e.g., lipopolysaccharide, LPS) in the blood.

- Endotoxic Shock
  - Similar to septic shock (hypotension and organ hypoperfusion despite fluids), but with specific involvement of bacterial endotoxins.

New Slide

# *Systemic Inflammatory Response Syndrome (SIRS)*

- A more general, inclusive term
- Systemic inflammatory response to a variety of severe clinical insults (e.g., infection, burns, trauma, pancreatitis)

# *Multiple Organ Dysfunction Syndrome (MODS)*

- Progressive distant organ failure following severe infectious or noninfectious insults

# *Morbidity/Mortality of Sepsis and Septic Shock*

- Most common cause of death in ICUs
- U.S. cases per year:
  - Sepsis = 400,000
  - Septic Shock = 200,000
  - Death = 100,000

J.E. Parrillo, N. Eng. J. Med. 328:1471-1477, 1993

# *Pathophysiology of Septic Shock*

## General Clinical Signs

- **Flu-like symptoms**
  - **chills followed by fever**
  - **general malaise, irritability, lethargy, mental confusion**
- **Warm skin (early sign)**
- **Site of infection may or may not be evident**

# *Pathophysiology Cont.*

## Cardiovascular

- **Systemic vasodilation and hypotension ($P_{sys}$ < 90 mmHg); increased SVR in late stages**
- **Tachycardia (>100 beats/min)**
- **Increased cardiac output (hyperdynamic), although contractility is depressed; hypodynamic in late shock**
- **Ventricular dilation; decreased ejection fraction**
- **Loss of sympathetic responsiveness**

# *Pathophysiology Cont.*

## Cardiovascular Cont.

- **Hypovolemia due to vascular leakage; central venous pressure may be decreased or increased depending upon fluid resuscitation**
- **Compromised nutrient blood flow to organs; decreased organ oxygen extraction**

| | Cardiogenic Shock | Hemorrhagic Shock | Septic Shock |
|---|---|---|---|
| Primary CV Origin | Cardiac | Volume | Vascular |
| Cardiac Output | ↓ | ↓ | ↑↓ |
| Vascular Resistance | ↑ | ↑ | ↓↑ |
| Blood Volume | ↑ | ↓ | ↓ |
| Management | Mechanical<br>Inotropes<br>Vasopressors<br>Vasodilators | IV Fluids/Blood<br>Vasopressors | IV Fluids<br>Antibiotics<br>Vasopressors<br>Inotropes |

# *Pathophysiology Cont.*

## Pulmonary & Renal

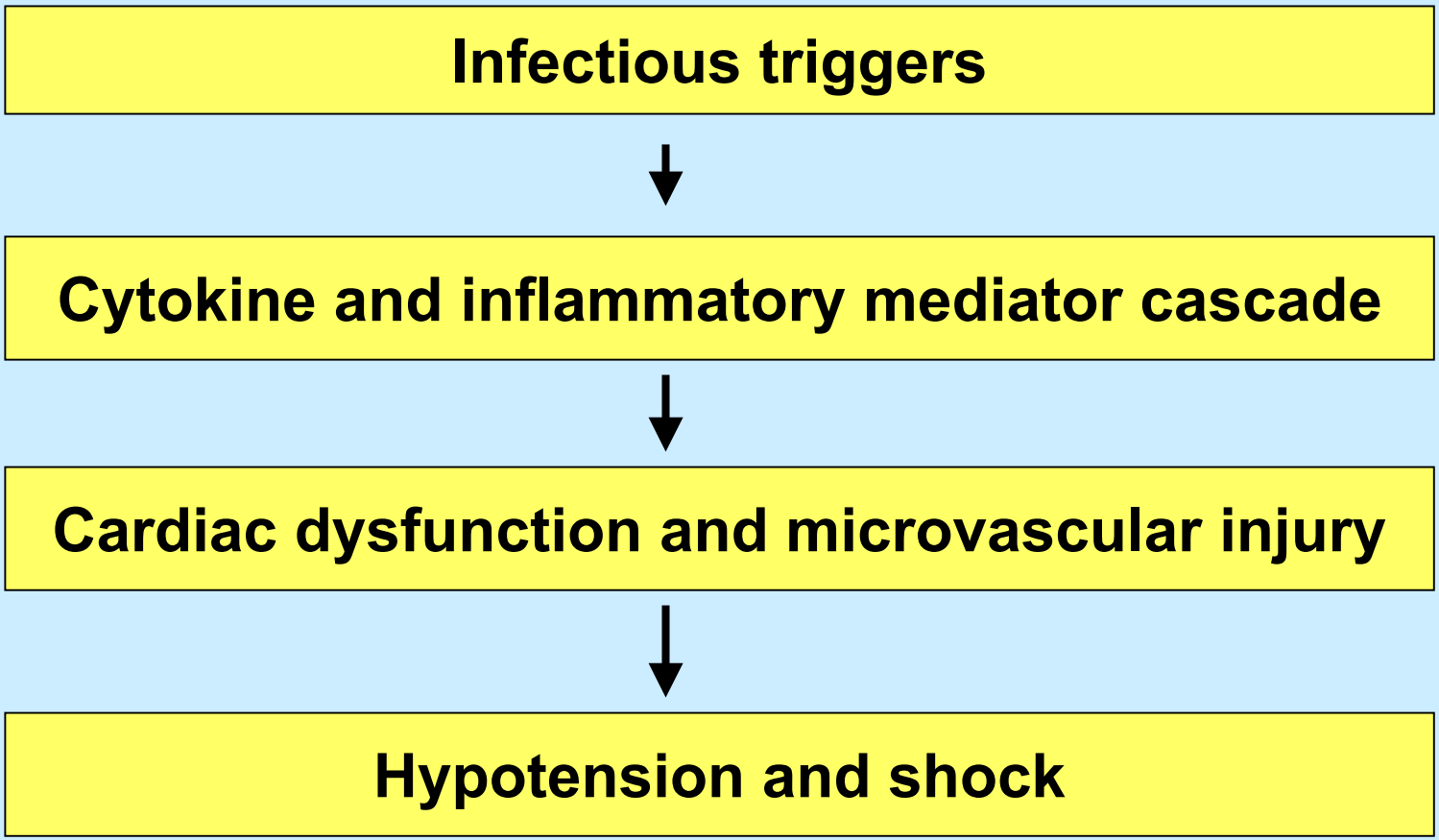
- **Hyperventilation with respiratory alkalosis**
- **Pulmonary hypertension and edema**
- **Hypoxemia (arterial $pO_2$ < 50 mmHg)**
- **Reduced pulmonary compliance; increased work**
- **Respiratory muscle failure**
- **Renal hypoperfusion and oliguria despite elevated cardiac output**
- **Acute tubular necrosis and renal failure**

# *Pathophysiology Cont.*

## Other

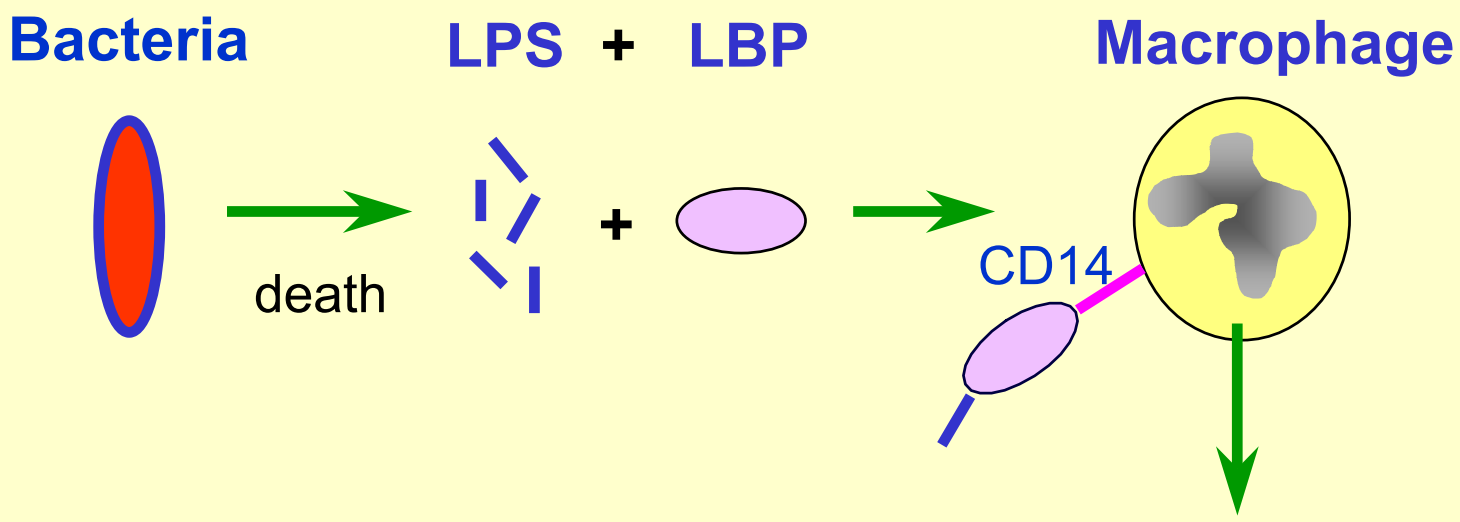
- **Disseminated intravascular coagulation (DIC)**
- **Blood dyscrasias**
  - **leukopenia**
  - **thrombocytopenia**
  - **polycythemia**
- **Central and peripheral nervous dysfunction**
- **Increased lactate occurs early**

# *Pathogenesis of Septic Shock*




Revised Slide

# *Bacterial-Mediated Sepsis*
## *(Gram-negative)*

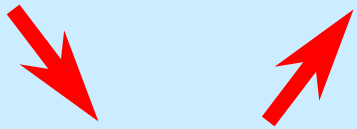



**LPS = Lipopolysaccharide**

**LBP = LPS-Binding Protein**

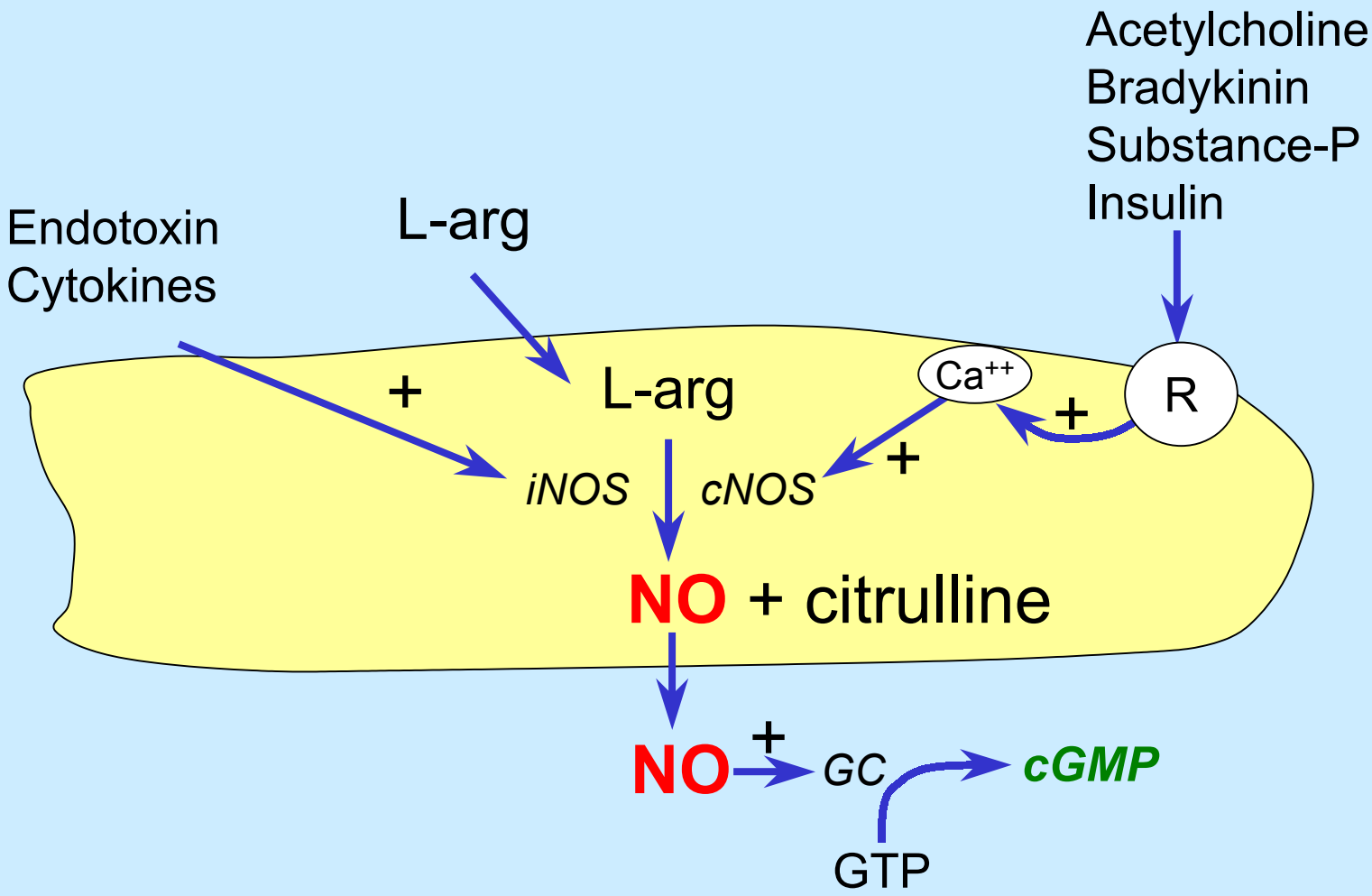
# ENDOTOXIN

Vasodilation
Cardiac Depression
Microvascular Leakage
Platelet Aggregation
Leukocyte Adhesion



Tumor Necrosis Factor
Interleukins
Gamma Interferon
Platelet Activating Factor
Leukotrienes
Thromboxanes
Prostaglandins
Histamine
Nitric Oxide
Oxygen Free Radicals

# Nitric Oxide Formation

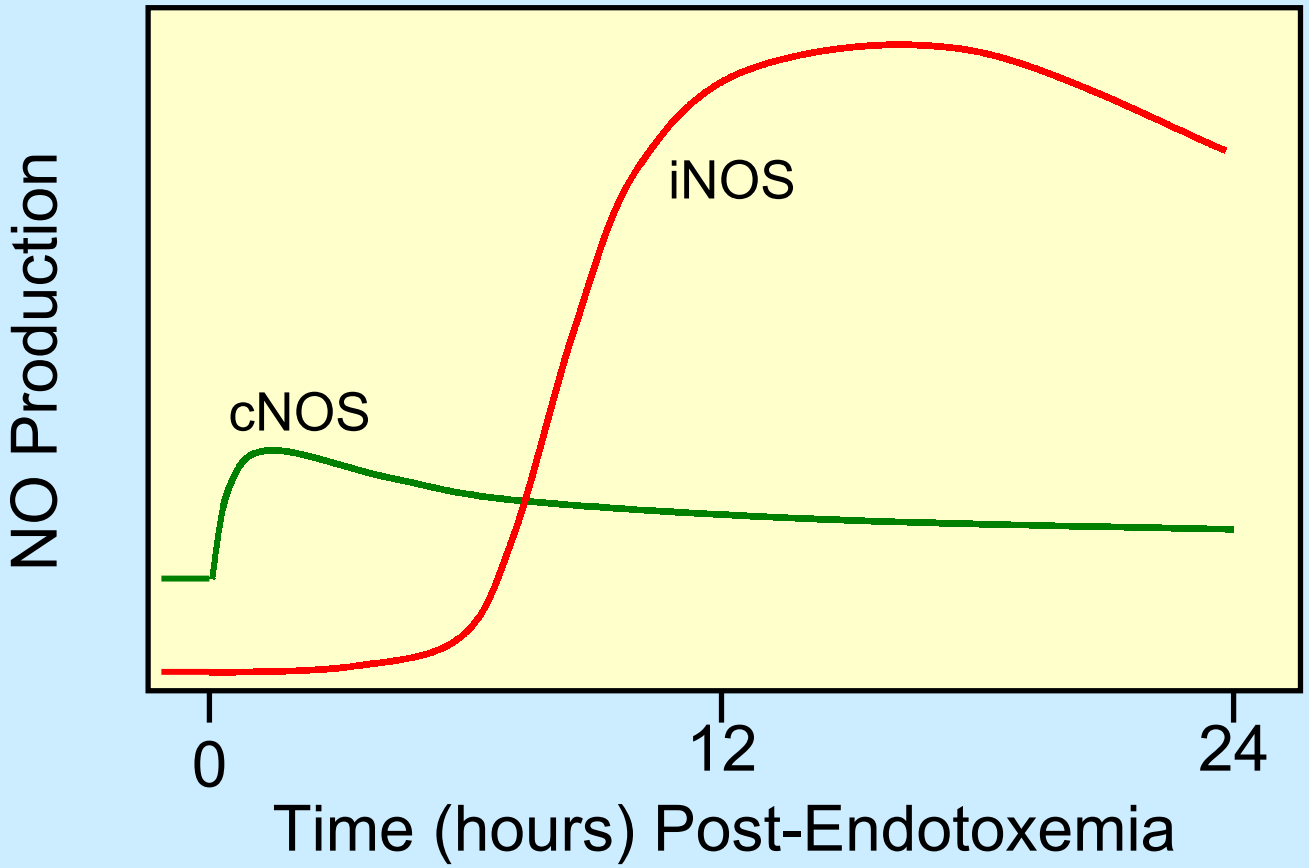


# *Actions of Nitric Oxide*

- Vasodilation (direct via cGMP and indirect via inhibition of NE and ET-1 release)
- Inhibits leukocyte-endothelial cell adhesion
- Inhibits platelet adhesion/aggregation
- Modulates vascular permeability
- Scavenges superoxide radicals
- High concentrations are cytotoxic

*What role does NO play in septic shock?*

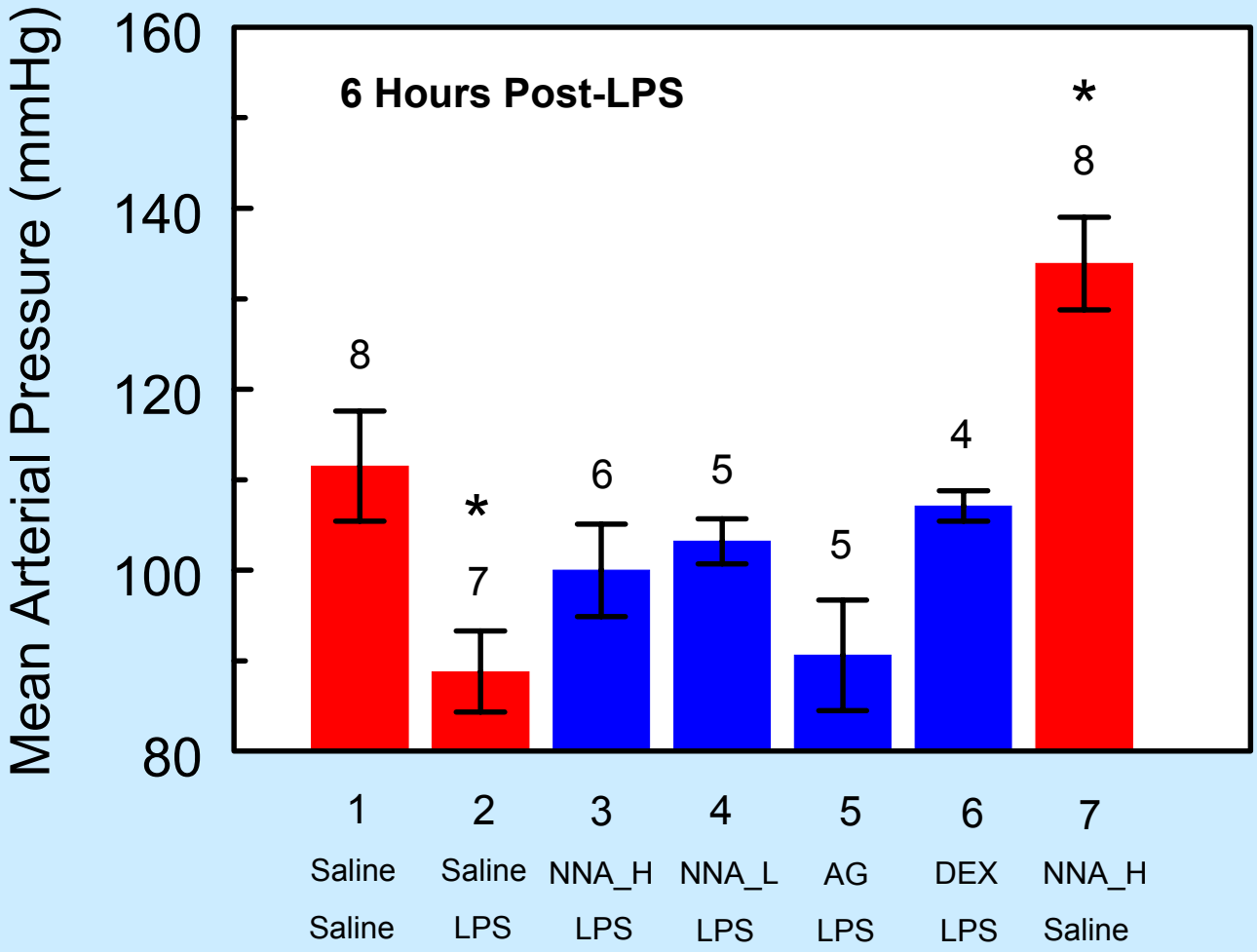
# NO Formation in Septic Shock



# *Pretreatment with NOS Inhibitors*

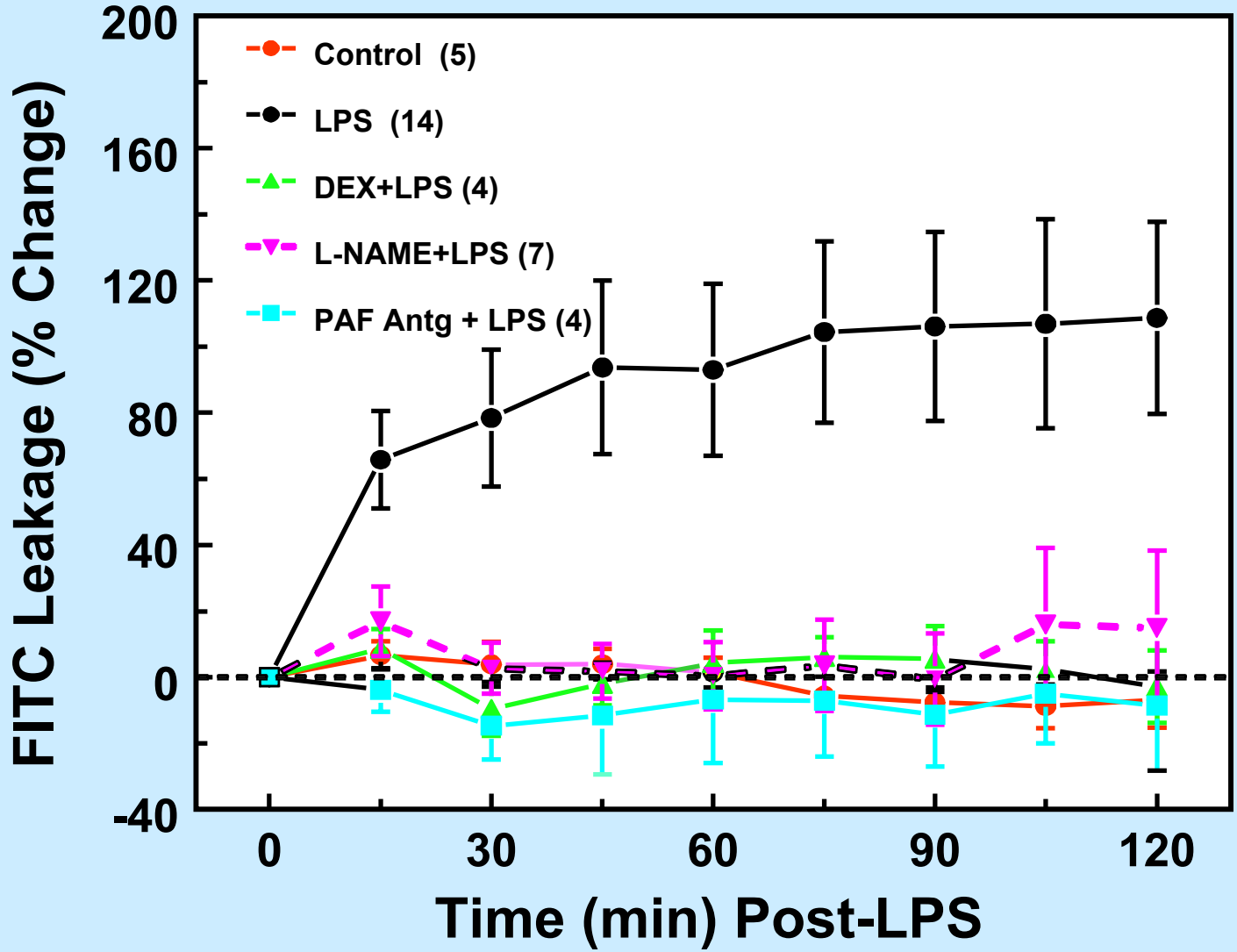
- Hypothesis:
  - Increased NO production during sepsis causes systemic vasodilation, cardiac depression, increased capillary permeability (leading to decreased blood volume) and hypotension.
- Therefore:
  - Pretreatment with a NOS inhibitor in animal models of sepsis should prevent NO-induced hypotension and edema.
  - Treatment with a NOS inhibitor during sepsis should reverse cardiovascular changes.

# Effects of Pretreatment with NOS Inhibitor




Klabunde and Coston. *Shock* 3:73-78, 1995.

# NO and Microvascular Leakage

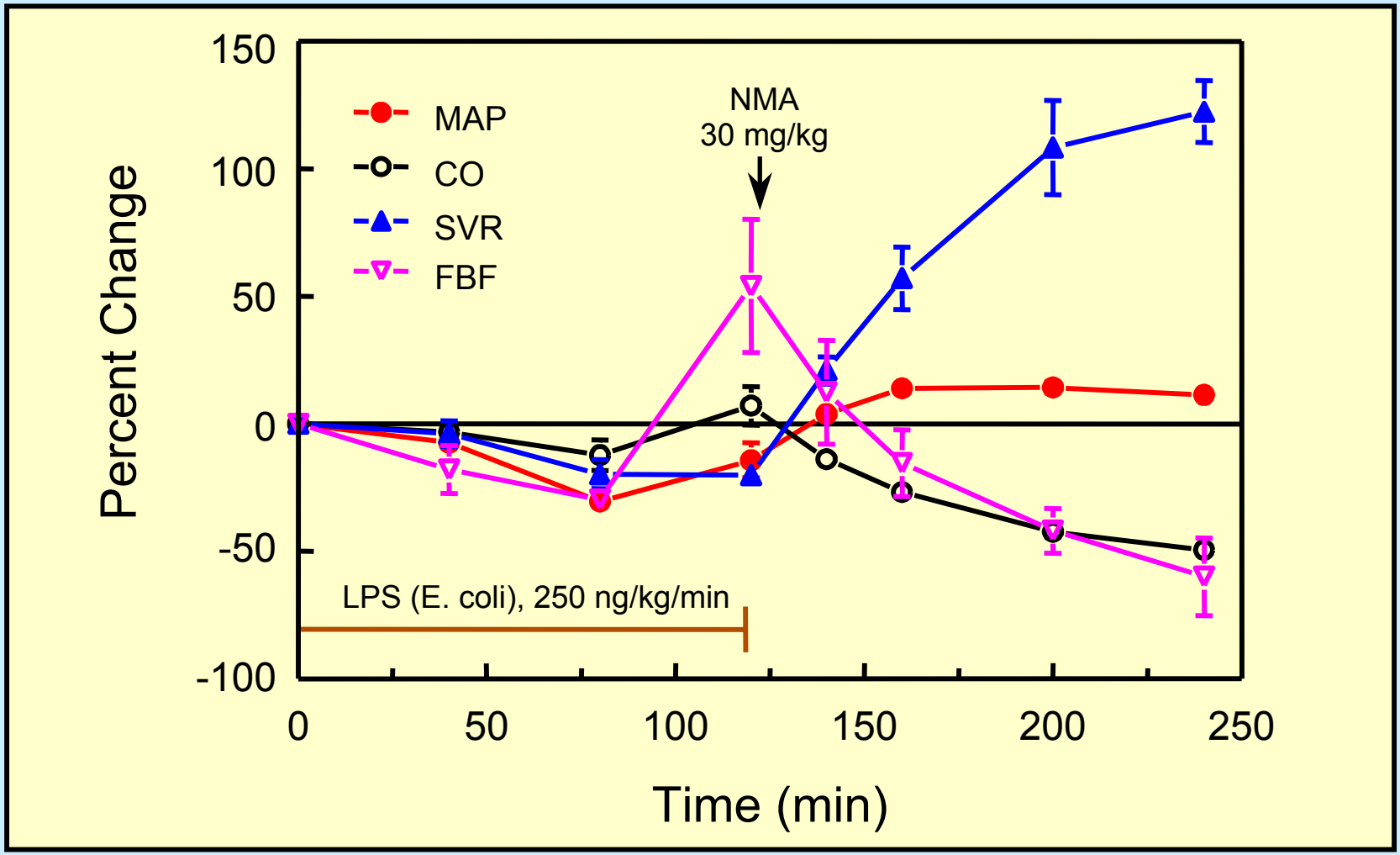



Laniyonu, Coston and Klabunde. *Shock* 7:49-54, 1997.

# *Other Studies*

- Pretreatment with NOS inhibitors:
  - Prevents fall in systemic vascular resistance
  - Prevents arteriolar hyporesponsiveness to catecholamines

# *Therapeutic Efficacy of NOS Inhibitors in Endotoxic Shock*




Klabunde and Ritger. *Biophys Biochem Res Commun* 178:1135-1140, 1991.

# *Summary of Role of NO in Septic Shock*

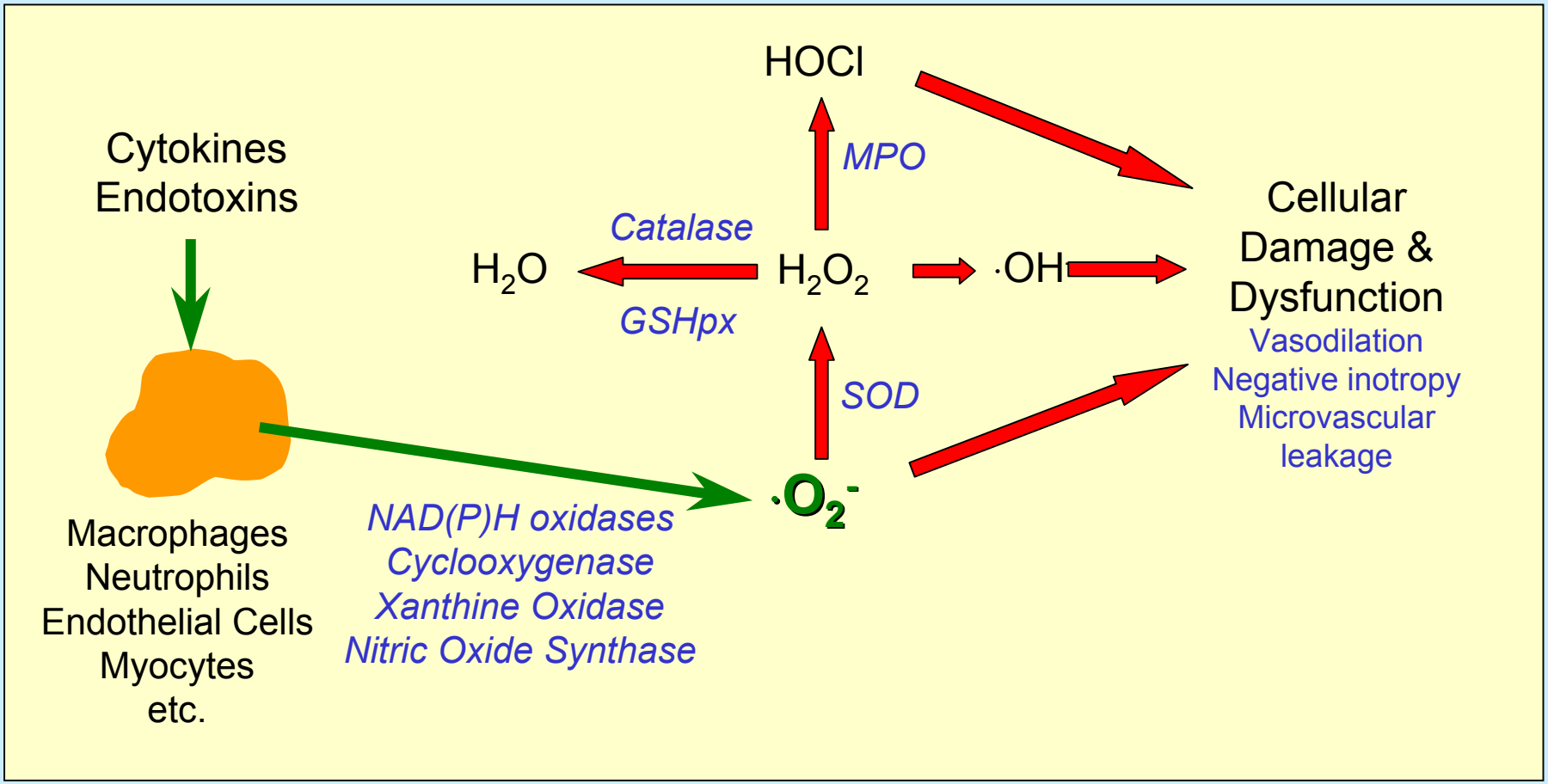
- Increased NO production decreases SVR and leads to hypotension.
- Non-specific NOS inhibition restores arterial pressure, but reduces cardiac output and organ perfusion
- NO is involved in microvascular leakage during endotoxemia
- Other studies:
  - NOS inhibition causes pulmonary dysfunction and increases mortality.
  - Selective iNOS inhibition being investigated for therapeutic potential; non-selective inhibitors increase mortality
  - Clinical studies show no benefit on survival

# *Are nitric oxide inhibitors good or bad in septic shock?*

- Bad – NOS inhibitors increase
  - Cardiac depression and organ hypoperfusion
  - Thrombosis
  - Leukocyte-endothelial adhesion and inflammation
  - Superoxide anion (NO normally scavenges superoxide)
  - Increase mortality in many studies
- Good – NOS inhibitors prevent
  - Excessive vasodilation and hypotension
  - Possible cardiac depression
  - Formation of new, damaging free radical species (e.g., peroxynitrite)

New Slide

# *Formation of Reactive Oxygen Species*



# *Free Radicals in Septic Shock*

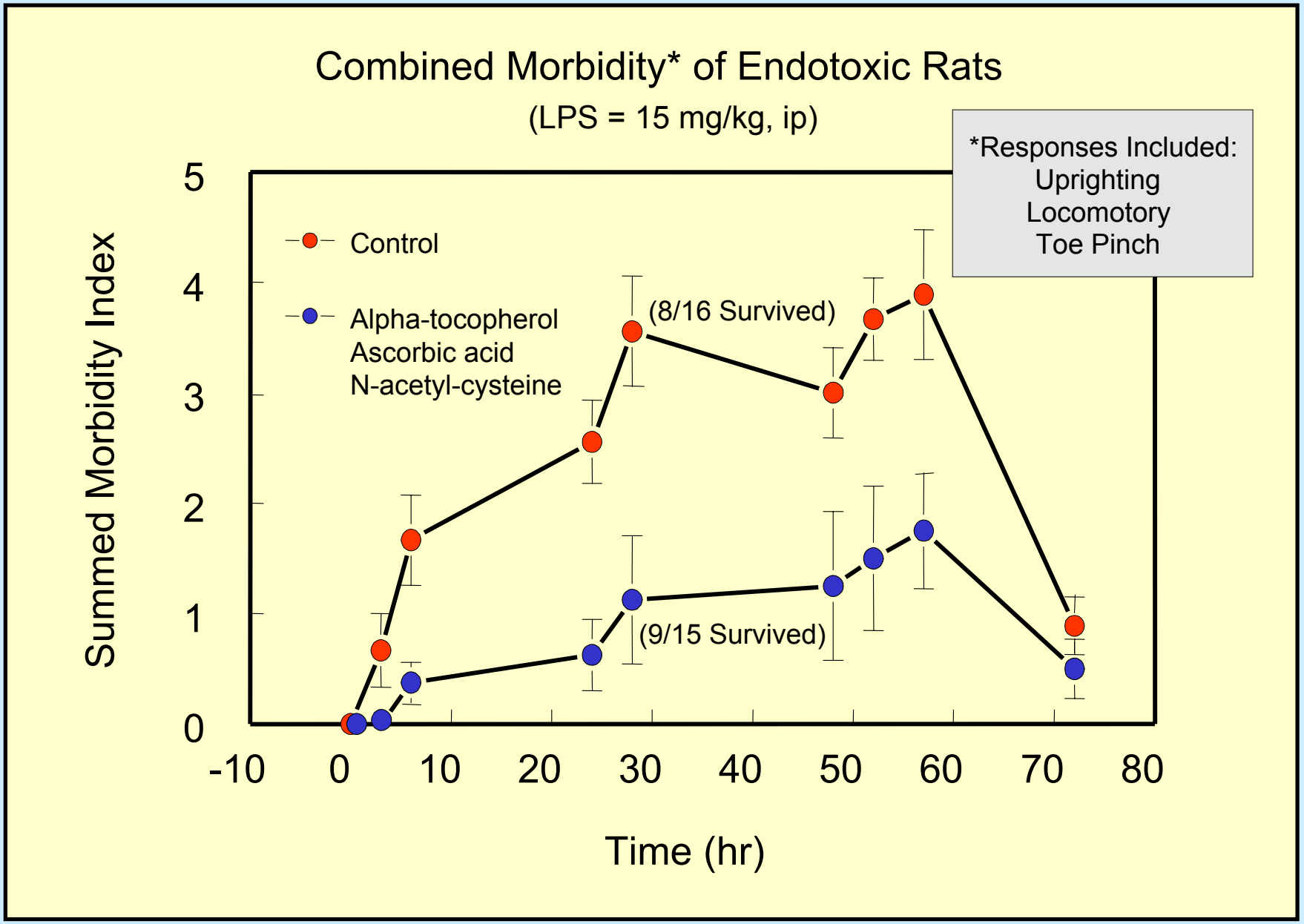
- Reactive oxygen species (ROS) are elevated in sepsis
  - In rat models of acute endotoxemia, superoxide anion increases 3-fold within 60 min and plateaus after about 4 hours
    (Brovkovych et al, J Physiol Pharmacol 48:633, 1997)

# *Free Radicals in Septic Shock* *cont.*

- Endogenous antioxidant and scavenging systems can become depleted in severe sepsis, particularly
  - Alpha-tocopherol
  - Ascorbic acid
  - β-carotene
- Survival in septic patients with organ dysfunction is inversely correlated with the reduction in plasma antioxidant potential
  (Cowley et al., Crit Care Med 24:1179, 1996)

# *Free Radicals in Septic Shock* cont.

- In animal studies, treatment with ROS scavengers can improve clinical status and decrease mortality
- Animal studies suggest that the following treatments may improve outcome:
  - N-acetyl-L-cysteine (scavenges hydroxyl radical and hypochlorus acid; replenishes glutathione)
  - Alpha-tocopherol
  - Ascorbic acid (treatment during bacteremia in rats prevents hypotension - Armour et al., J Appl Physiol 90:795, 2001)
  - TEMPO (SOD mimic)


Combined Morbidity* of Endotoxic Rats
(LPS = 15 mg/kg, ip)
*Responses Included:
Uprighting
Locomotory
Toe Pinch
Summed Morbidity Index
Control
Alpha-tocopherol
Ascorbic acid
N-acetyl-cysteine
(8/16 Survived)
(9/15 Survived)
0
1
2
3
4
5
-10
0
10
20
30
40
50
60
70
80
Time (hr)

# Effects of ROS Scavenging on LPS-Induced Microvascular Leakage

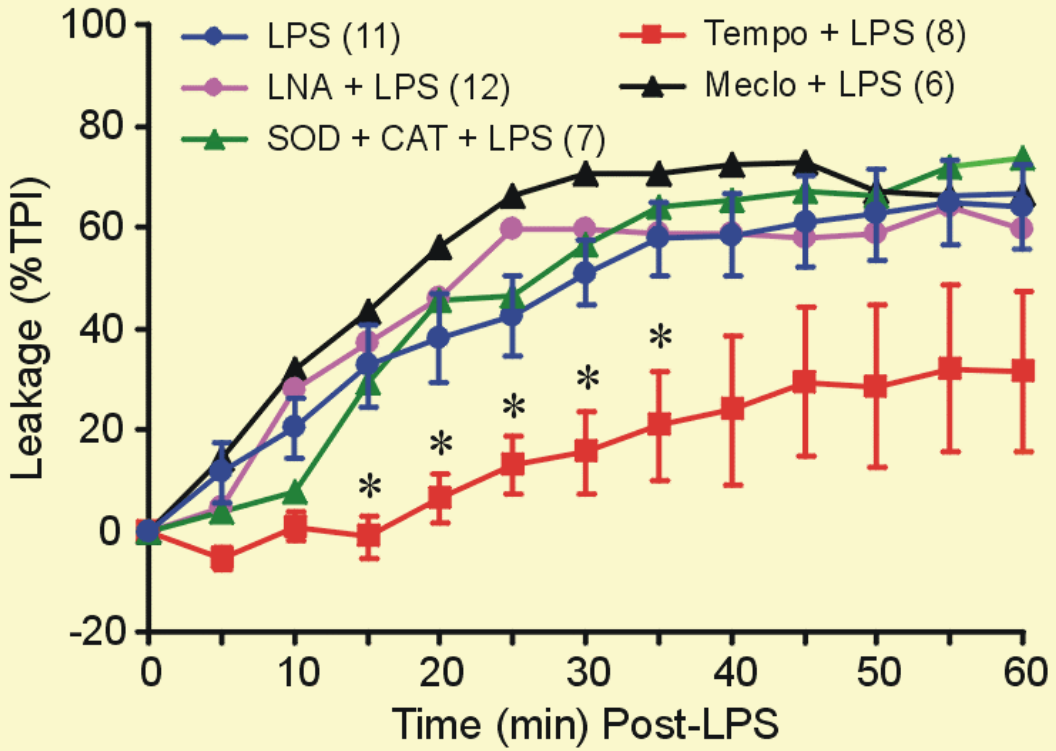
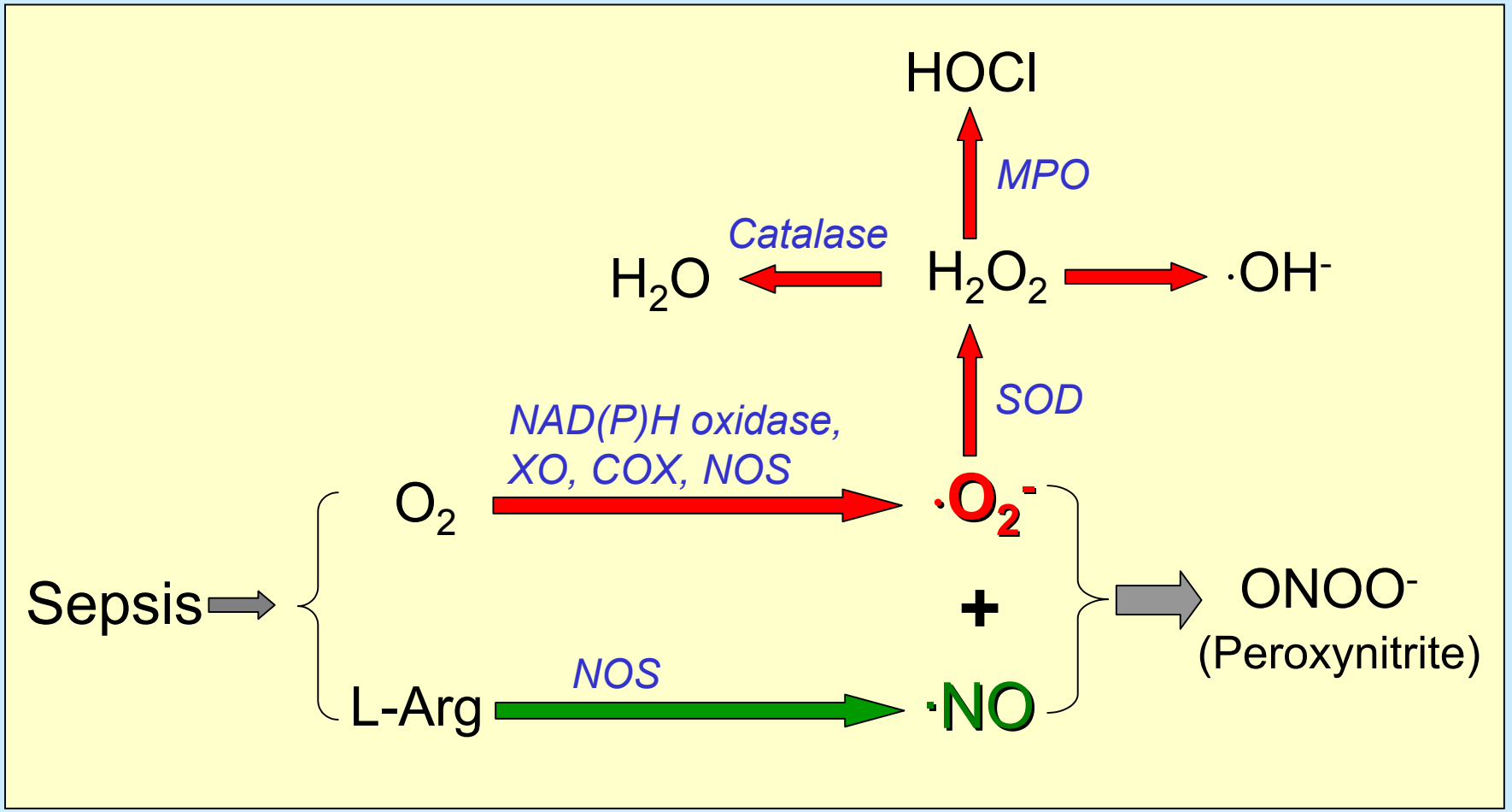



***Figure 2.*** Inhibition of LPS-induced leakage by pretreatment with tempo. Vertical bars, S.E. (only shown for LPS and Tempo + LPS groups. Number of experiments in parentheses. * $p<0.05$ compared to all

# *Unresolved Issues with Antioxidant Therapy*

- Is there one antioxidant that is best or should a cocktail be use?
- What is the therapeutic window for antioxidant therapy?
- Do antioxidants increase survival?
- Is there a therapeutic role for inhibitors on ROS production? *e.g.,* xanthine oxididase inhibitors (allopurinol), NAD(P)H oxidase inhibitors

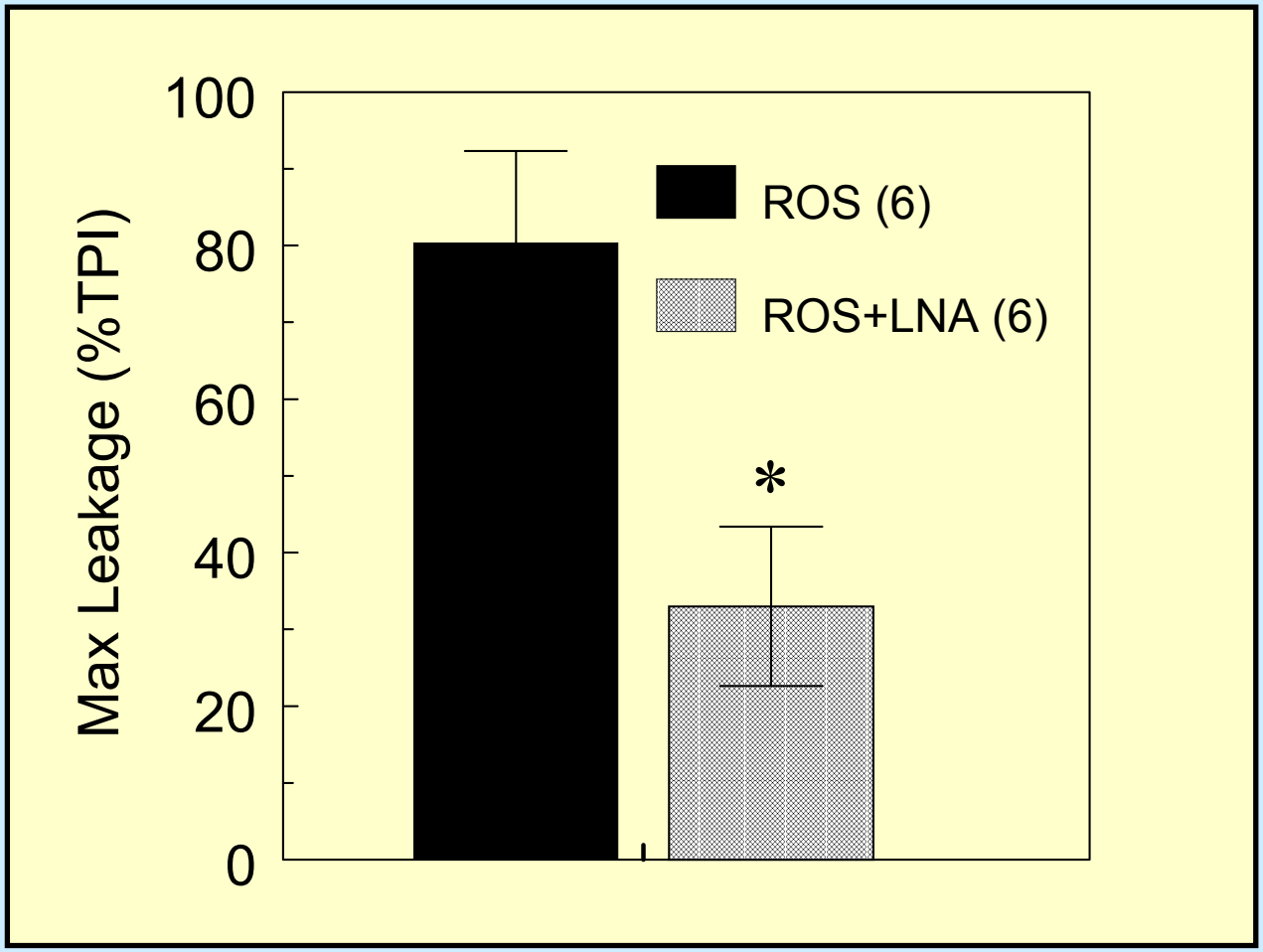
# Nitric Oxide and Superoxide Anion Interactions



# *Superoxide Scavenging of NO and the Formation of Peroxynitrite*

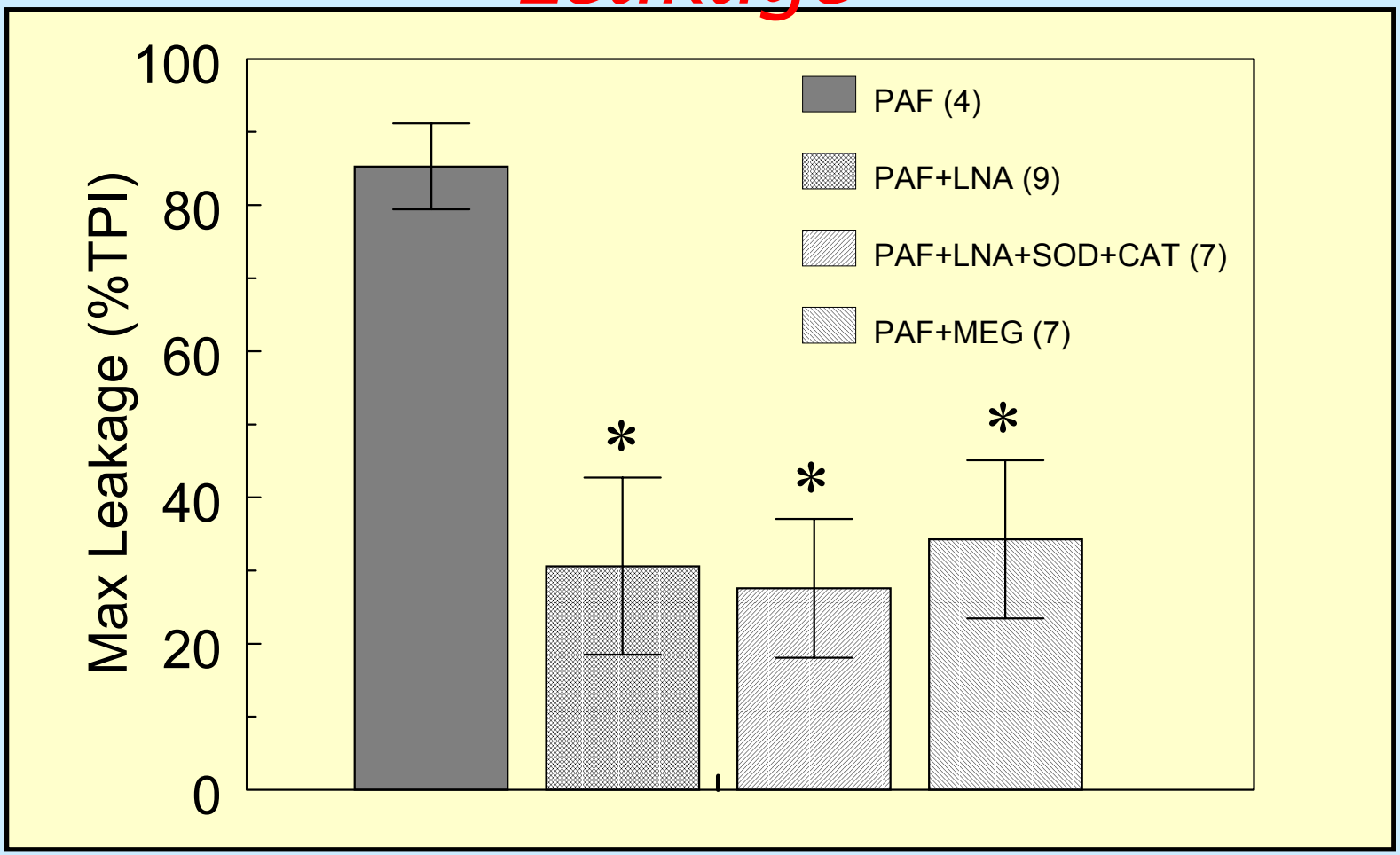
- Increased ROS decreases NO, which may contribute to:
  - Leukocyte and platelet adhesion (leading to vascular plugging)
  - Vasoconstriction (leading to impaired perfusion)
- Peroxynitrite formation can cause vasodilation and cardiac depression, as well as cellular damage in general
- Decreasing ROS by antioxidants, for example, may enhance hypotension by increasing NO bioavailability.
  - Therefore, therapy may need to be directed against both ROS and NO.

# Nitric Oxide Contributes to ROS-Induced Microvascular Leakage




Klabunde and Anderson, *J Vasc Res* 39:338, 2002.

# Peroxynitrite Formation Contributes to PAF-Induced Microvascular Leakage




Klabunde and Anderson, *J Vasc Res* 39:338, 2002.

# *Summary*

- Cardiovascular manifestations of septic shock include:
  - Cardiac depression
  - Systemic vasodilation, hypovolemia and hypotension
  - Reduced organ perfusion
- NO and ROS are implicated in contributing to these cardiovascular changes
- ROS scavengers and selective iNOS inhibitors may provide a new therapeutic approach to improving survival in septic patients