



## **Main biological properties of albumin**

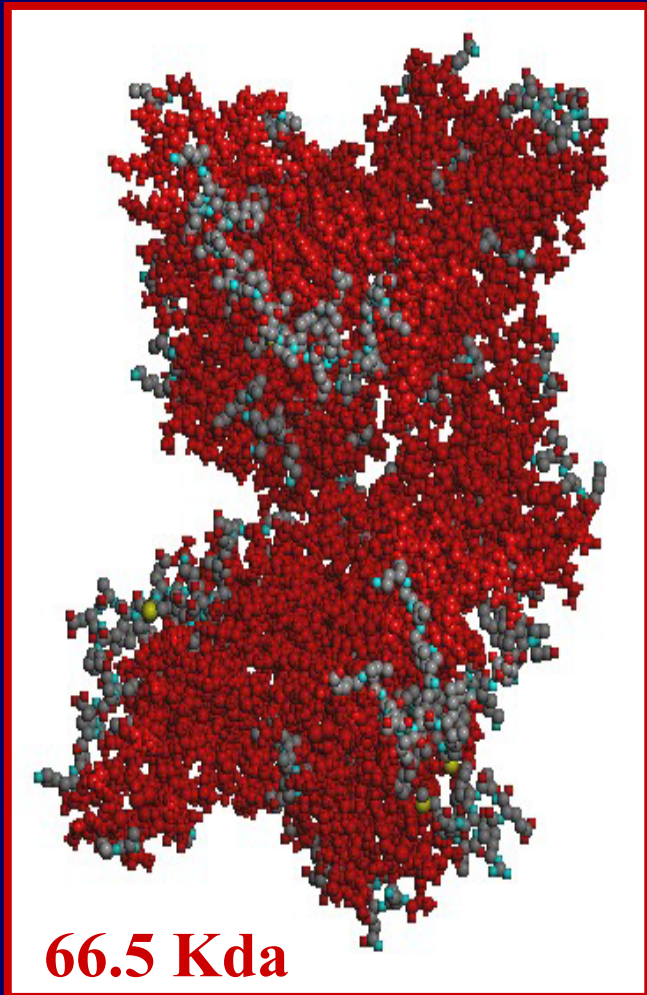
**P. Angeli**

**Dept. of Clinical and  
Experimental Medicine  
University of Padova**

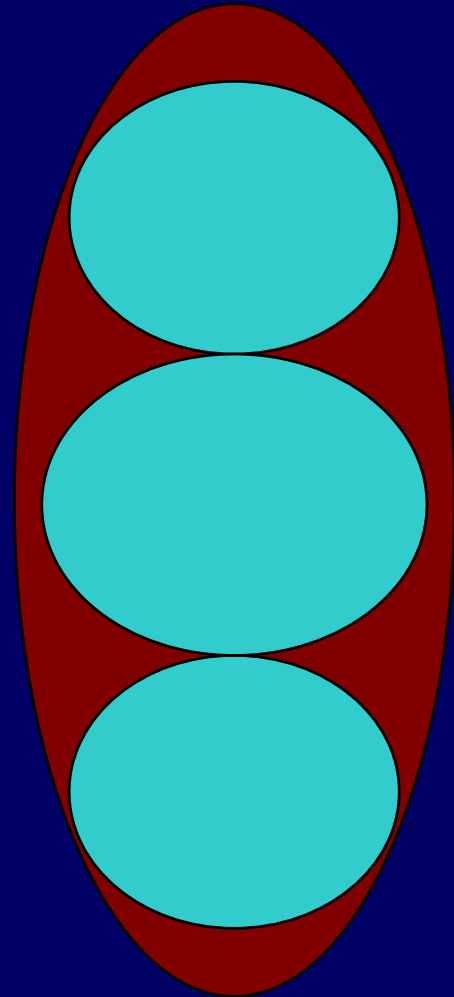
***XXIX Congresso SIFO***

***Napoli 12-15 Ottobre 2008***

# HUMAN ALBUMIN



The structure of albumin



The elipsoid structure of albumin in solution

## HUMAN ALBUMIN

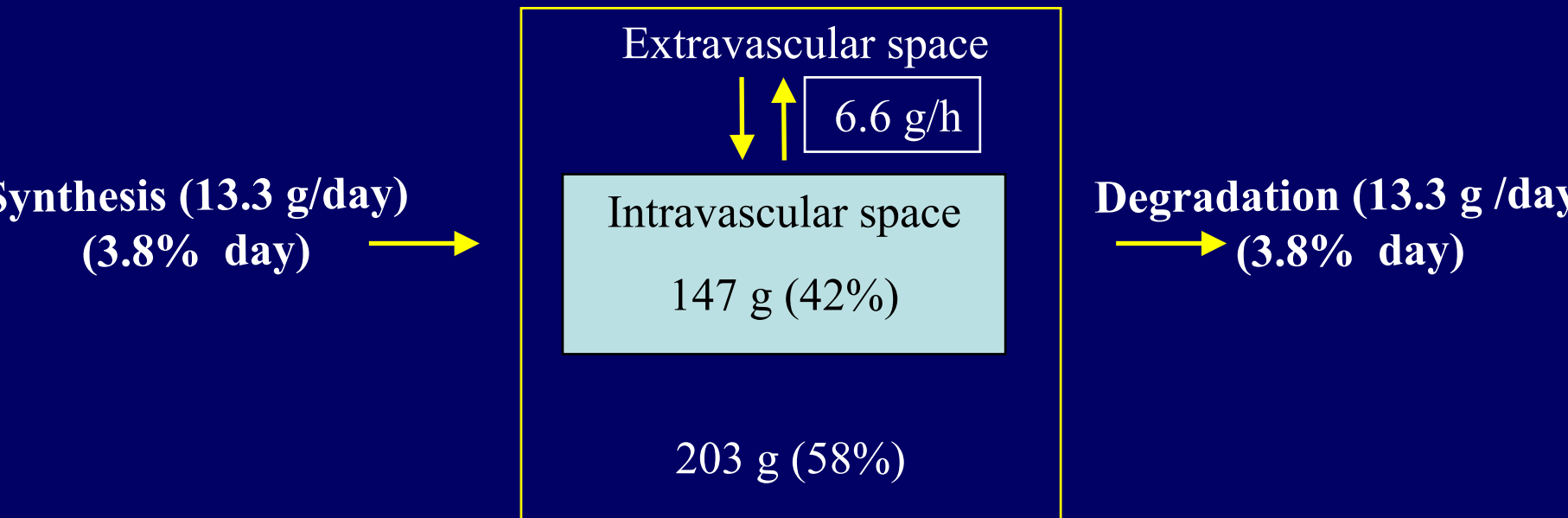
### Synthesis of albumin

- In humans albumin synthesis takes place only in the liver (10-25 g of albumin per day).
- The liver can increase albumin synthesis to only 2-2.7 times normal because most of the liver's synthetic activity is already devoted to albumin at rest.
- Albumin is not stored by the liver but is secreted into the portal circulation as soon as it is manufactured.

*DC. Carter, et al. Adv. Protein Chem. 1994 ; 45 : 153-203.*

# HUMAN ALBUMIN

**Albumin distribution in a healthy 70 kg adult with a total albumin pool of 350 g (5g/kg B.W.)**



*Y. Takeda, et al. J. Lab. Clin. Med. 1963 ; 61 : 183-202.*

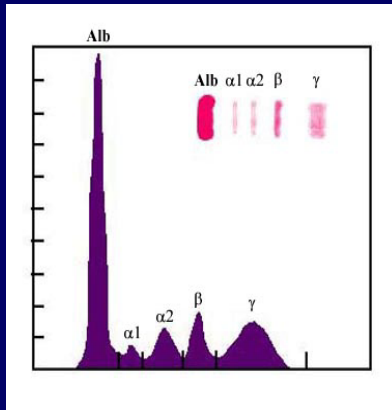
## HUMAN ALBUMIN

### Main biological properties of albumin

- Albumin accounts for 75-80% of plasma colloid oncotic pressure

# HUMAN ALBUMIN

## Albumin and plasma colloid oncotic pressure



### High concentration (2/3)

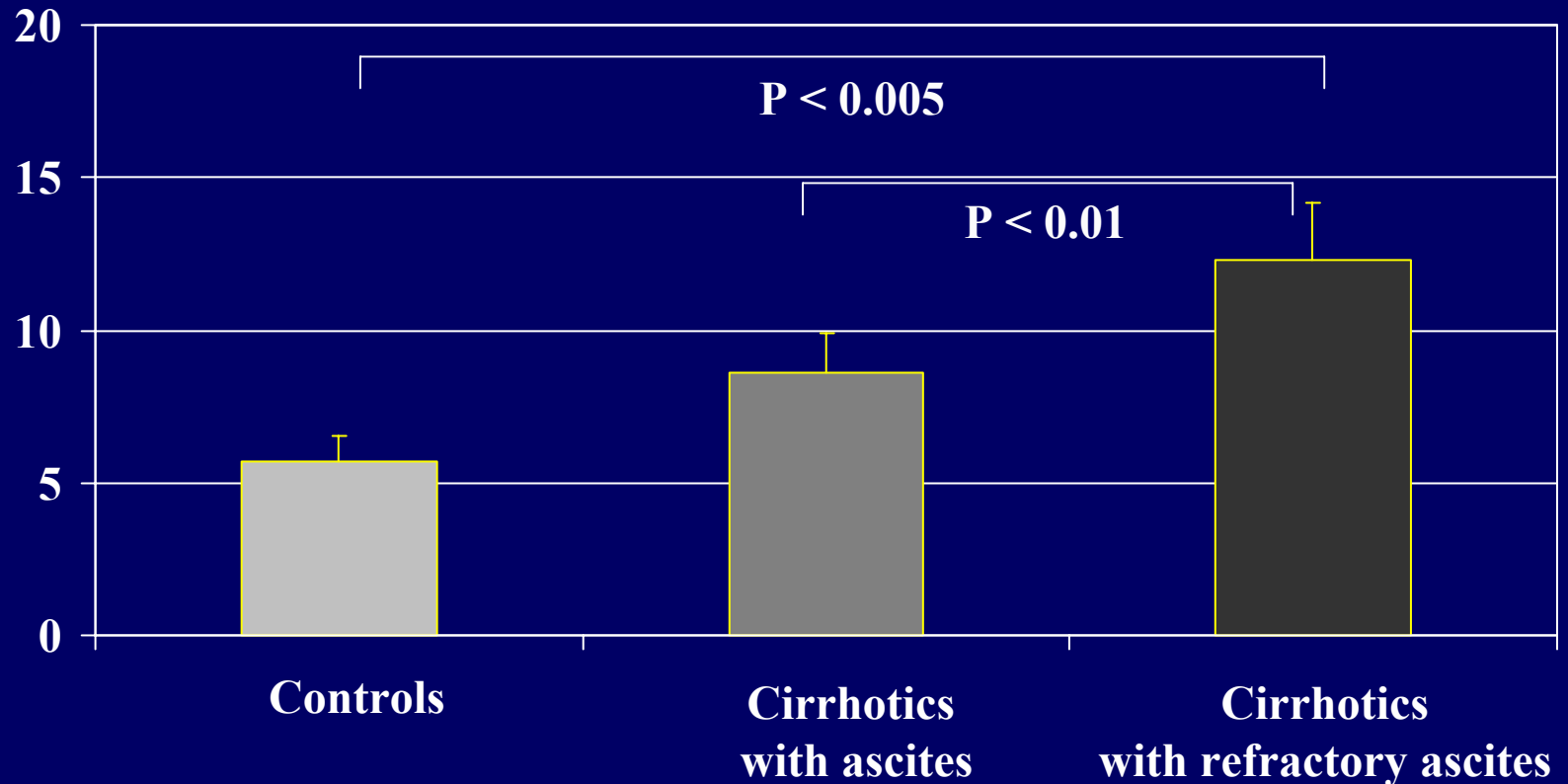
### Gibbs-Donnan effect (1/3)



$\text{Na}^+$

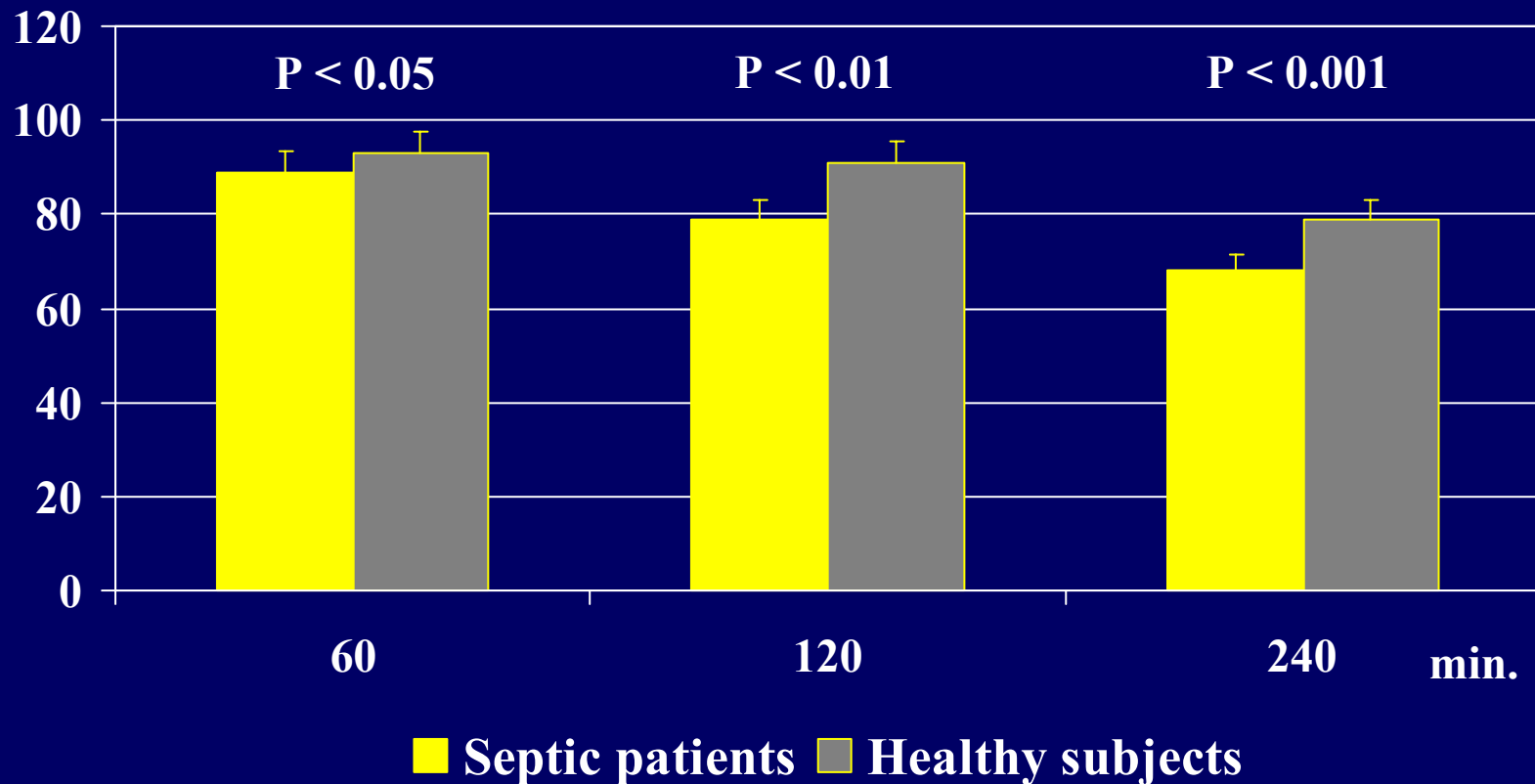
# Overall transvascular transport of albumin in cirrhosis

(% IVM  $\cdot$  h<sup>-1</sup>)



# HUMAN ALBUMIN

**Changes in the albumin increment (% of initial value) following a bolus of albumin 20%**



*M.P. Margaron, et al. B.J.A. 2004 ; 92 : 821-826.*



## **HUMAN ALBUMIN**

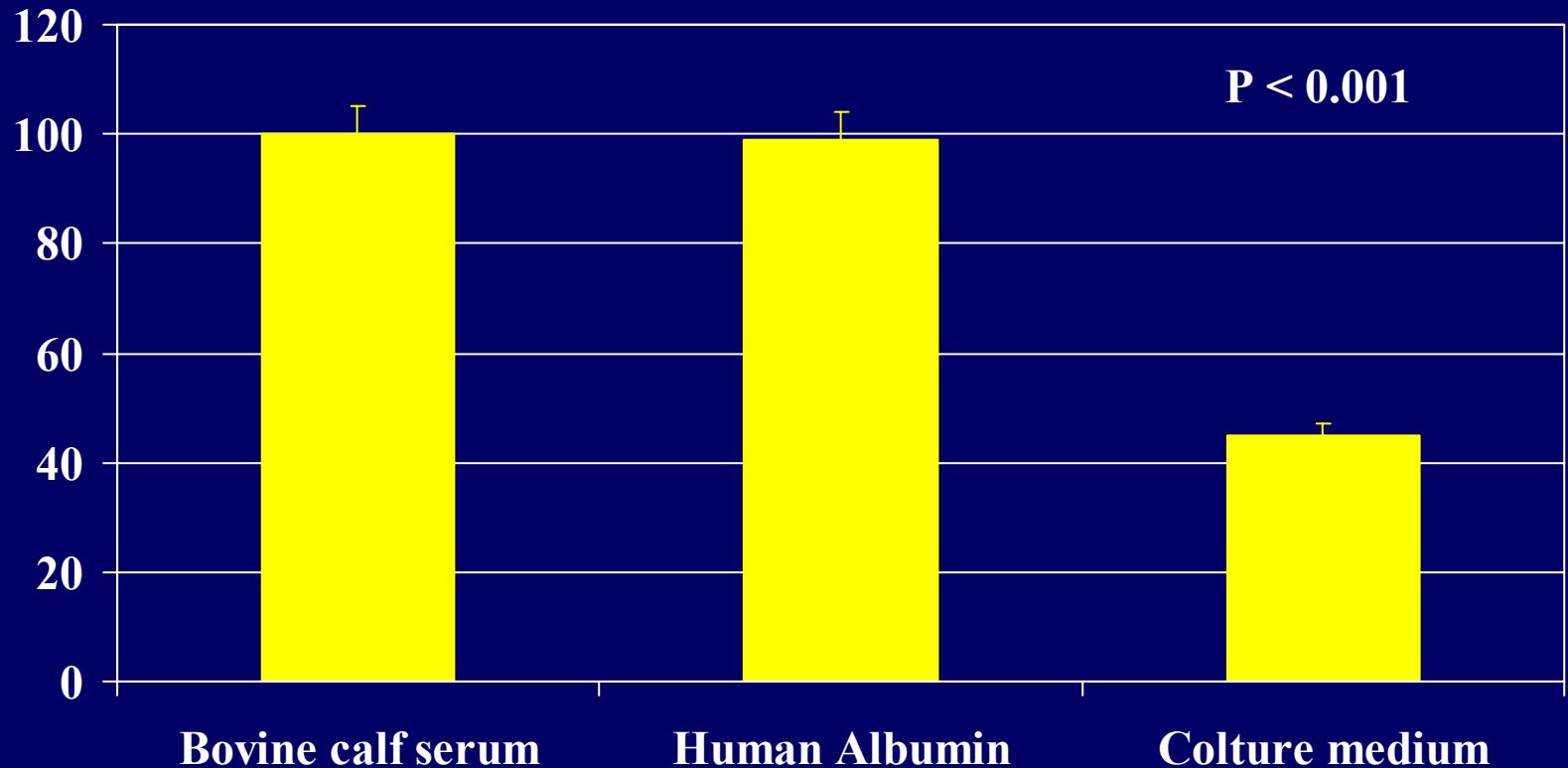
### **Main properties of albumin**

- **Albumin accounts for 75-80% of plasma colloid oncotic pressure**

- **Effect on endothelial function**

# HUMAN ALBUMIN

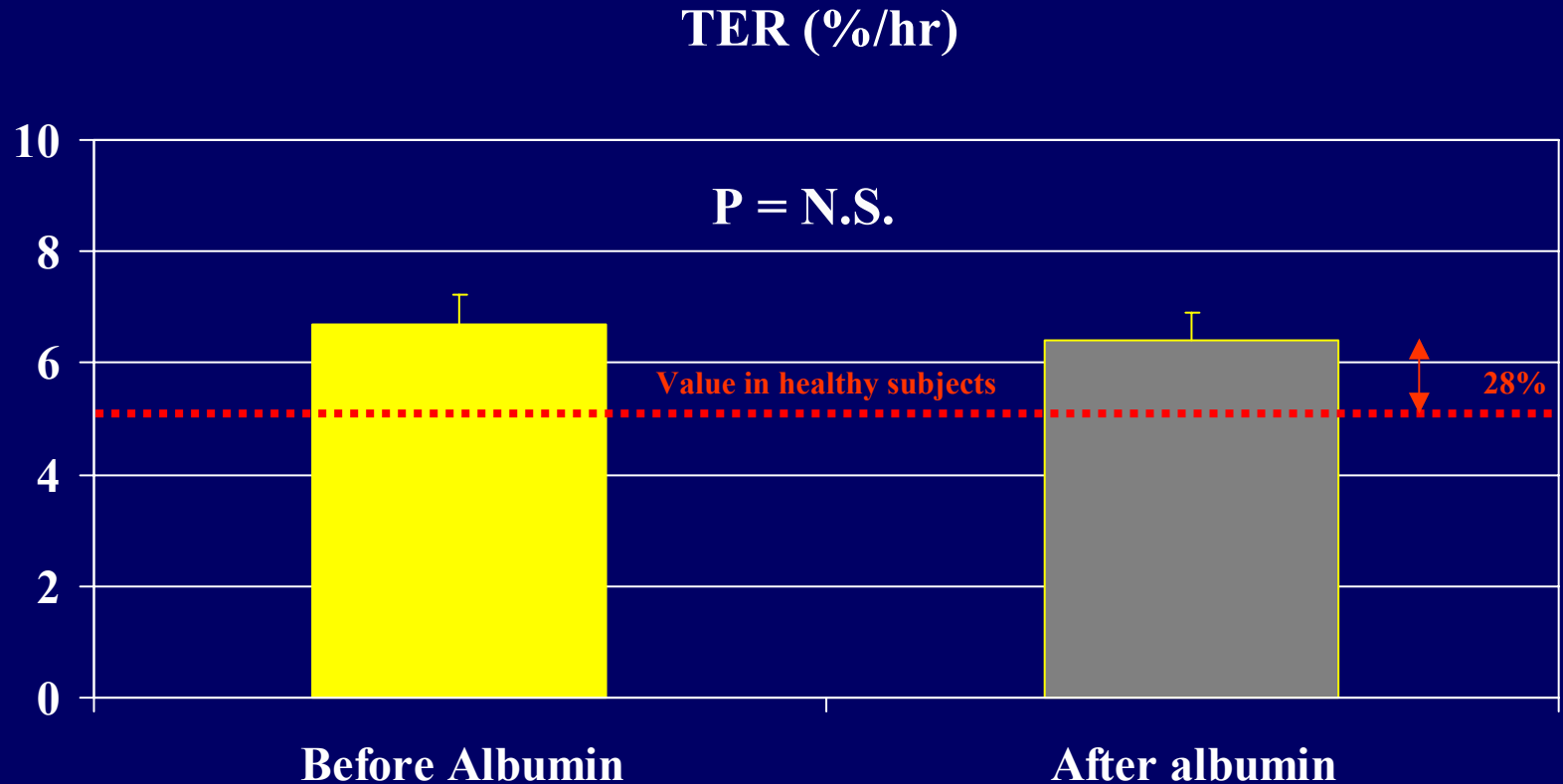
**Relative protection from apoptosis for cultured human endothelial cells**



*M.P. Margaron, et al. B.J.A. 2004 ; 92 : 821-826.*

# HUMAN ALBUMIN

Effect of albumin supplementation on transvascular escape rate (TER) of  $^{125}\text{I}$ -labeled albumin in pigs with septic shock.



*M. Margason et al. J. Appl. Physiol. 2002 ; 92 : 2139-2145.*

## **HUMAN ALBUMIN**

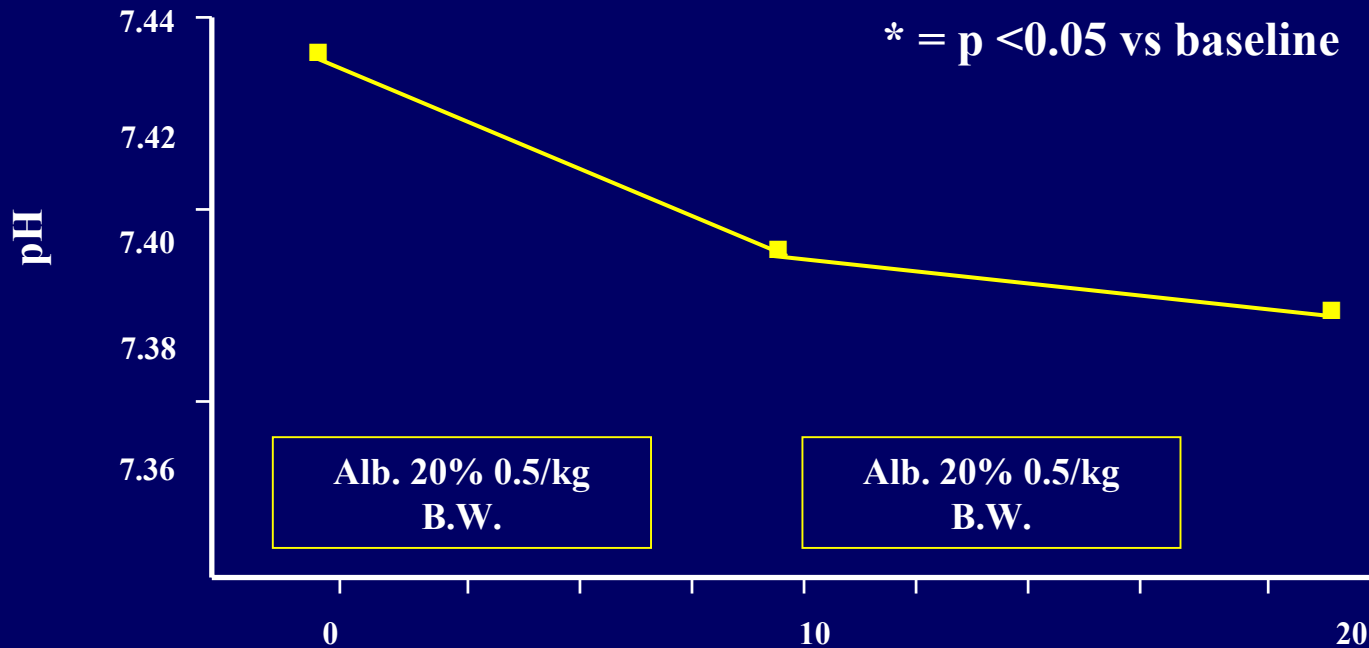
### **Main properties of albumin**

- **Albumin accounts for 75-80% of plasma colloid oncotic pressure**

- **Effect on endothelial function**
- **Regulation of acid-base balance**

# HUMAN ALBUMIN

## Effect of two boluses of 20% albumin solution (0.5 g/kg B.W.) on acid-base balance

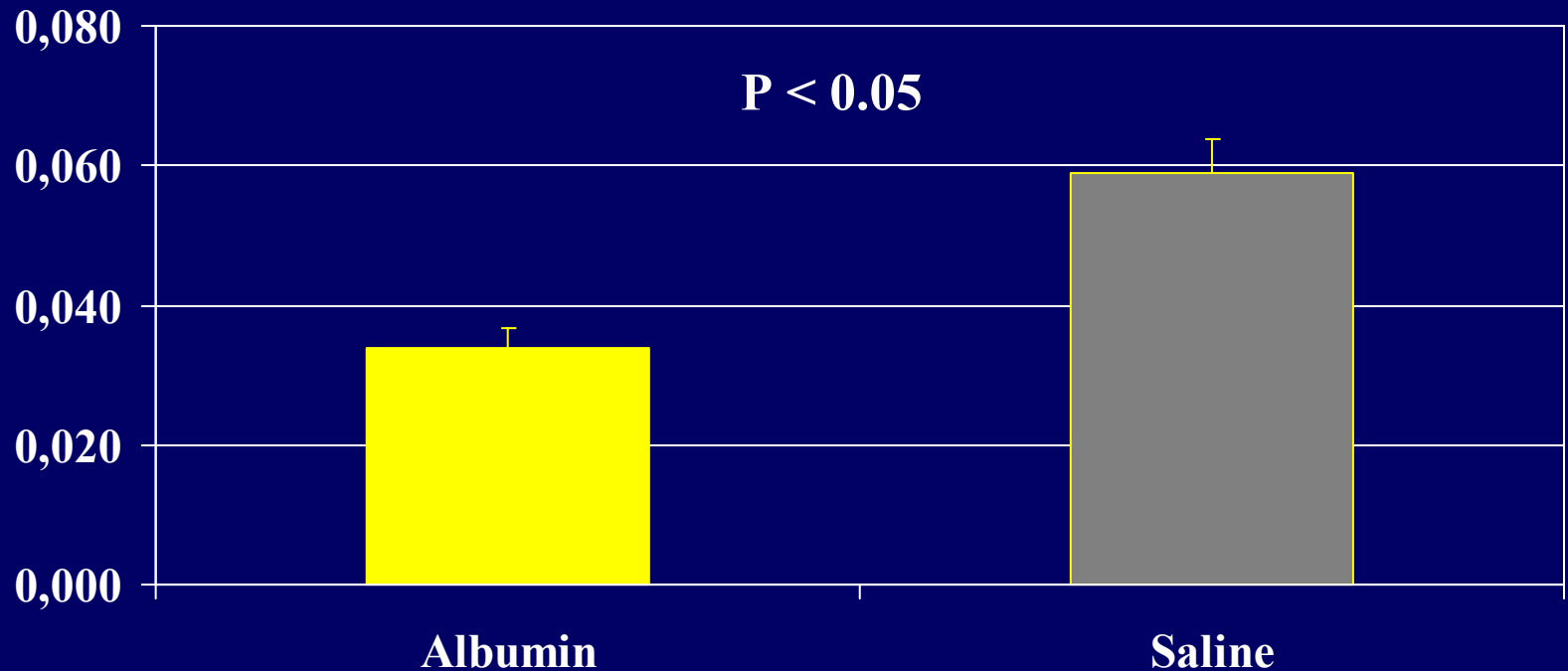


*D. Bruegger, et al. Intens. Care. Med. 2005 ; 31 : 1123-1127.*

# HUMAN ALBUMIN

Mean difference in pH between baseline value and mean value during albumin or saline resuscitation in critically ill patients

(pH)



*R. Bellomo et al. Crit. Care Med. 2006 ; 34 : 2891-2897.*

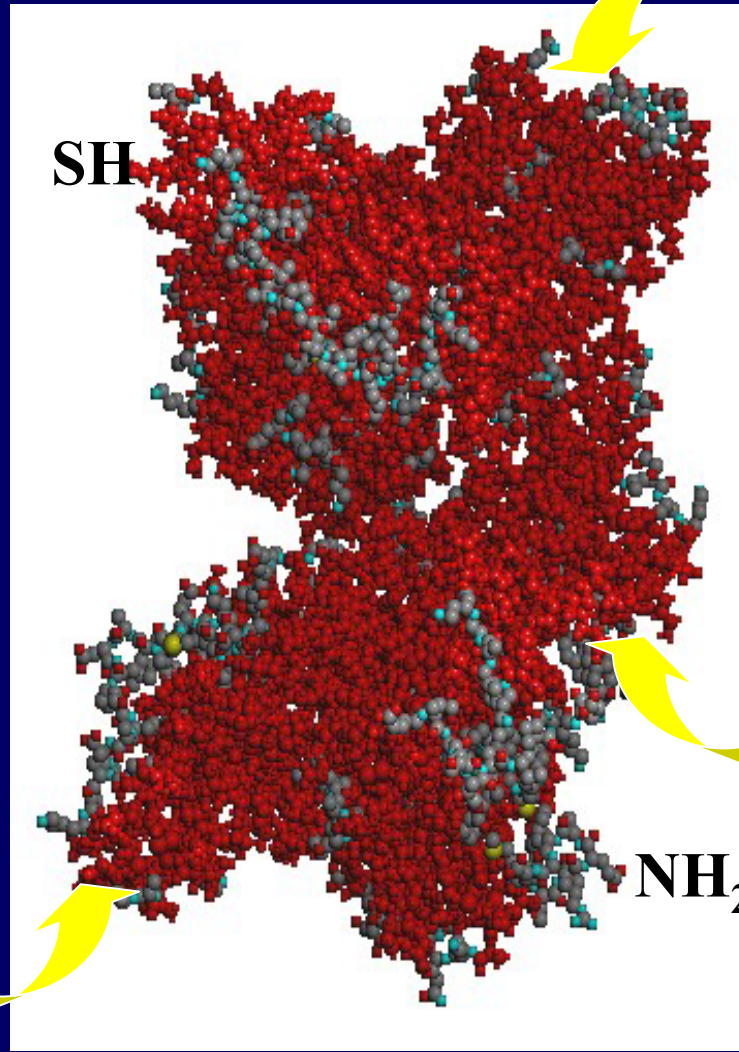
## HUMAN ALBUMIN

### Main biological properties of albumin

- **Albumin accounts for 75-80% of plasma colloid oncotic pressure**

- **Effect on endothelial function**
- **Regulation of acid-base balance**
- **Ligand-binding**

# HUMAN ALBUMIN



## Endogenous molecules

Bilirubin  
Biliary salts  
LCFA

## Exogenous molecules

Benzene  
Aflatoxina G

## DRUGS

NSAIDs  
Warfarin  
Benzodiazepines  
Furosemide



## **HUMAN ALBUMIN**

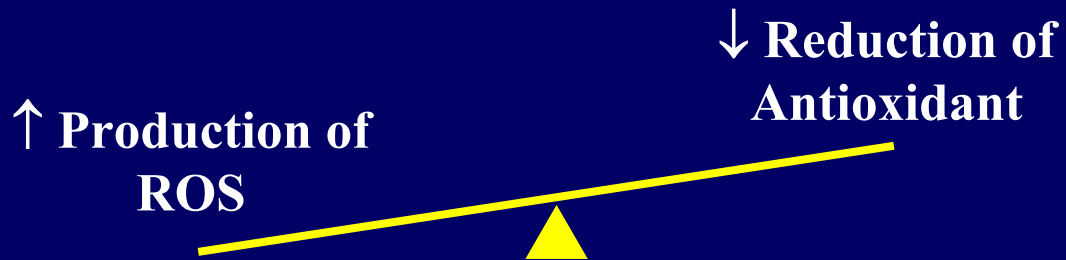
### **Main biological properties of albumin**

- **Albumin accounts for 75-80% of plasma colloid oncotic pressure**

- **Effect on endothelial function**
- **Regulation of acid-base balance**
- **Ligand-binding**
- **ROS/RNS scavenging and antioxidant function**

## ALBUMIN AND OXIDATIVE STRESS

# Oxidative stress



# ALBUMIN AND OXIDATIVE STRESS

## Cellular sources of ROS in oxidative stress

Inflammation, endotoxins

**NADPH oxidases**

Oxidative stress due to cellular surface modifications

Endotoxins, exogenous toxins

**NADH dehydrogenase  
Cytochrome oxidase**

Oxidative stress due to inefficiency of respiratory chain

Reduced tissue perfusion

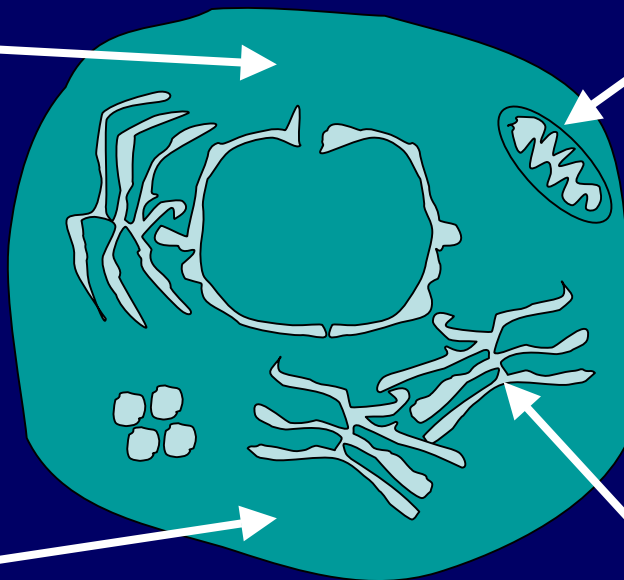
**Xanthine oxidase**

Oxidative stress due to change in intracellular  $pO_2$

Induction

**Cytochrome P<sub>450</sub>  
Cytochrome b<sub>5</sub>**

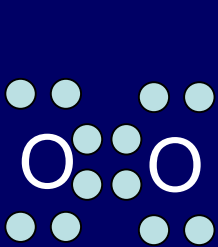
Oxidative stress due to metabolic or drug induction



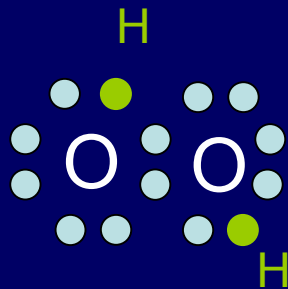
## ALBUMIN AND OXIDATIVE STRESS

### Reactive oxygen species (ROS)

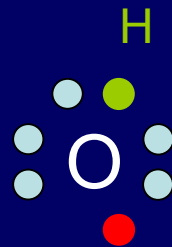
- Molecules like hydrogen peroxide
- Radicals like the hydroxyl radical: the most reactive
- The superoxide anion which is both ion and radical



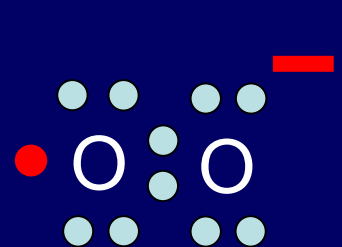
Oxygen O<sub>2</sub>



Hydrogen Peroxide



Hydroxyl Radical



Superoxide anion

## **Biological anti-oxidant systems**

### **1. INTRACELLULAR**

**SOD**

**Catalase**

**Peroxidase**

**Glutathione**

**Selenium**

### **2. MEMBRANE**

**Vitamin E**

**β Carotene**

**Ubiquinone**

### **3. EXTRACELLULAR (PLASMA)**

**Metal-Binding Proteins**

**Caeruloplasmin, Transferrin**

**Albumin**

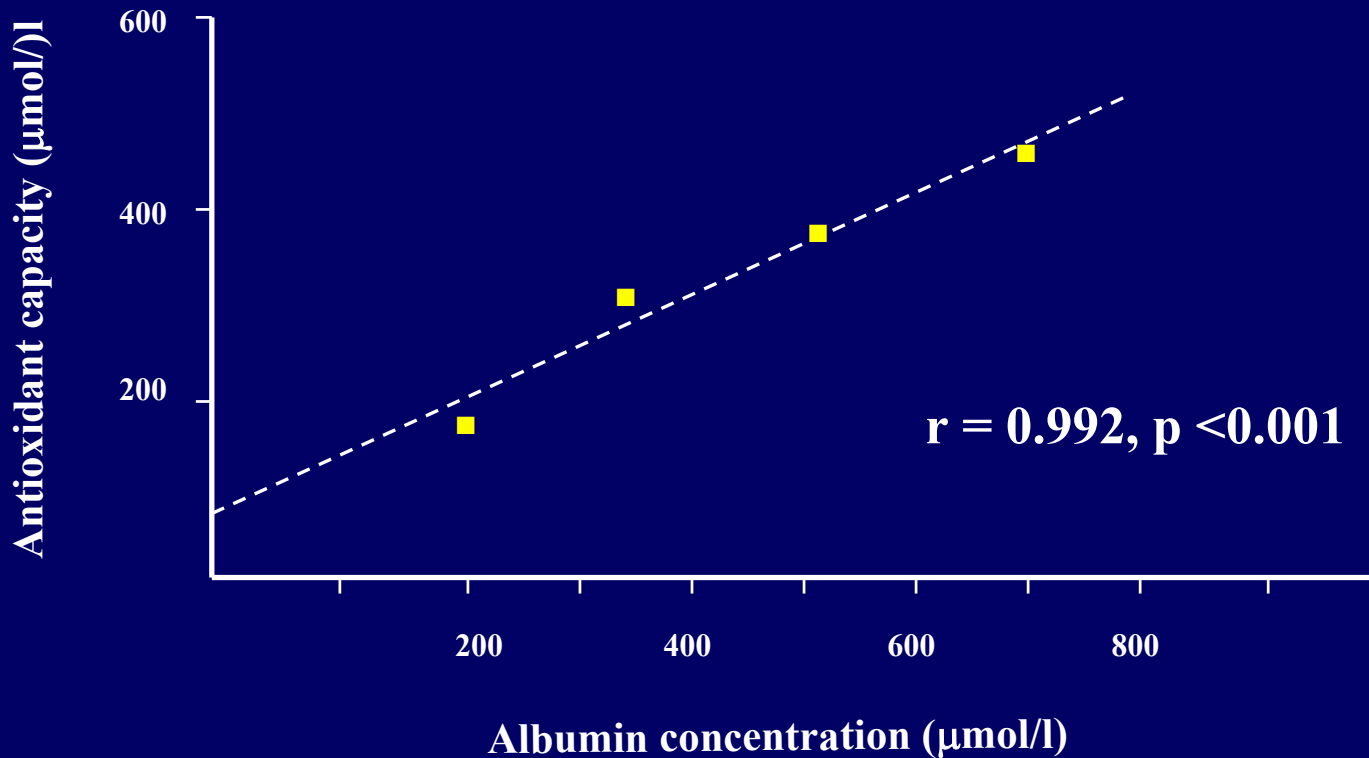
**Uric acid**

**Vitamin E**

**Vitamin C**

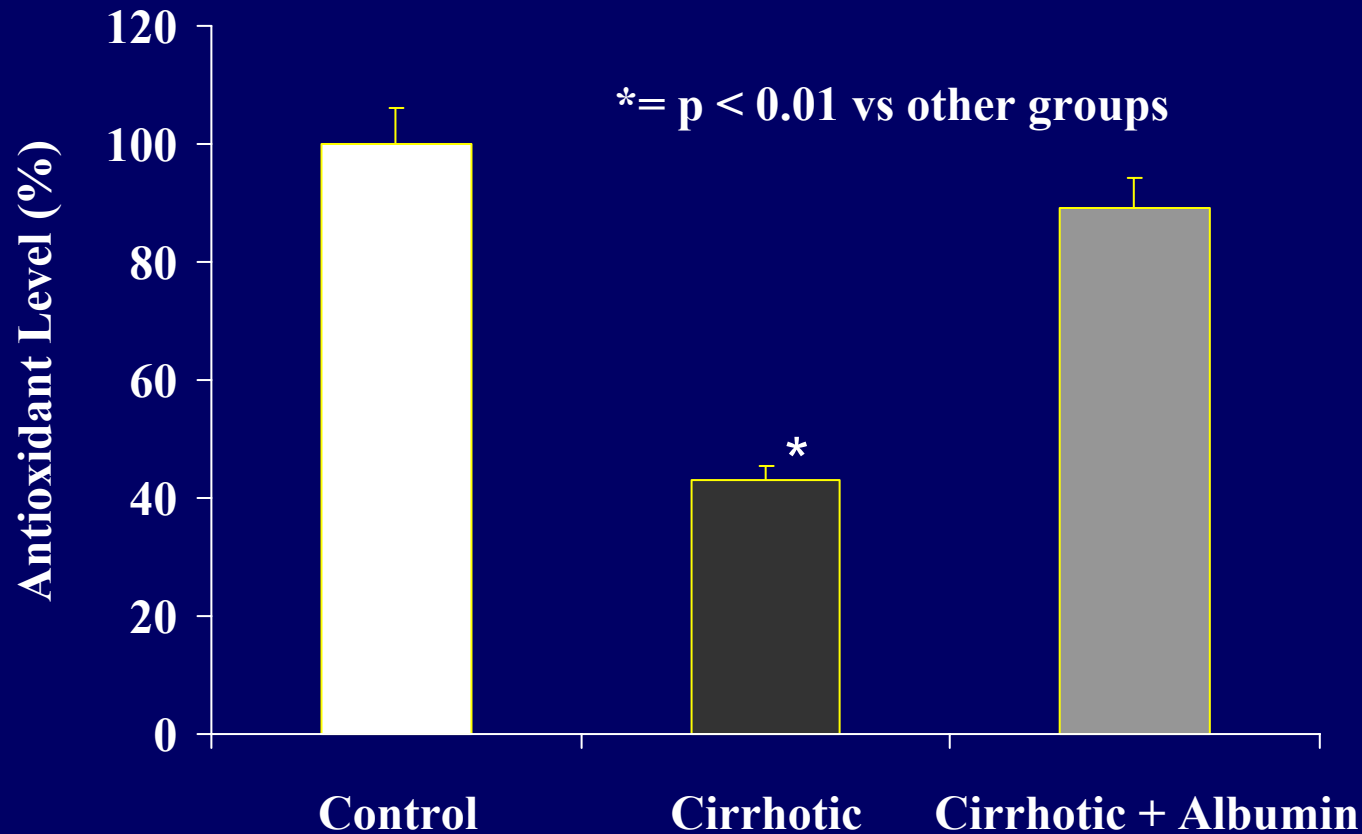
## ALBUMIN AND OXIDATIVE STRESS

**Plot of the total antioxidant capacity of human serum  
as a function of the albumin concentration**



*H. Ihara, et al. J. Clin. Lab. Anal. 2004 ; 18 : 45-49.*

Effect of albumin on the plasma antioxidant level in cirrhotic rats level



# ALBUMIN AND OXIDATIVE STRESS

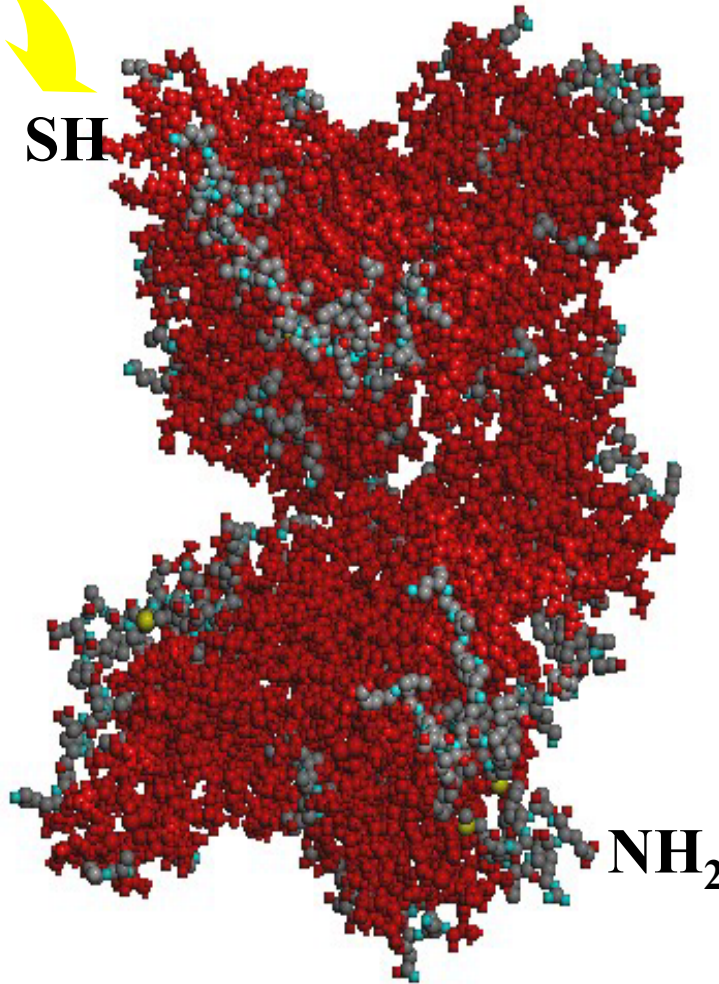
ROS/RNS

$H_2O_2$

$ONOO^-$

Lipid hydroperoxide

SH

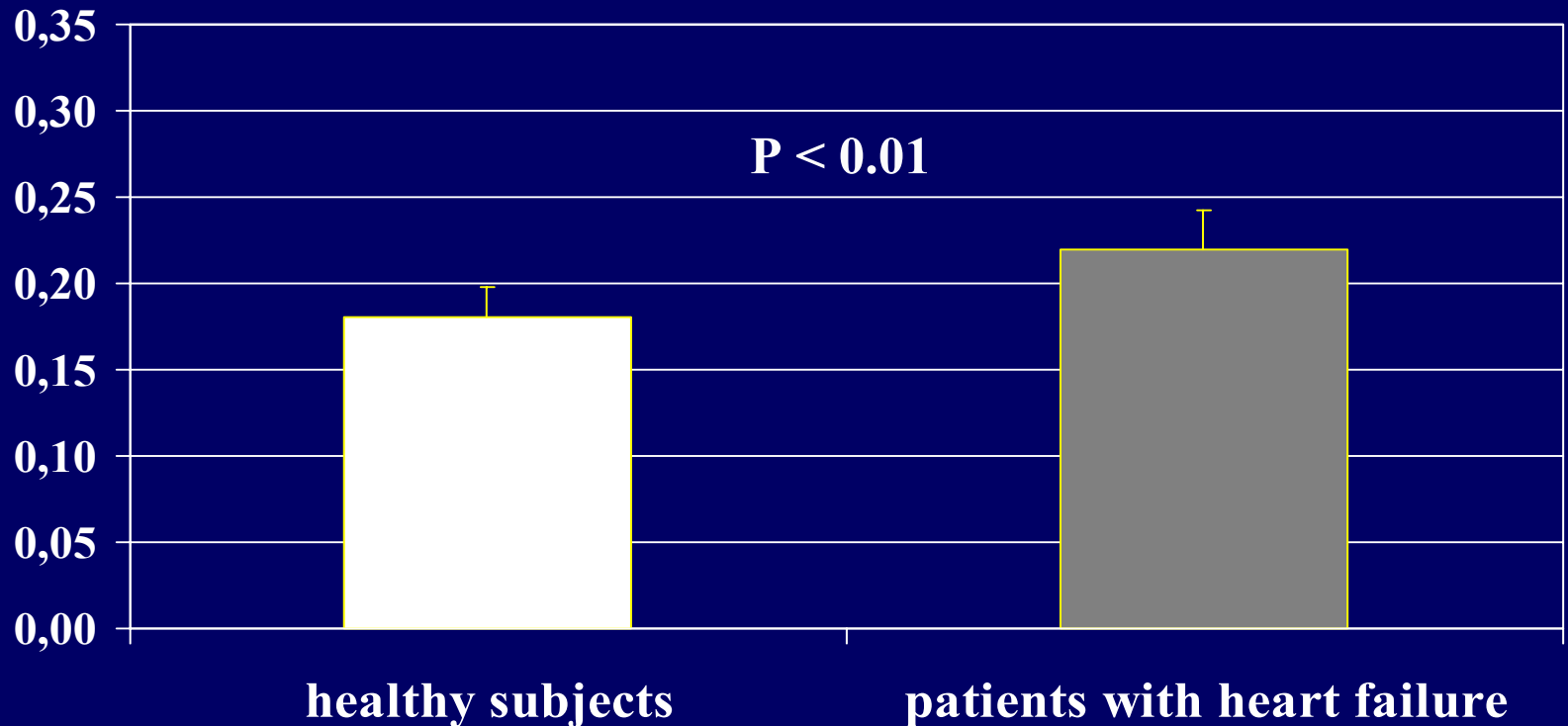


$NH_2$



# Plasma levels of oxidized proteins in patients with heart failure

(nmoles/mg protein)

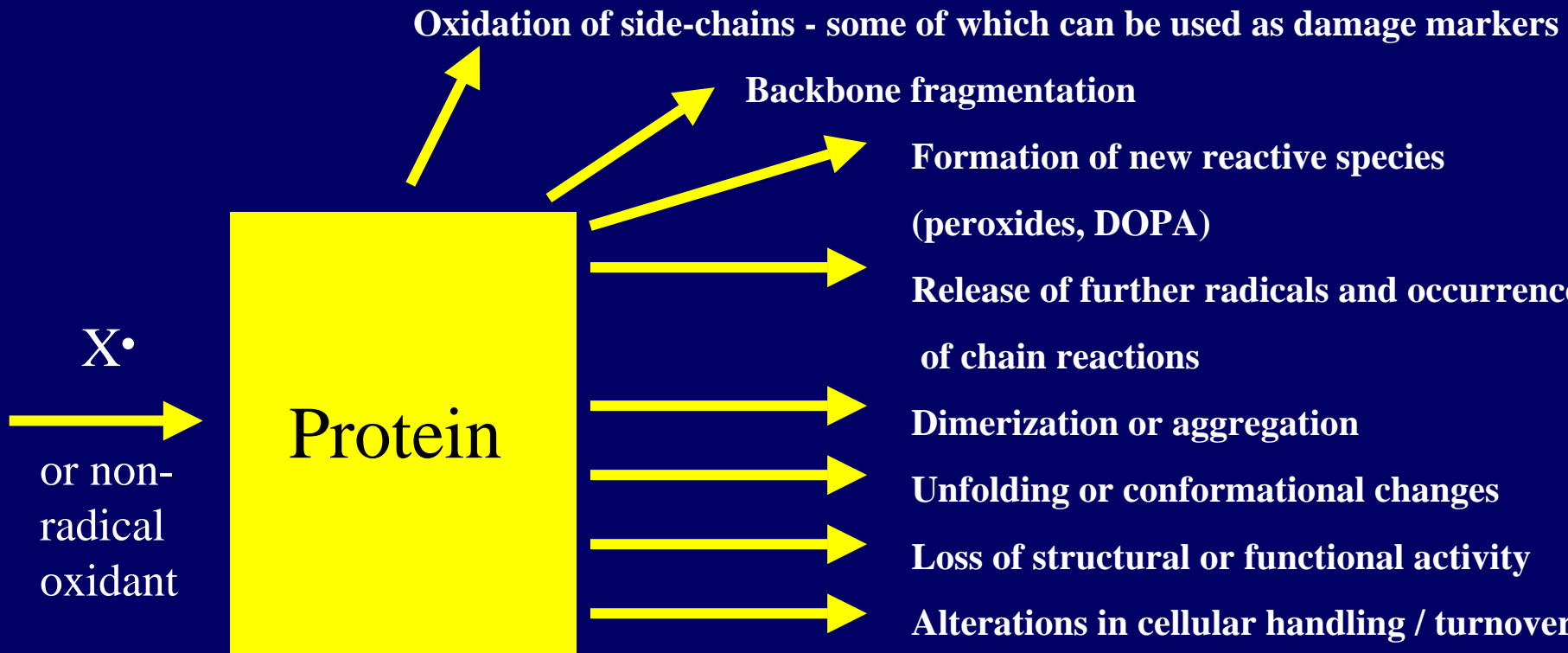


## ALBUMIN AND OXIDATIVE STRESS

### Oxidative stress

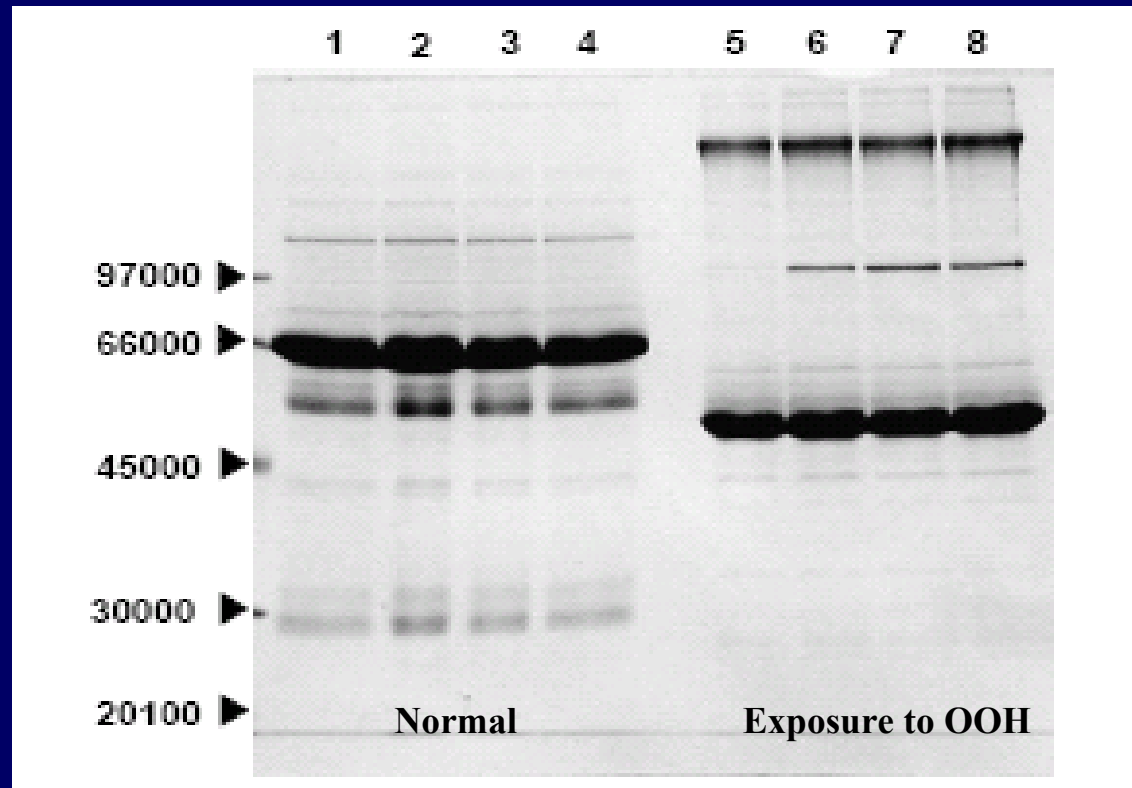
- Effects of ROS on albumin structure and functions
- Effects of albumin infusion on:
  - ROS production
  - ROS-related organ dysfunction

## Consequences of oxidation of proteins



## ALBUMIN AND OXIDATIVE STRESS

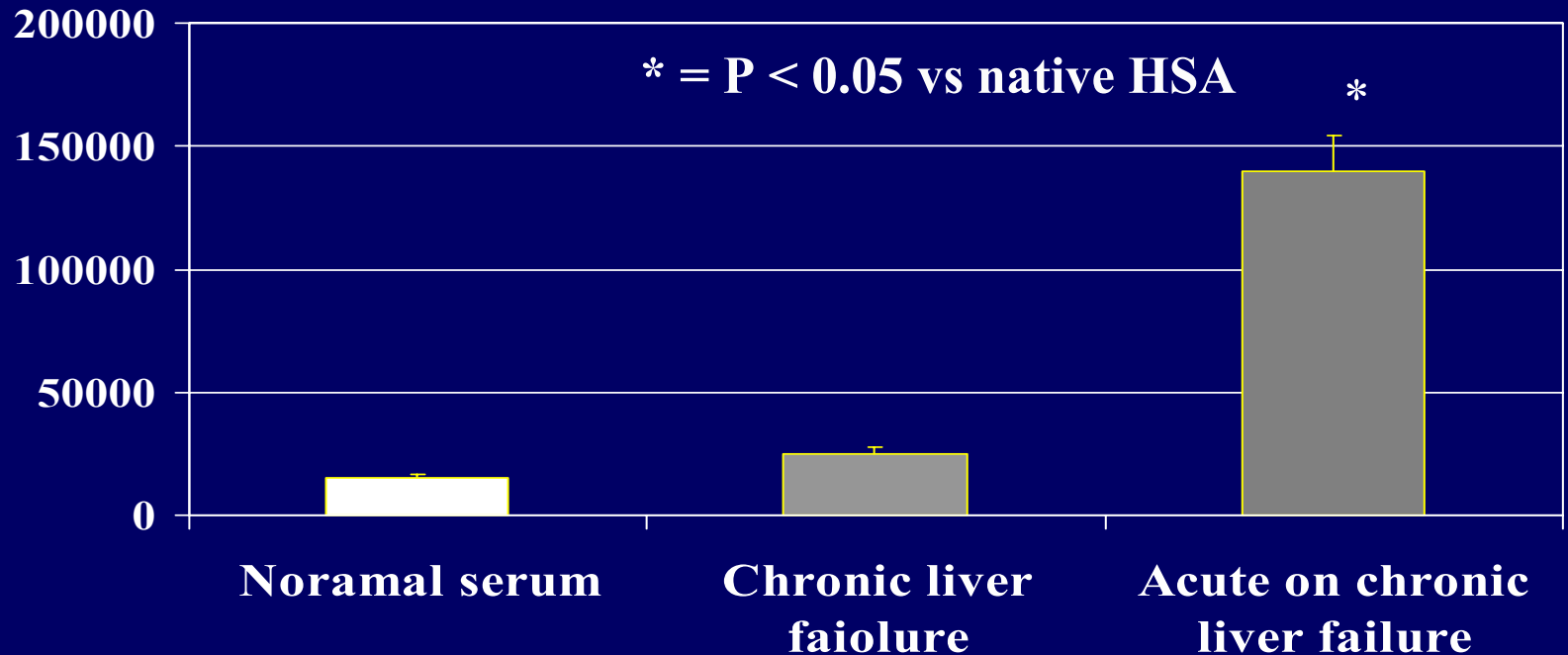
# Albumin dimers induced by peroxides in human plasma



*Y. Ogasawara, et al. Biochim. Biophys. Res. Com. 2006 ; 340 : 353-358.*

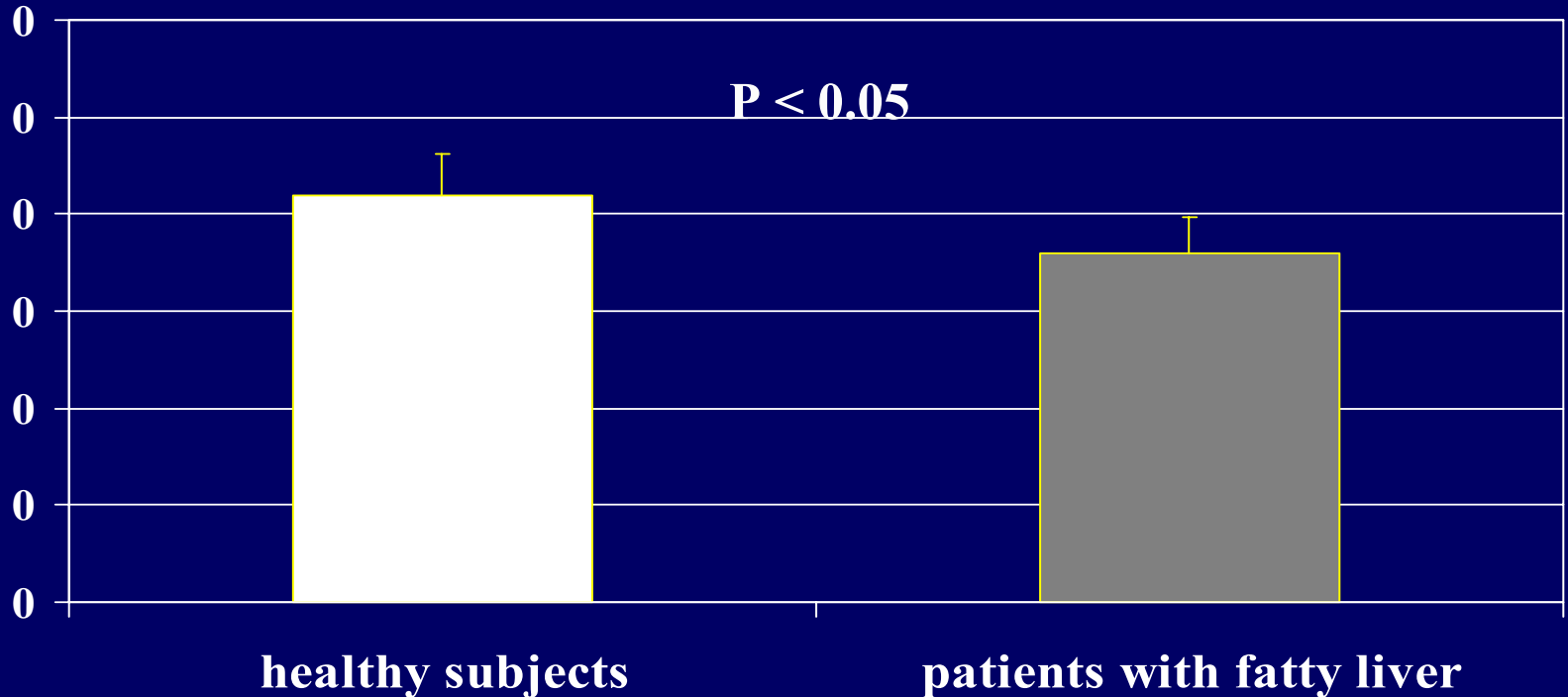
# Carbonyl groups in albumin of serum from patients with liver failure

(luminescence counts)



# Cobalt binding capacity of albumin in patients with fatty liver

(Co bound/g albumin)



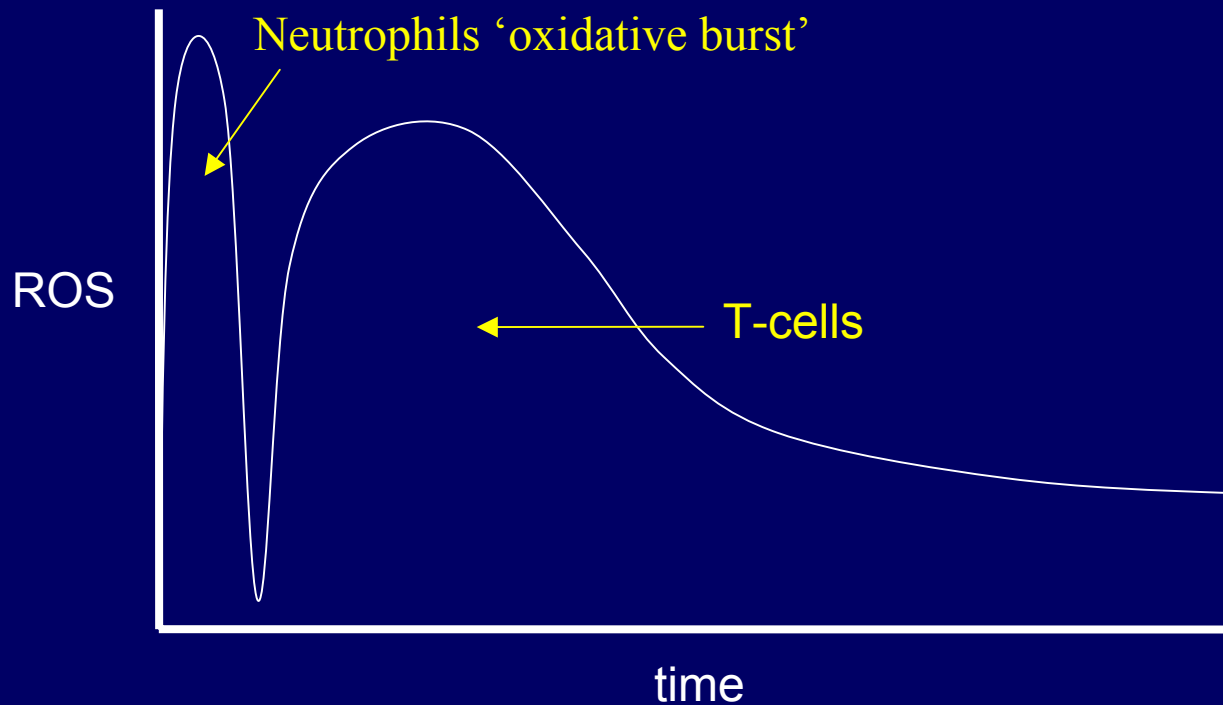
*GJ. Amirtharaj, et al. Biochim Biophys. Acta 2008 (in press).*

## ALBUMIN AND OXIDATIVE STRESS

### Oxidative stress

- Effects of ROS on albumin structure and functions
- Effects of albumin infusion on:
  - ROS production
  - ROS-related organ dysfunction

## Cellular sources of ROS in oxidative stress due to sepsis

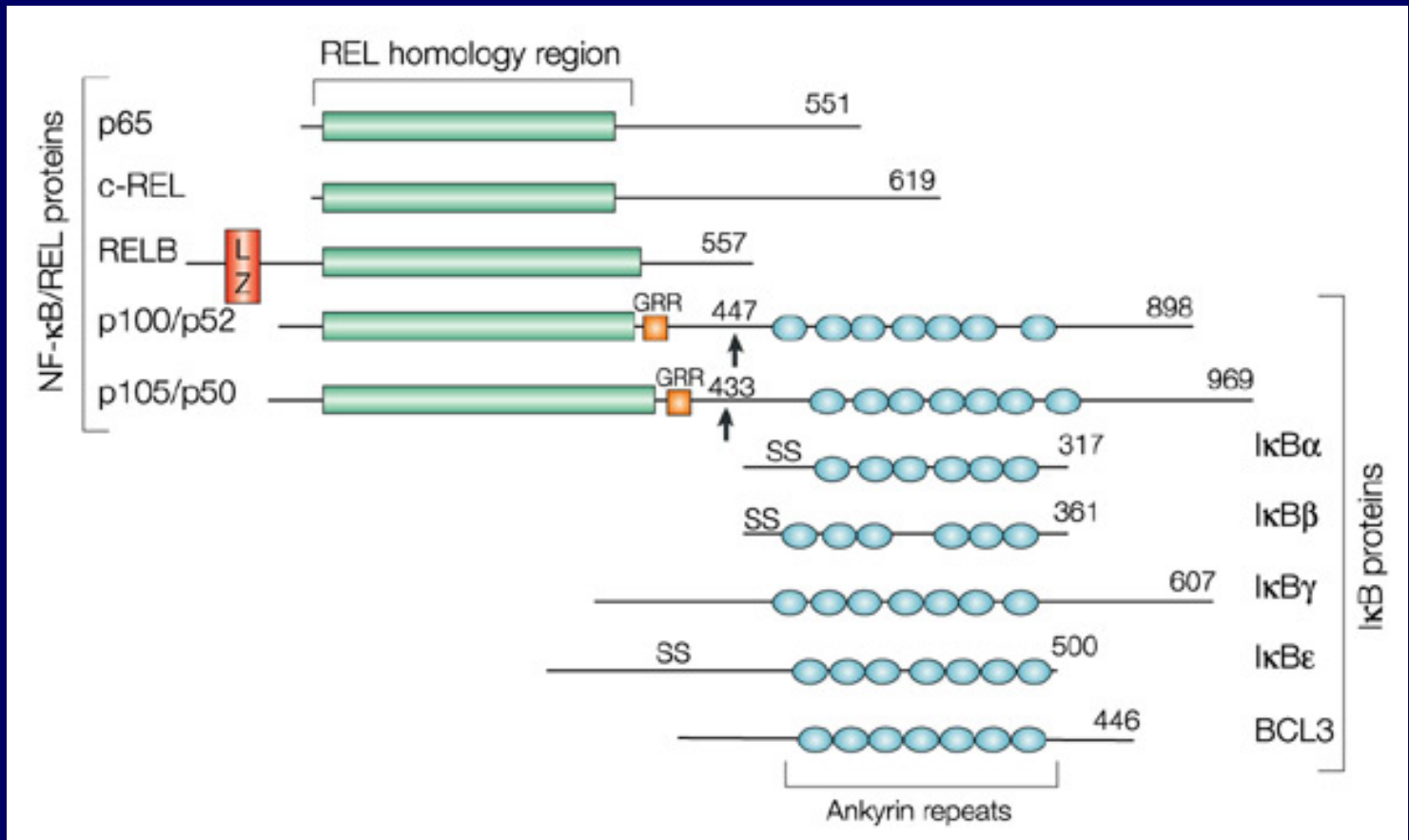


- Induction of iNOS and overproduction of nitric oxide (NO) and reactive nitrogen species (RNS)

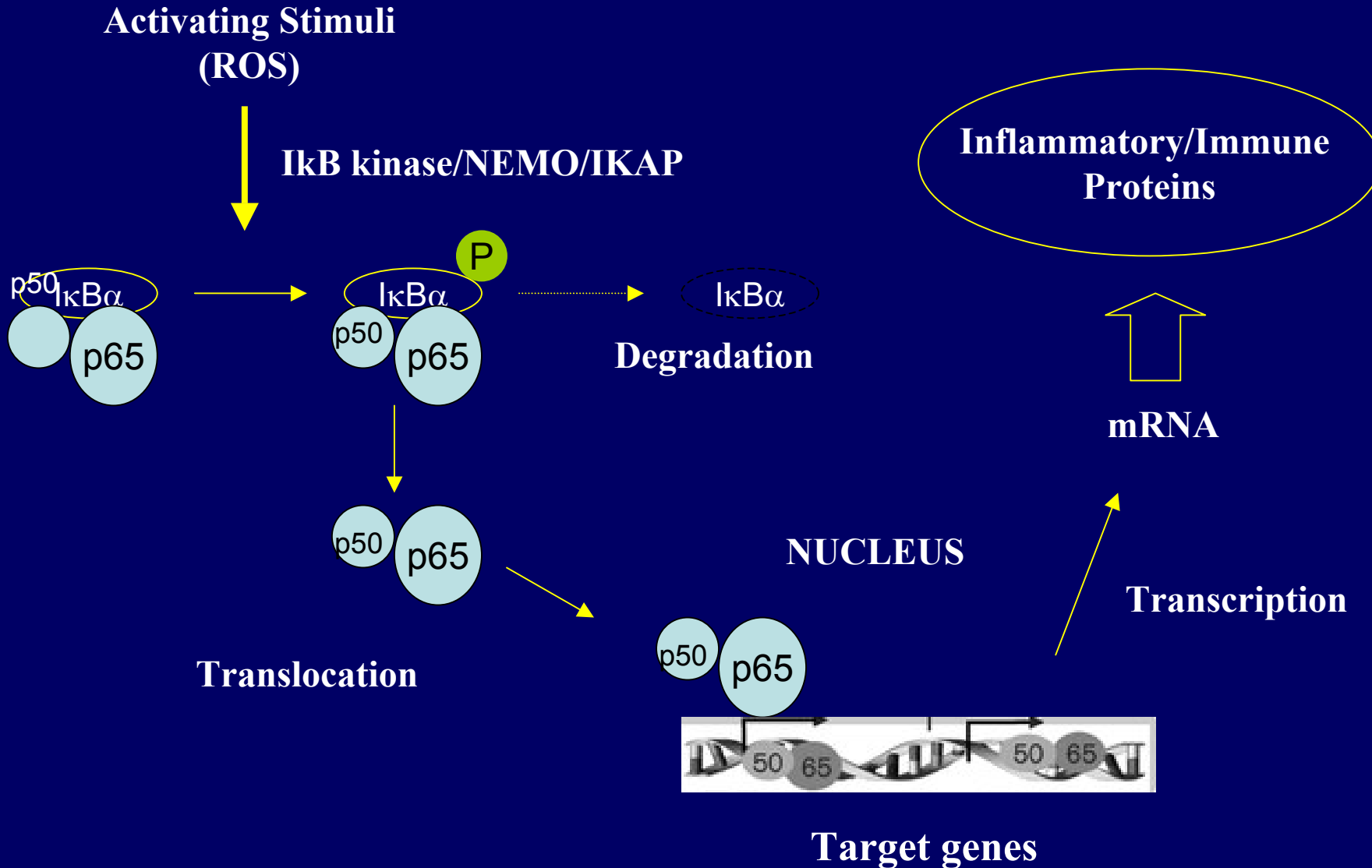


## ALBUMIN AND OXIDATIVE STRESS

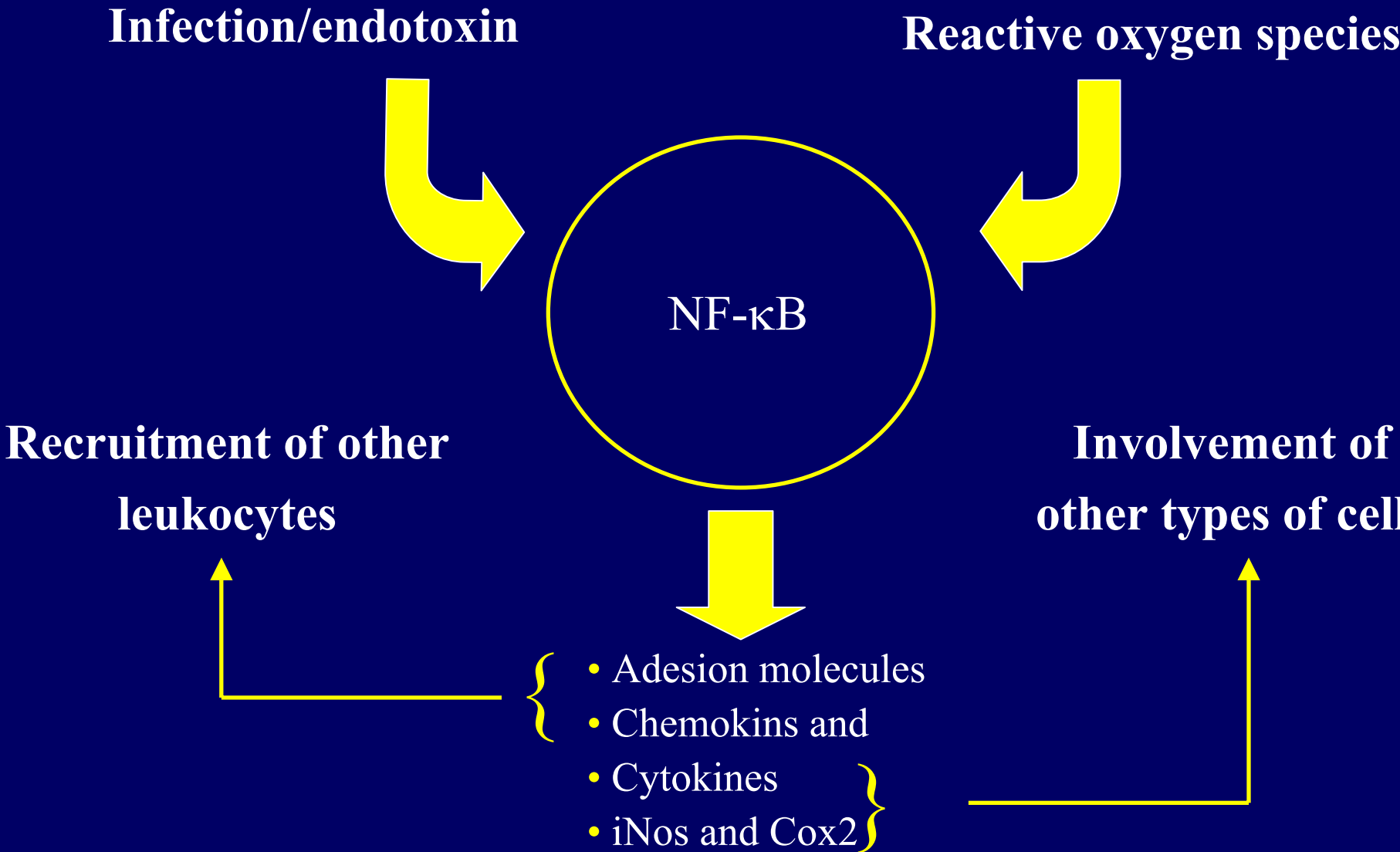
### Role of NF- $\kappa$ B in oxidative stress



# Mechanism of action of NF- $\kappa$ B



## Role of NF-κB in oxidative stress due to sepsis

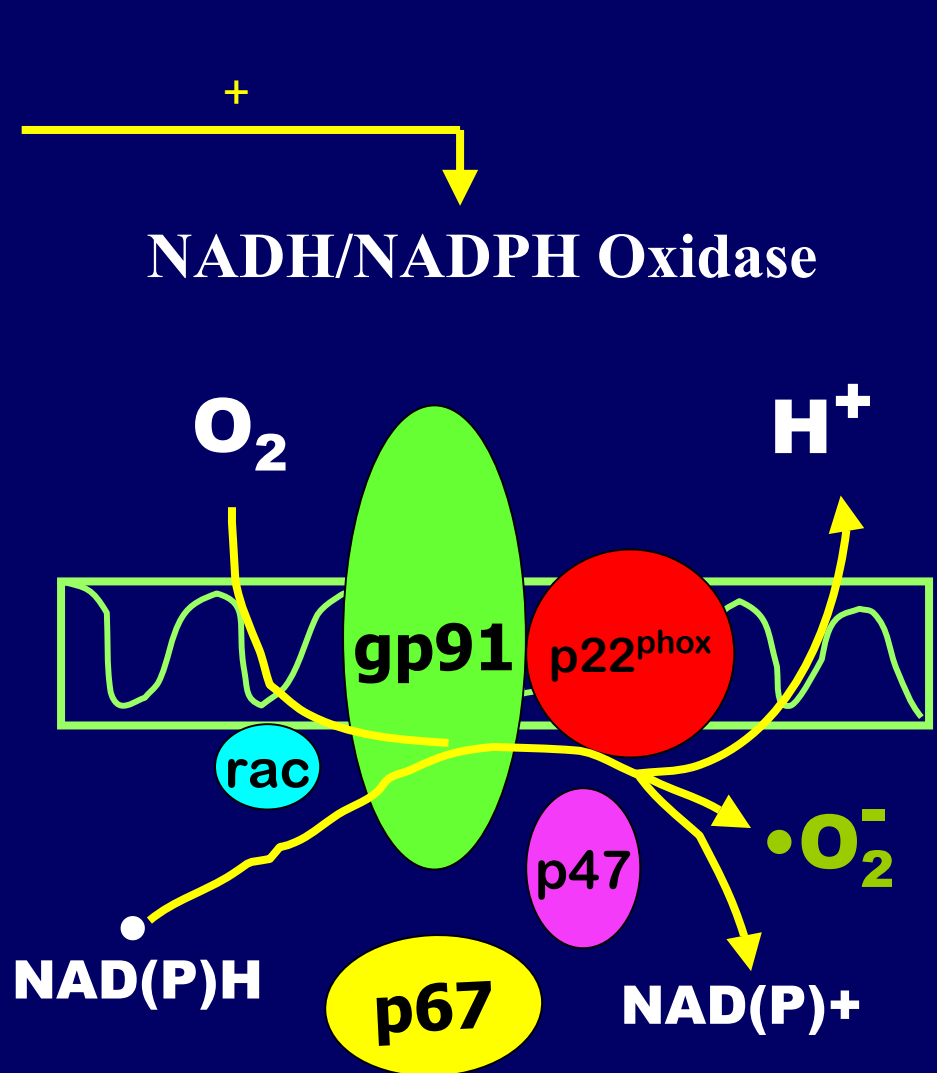


## ALBUMIN AND OXIDATIVE STRESS

### Cellular sources of ROS in oxidative stress due to sepsis

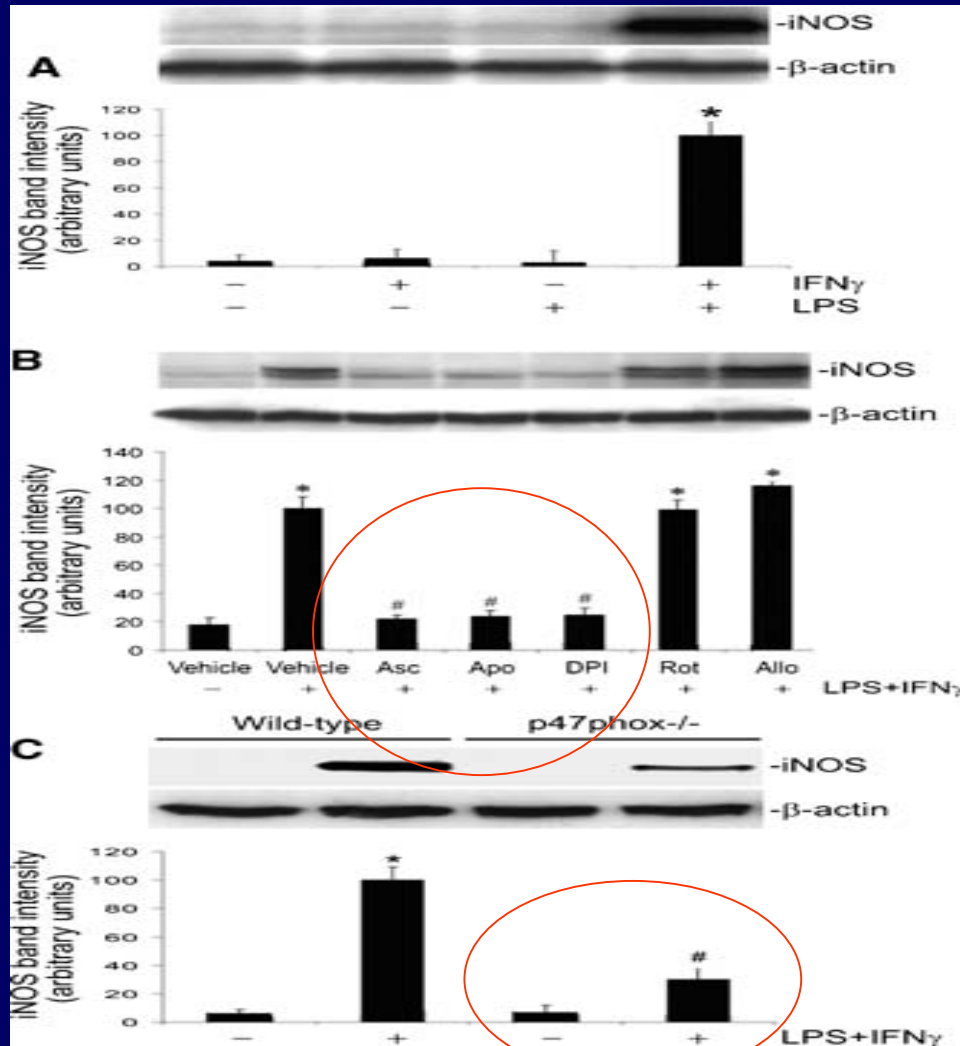
- TNF- $\alpha$
- Thrombin
- Angiotensin II
- Platelet-derived growth factor

- Endothelial cells
- Vascular smooth muscle cells
- Cardiac myocytes



## ALBUMIN AND OXIDATIVE STRESS

### Effect of endotoxin and cytokines on iNOS in endothelial cells



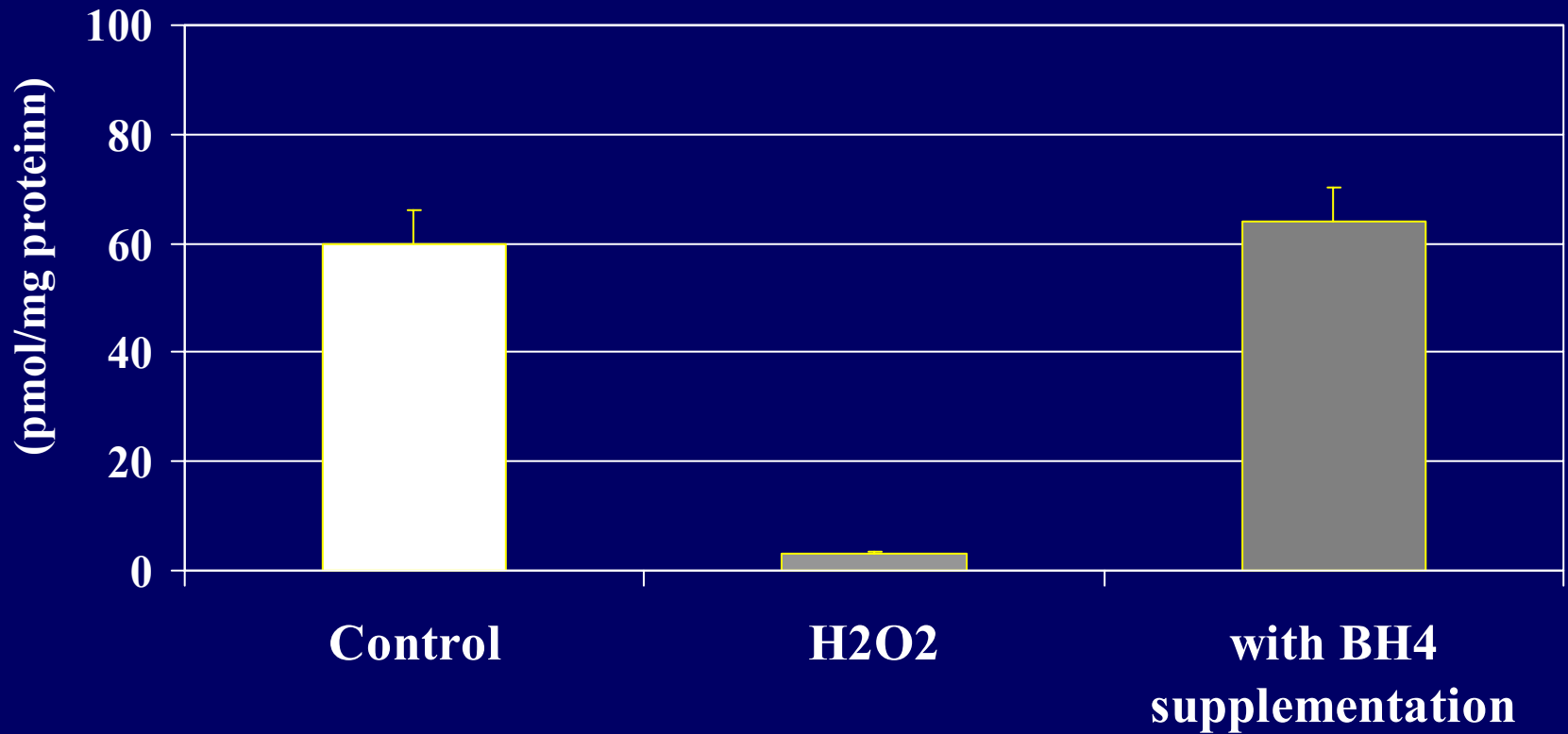
NADH inhibitors

Endothelial cells  
knock out for  
NADH

## ALBUMIN IN OXIDATIVE STRESS

The role ROS in the pathogenesis of endothelial dysfunction in sepsis

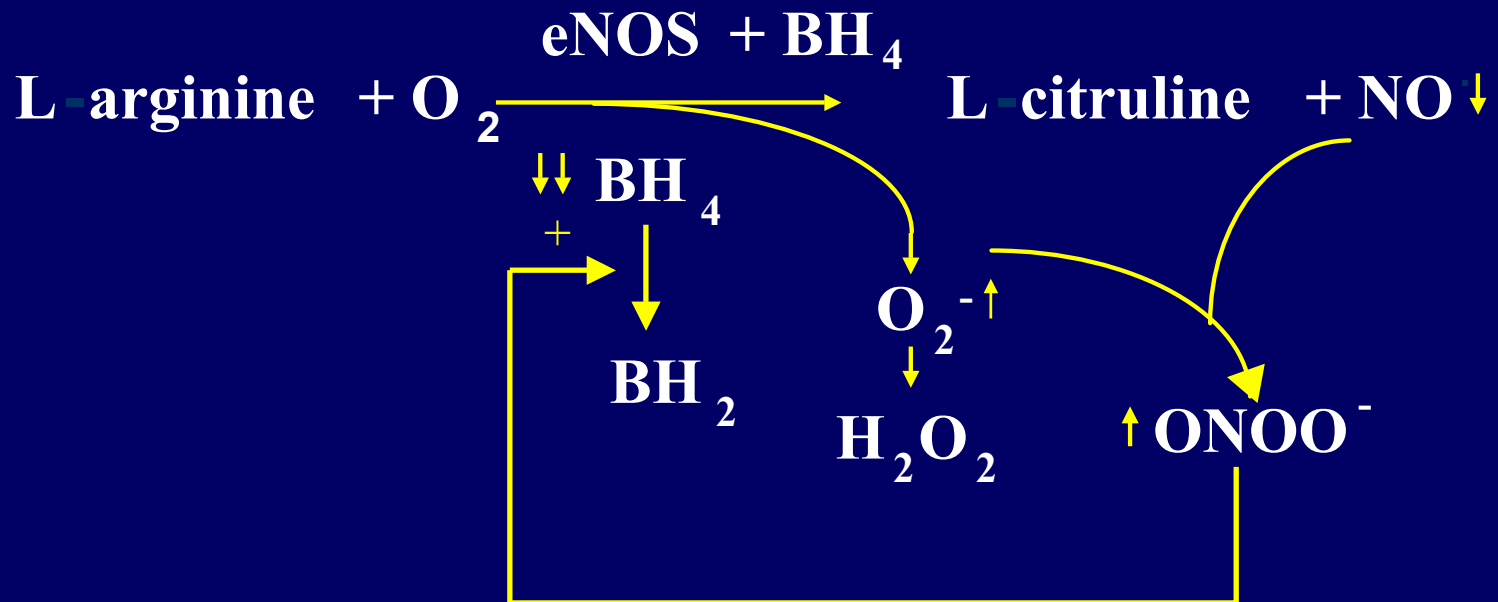
Endothelial availability of BH4



*BM. Boulden, et al. Free Rad. Biol. Med. 2006 ; 41 : 810-817.*

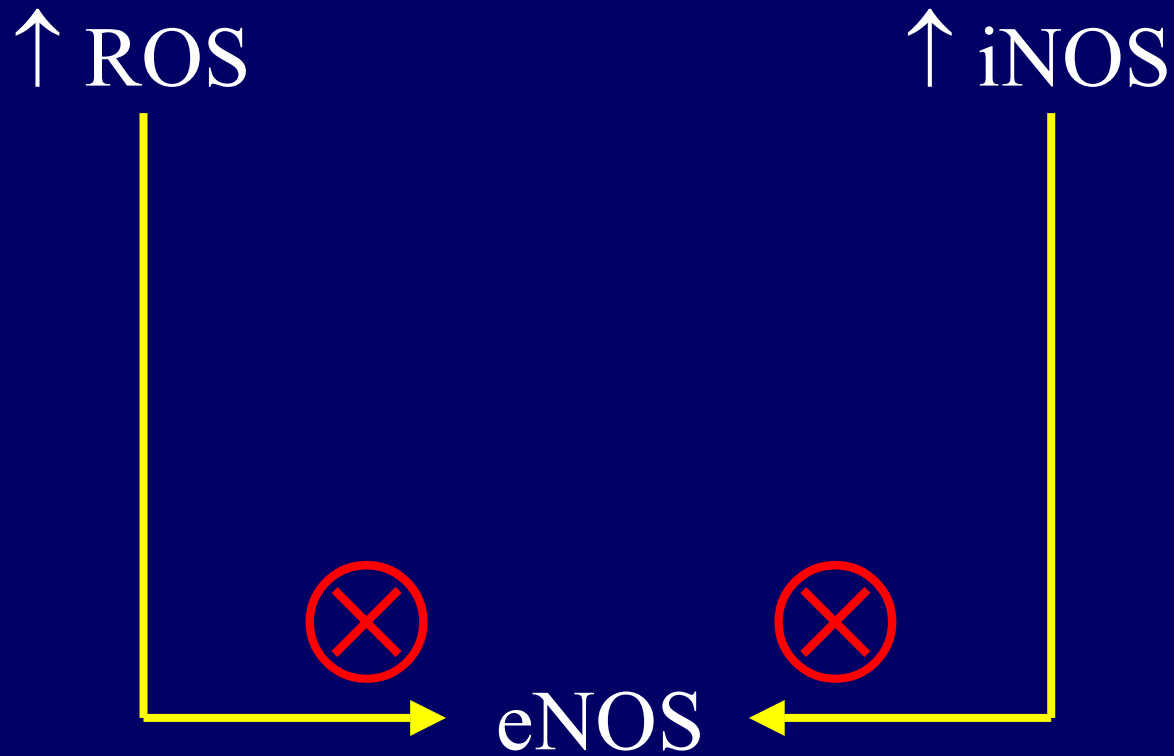
## ALBUMIN AND OXIDATIVE STRESS

**Decreased BH4 reduces NO bioavailability in endothelial cells by causing eNOS uncoupling**



## ALBUMIN AND OXIDATIVE STRESS

Effect of ROS and iNOS on eNOS in endothelial cells





## **ALBUMIN AND OXIDATIVE STRESS**

### **Microvascular dysfunction**

- **Reduced microcirculatory flow rate**
- **Increased flow heterogeneity**
- **Decrease capillary density**

**Organ failure**

**Multiorgan failure  
(MOF)**

**Effects of topical acetylcholine administration in patients with sepsis**

<b>Parameter</b>	<b>Patients with sepsis</b>		<b>Control subjects</b>
	<b>Baseline</b>	<b>Acetylcholine</b>	
<b>Total n° of vessels (n/mm)</b>	<b>4.9 (4.1-5.7)</b>	<b>6.0 (4.7-6.4)</b>	<b>5.4 (5.4-6.3)</b>
<b>Proportion of vessels perfused (%)</b>	<b>83 (77-96)</b>	<b>99 (98-100)</b>	<b>98 (97-99)</b>
<b>Proportion of capillaries perfused (%)</b>	<b>44 (24-60)</b>	<b>94 (77-96)</b>	<b>94 (92-95)</b>
<b>Absent flow (capillaries) (%)</b>	<b>29 (8-44)</b>	<b>1 (0-3)</b>	<b>3 (2-5)</b>
<b>Intermittent flow (capillaries) (%)</b>	<b>24 (19-38)</b>	<b>8 (3-19)</b>	<b>5 (3-6)</b>

## **ALBUMIN AND OXIDATIVE STRESS**

### **Potential effects of albumin in sepsis**

- **Scavenging of ROS and RNS**
- **Reduced ROS and RNS production**
- **Improvement of microvascular dysfunction**
- **Improvement of cardiac dysfunction**

## ALBUMIN AND OXIDATIVE STRESS

### Mean plasma levels of nitric oxide and S-nitrosothiols in humans

	Mean level, $\mu\text{M}$
Free nitric oxide	$0.0034 \pm 0.00058$
S-nitrosothiol	$7.19 \pm 5.73$
S-nitrosoprotein	$7.92 \pm 5.45$

*J. S. Stamler, et al. Proc. Natl. Acad. Sci 1992 ; 89 : 7674-7677.*

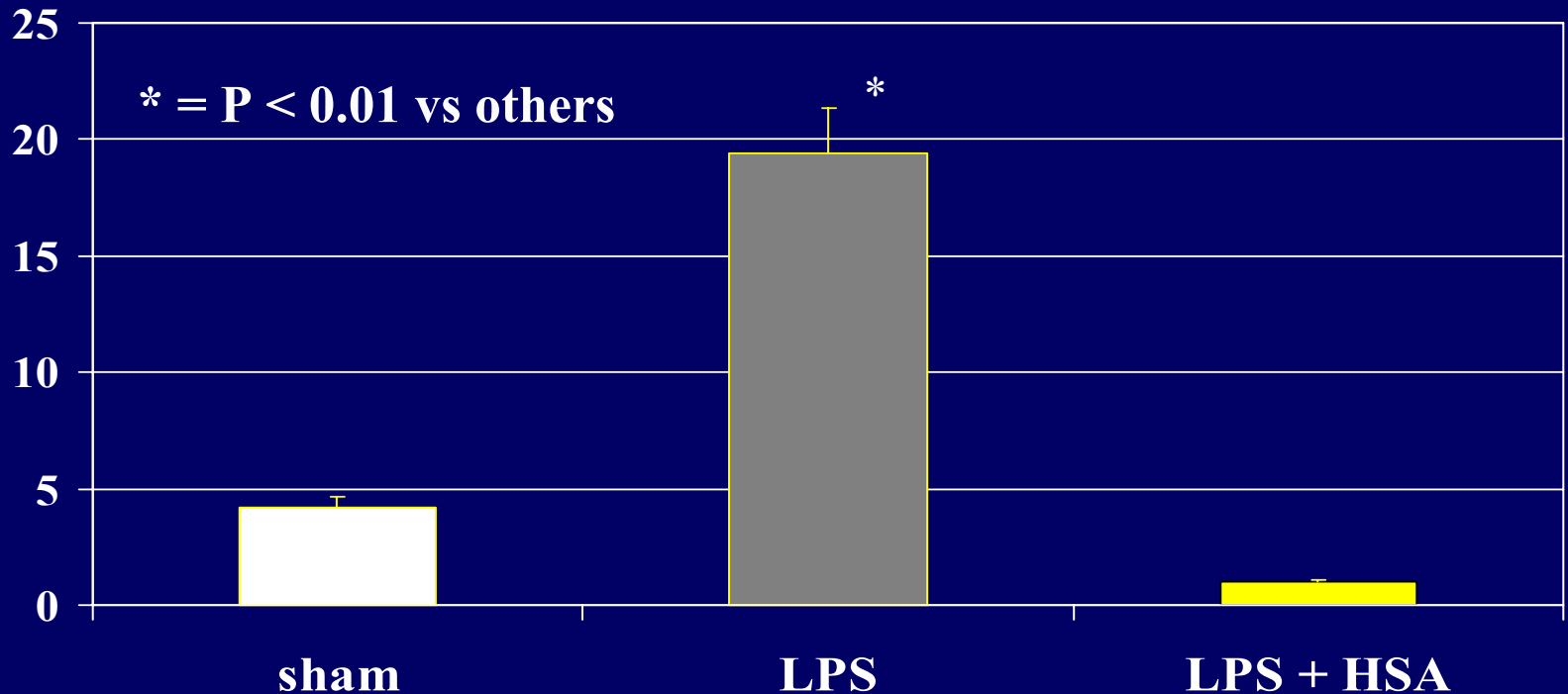
## **ALBUMIN AND OXIDATIVE STRESS**

### **Potential effects of albumin in sepsis**

- **Scavenging of ROS and RNS**
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Staining of the p65 subunit of NF- $\kappa$ B in aorta in septic mice

(arbitrary units)



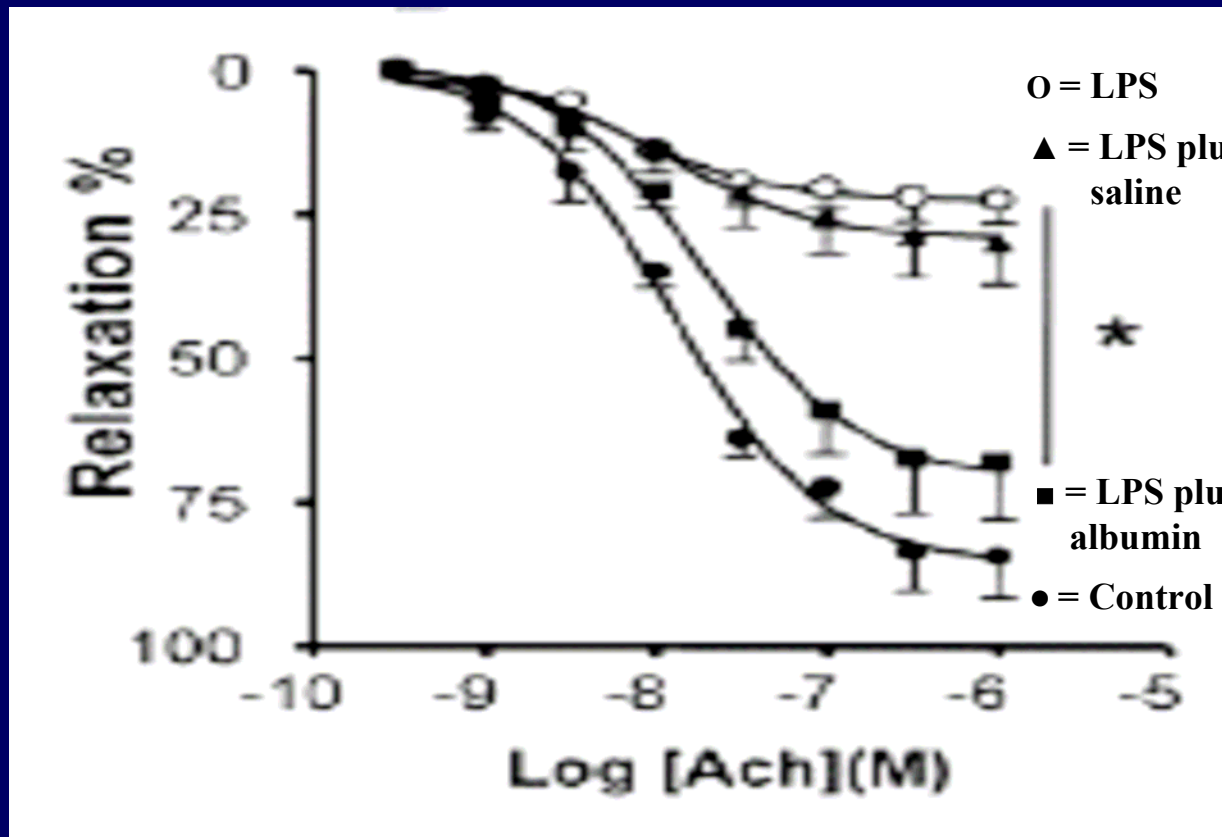
*F. Meziani, et al. Am. J. Pathol. 2007 ; 171 : 1753-1761.*

## **ALBUMIN AND OXIDATIVE STRESS**

### **Potential effects of albumin in sepsis**

- **Reduced ROS and RNS production and/or availability**
- **Improvement of microvascular dysfunction**
- **Improvement of cardiac dysfunction**

## Response to acetylcholine (Ach) in mesenteric arteriola of septic mice



*F. Meziani, et al. Am. J. Pathol. 2007 ; 171 : 1753-1761.*

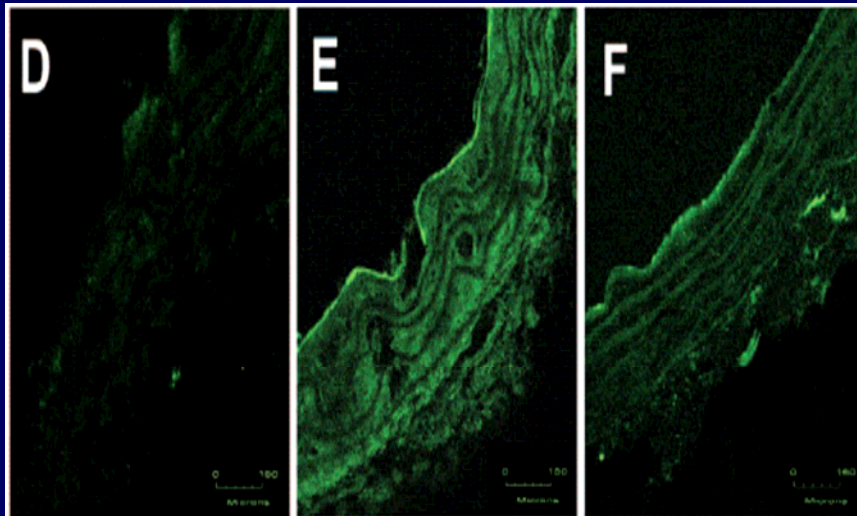


## ALBUMIN AND OXIDATIVE STRESS

# Effects of albumin on expression of inducible NOS (iNOS) in aorta of septic mice

Immunohistochemical staining for iNOS

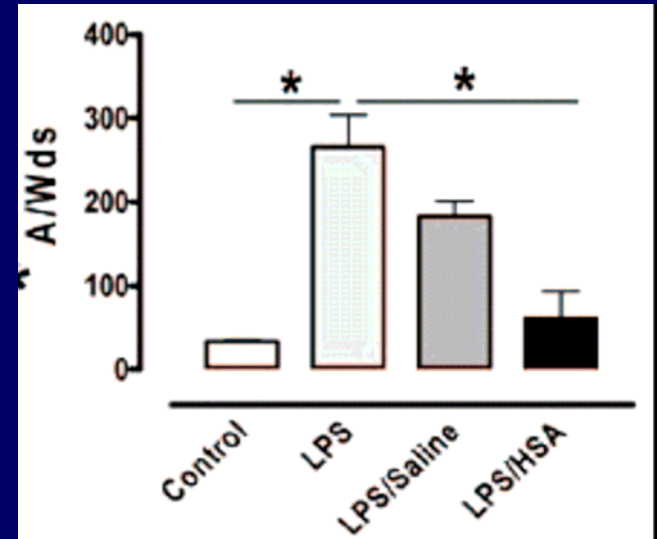
Quantification for NO synthesis



Control

LPS

Albumin +  
LPS



## **ALBUMIN AND OXIDATIVE STRESS**

### **Potential effects of albumin in sepsis**

- **Reduced ROS and RNS production and/or availability**
- **Improvement of microvascular dysfunction**
- **Improvement of cardiac dysfunction**

## ALBUMIN AND OXIDATIVE STRESS

### **Mechanisms of sepsis-induced cardiac dysfunction**

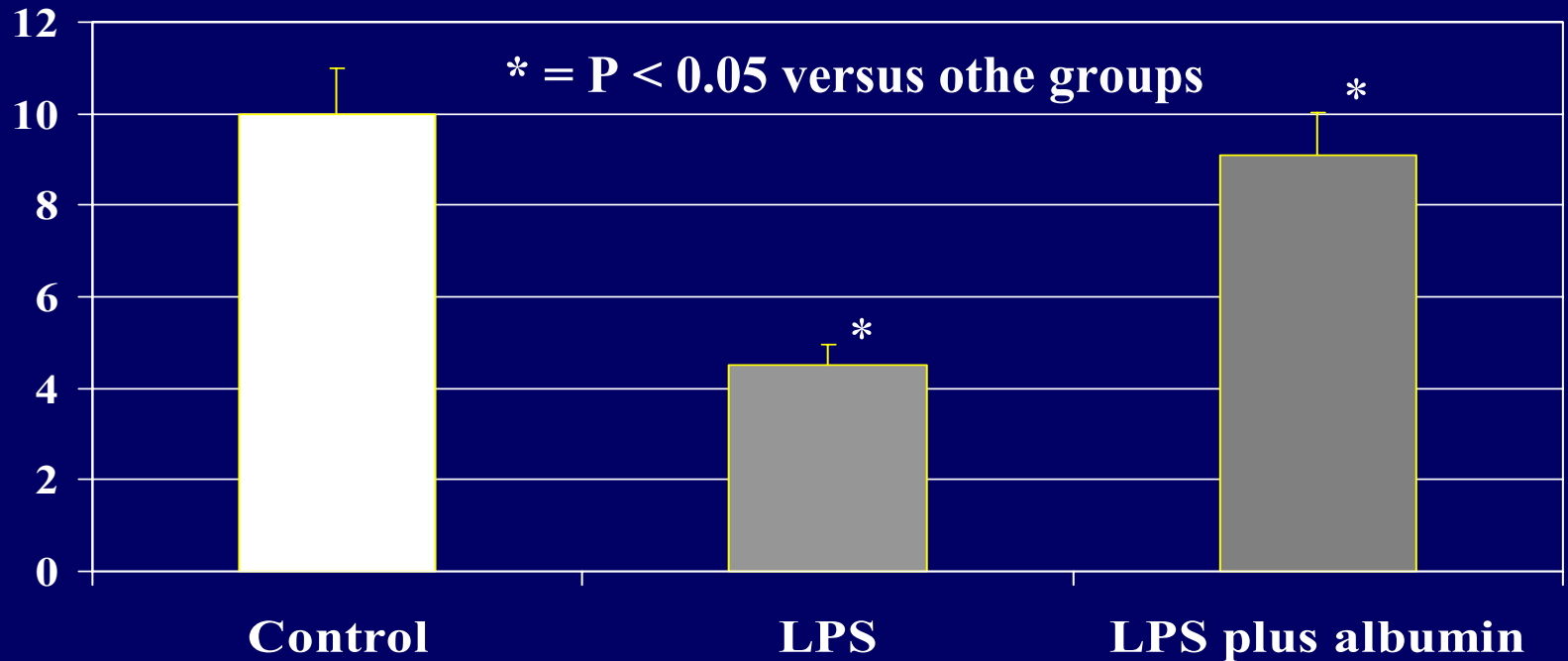
- **Microvascular dysfunction**
- **Depressed postreceptor signaling pathway**
- **Impaired calcium liberation from the sarcoplasmic reticulum through over-expression of S100A8 and S100A9**
- **Impaired electromechanical coupling at the myofibrillar level**
- **Cell death**

*A. Rudiger et al. Crit. Care Med. 2007 ; 35 : 1599-1608.*

## ALBUMIN IN OXIDATIVE STRESS

# Effect of albumin resuscitation on myocardic oxygenation in endoxemic rats

(Tissue PO<sub>2</sub> mm Hg)



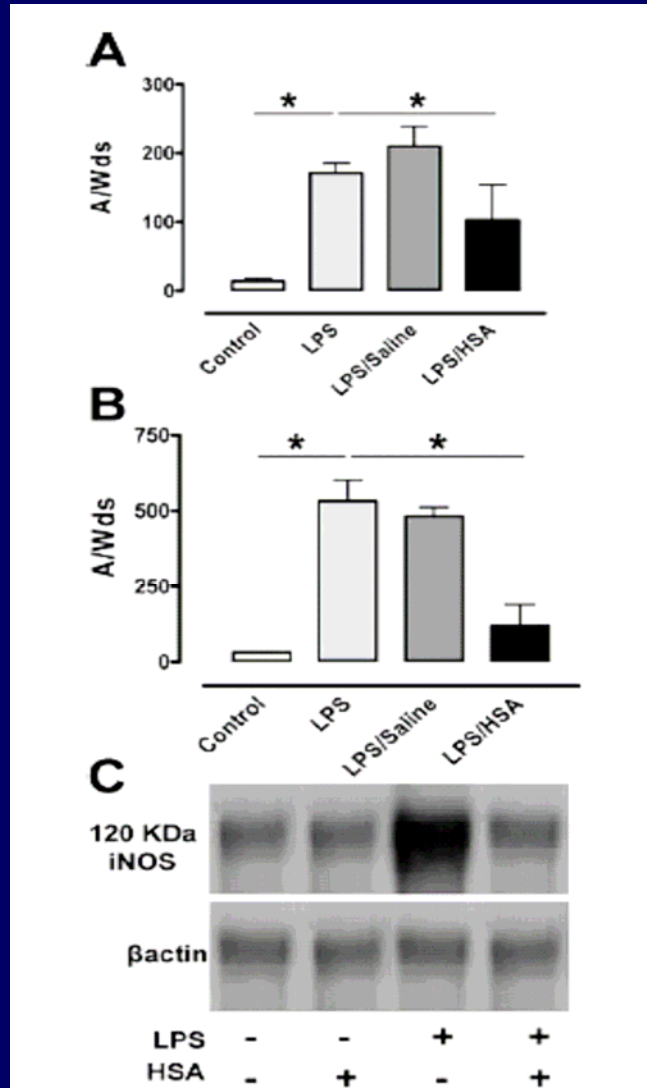
*C. Tokunaga, et al. J. Exp. Crit. Care Med. 2007 ; 35 : 1341-1347.*

## **Mechanisms of sepsis-induced cardiac dysfunction**

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# Effects of albumin on expression of inducible NOS (iNOS) in aorta of septic mice



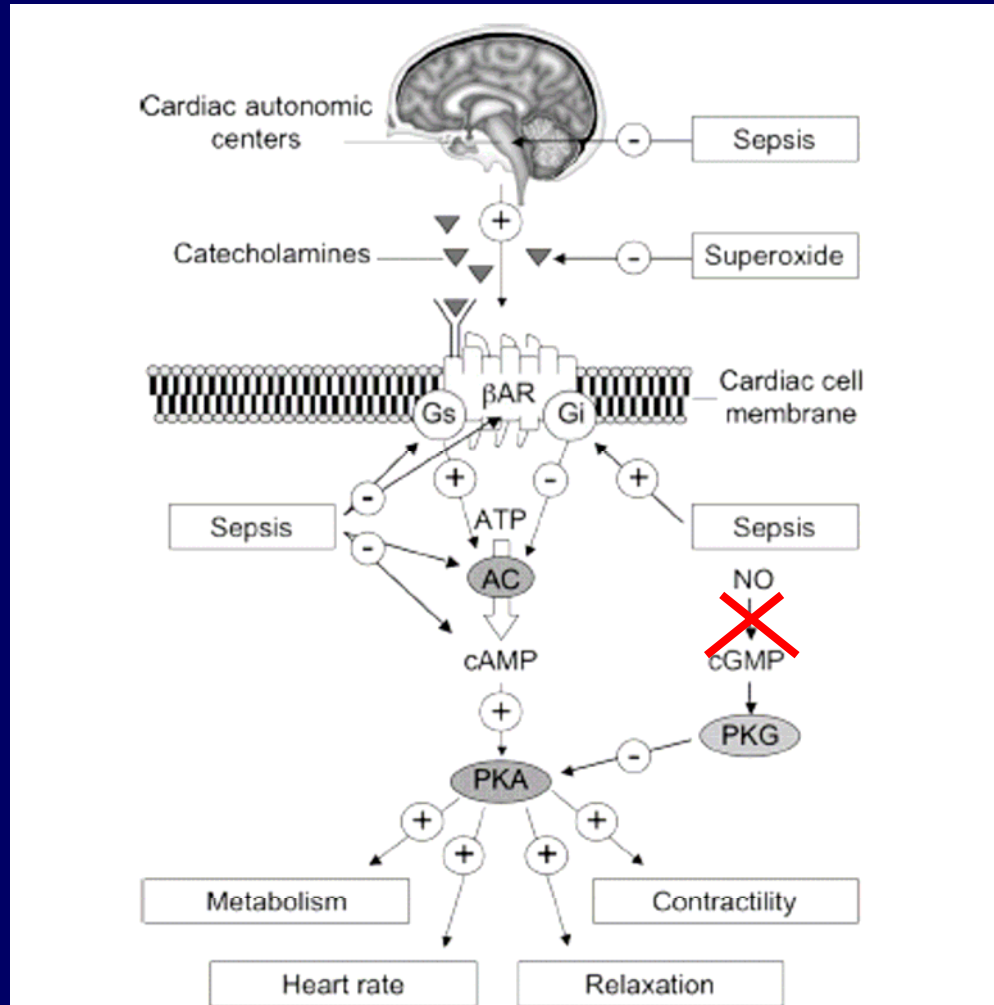
Albumin reduces NO overproduction in heart

Albumin reduces NO overproduction in lung

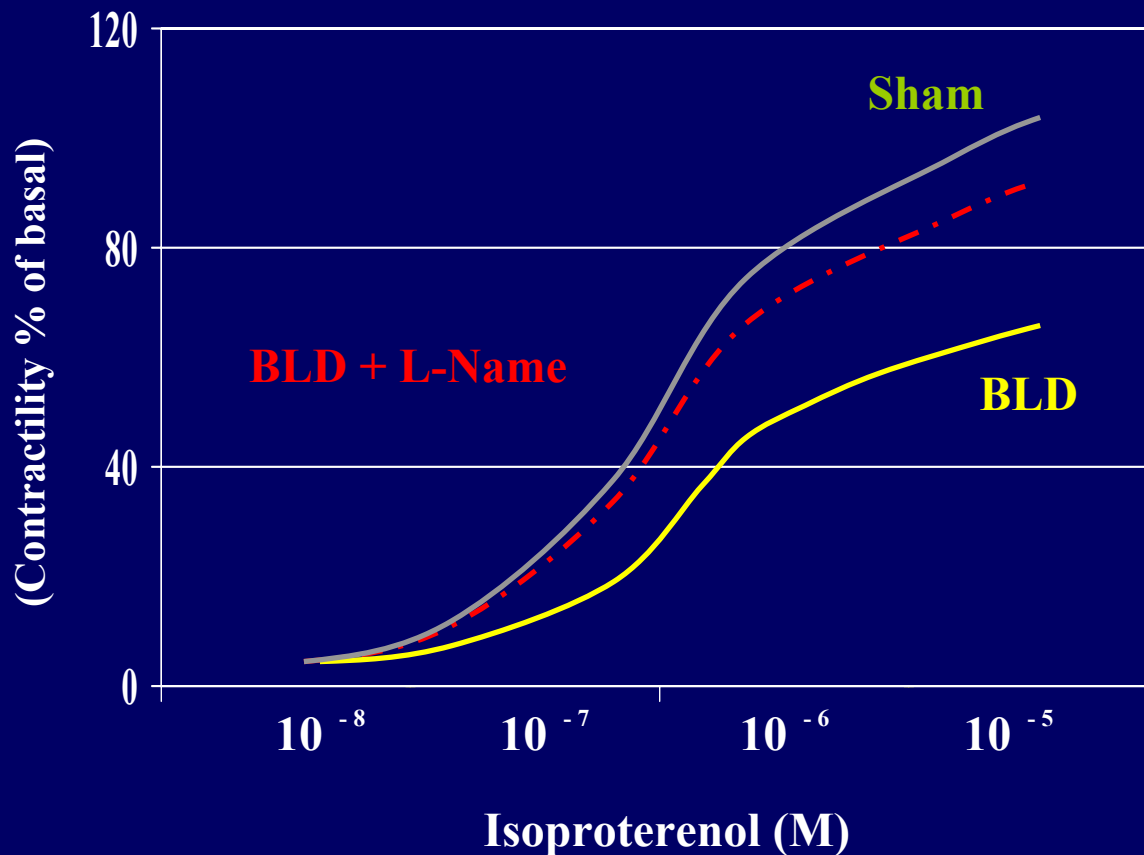
Albumin reduces iNos expression in heart

## ALBUMIN AND OXIDATIVE STRESS

# Albumin $\beta$ -adrenergic signaling in cardiac tissue



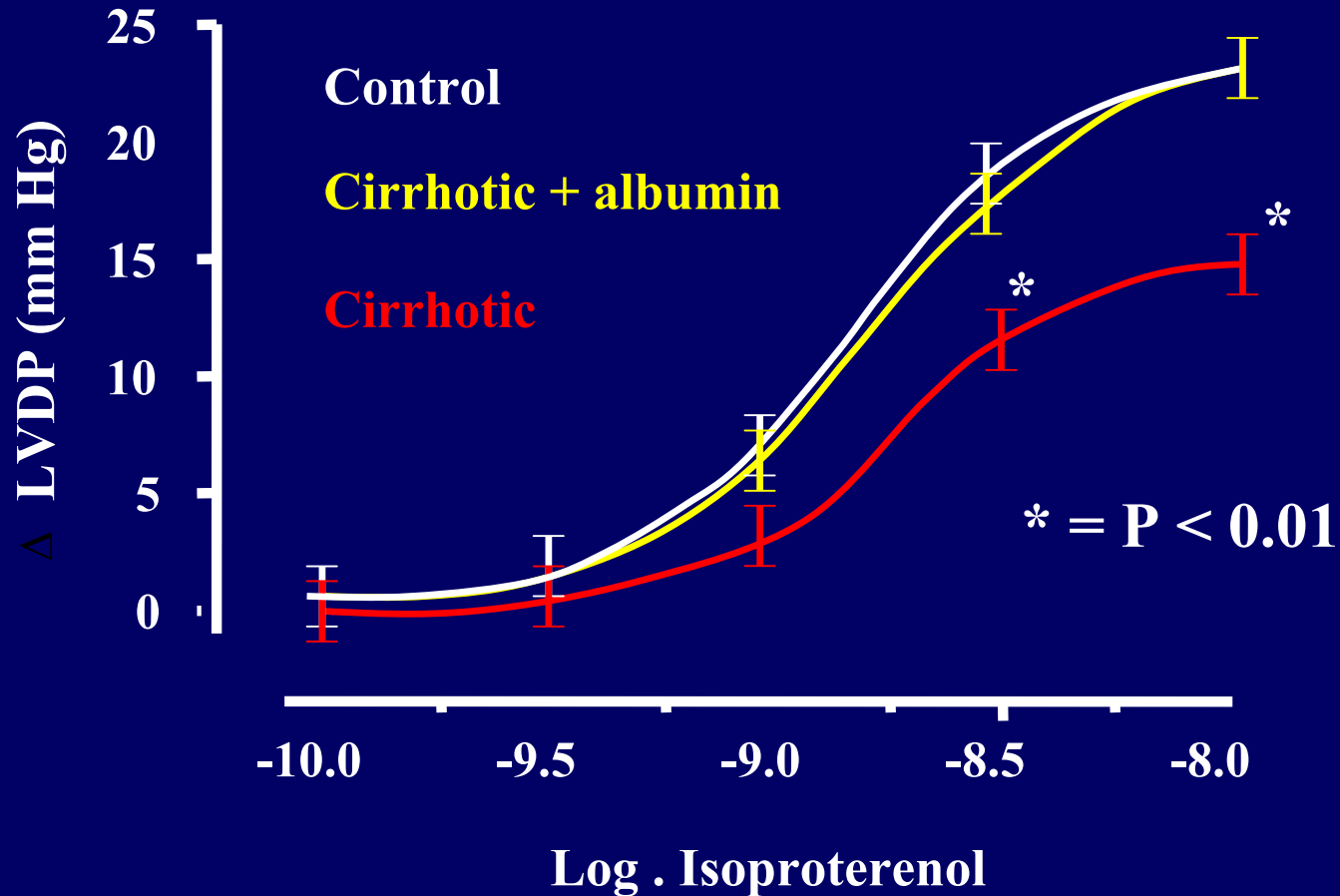
**Dose-responses to isoproterenol in isolated left ventricular papillary muscles from bile duct ligated- (BLD) rats and sham-operated rats**





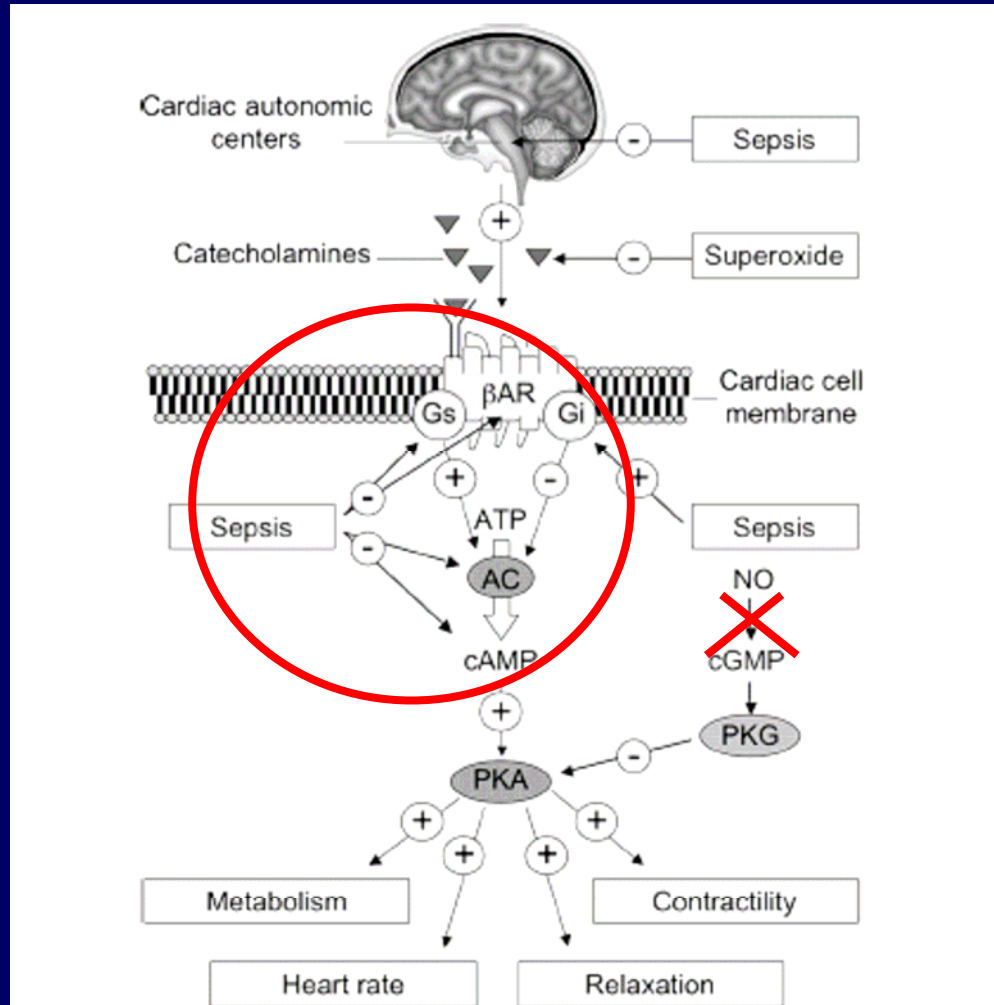
## ALBUMIN AND OXIDATIVE STRESS

### Effects of albumin on cardiac contractility in cirrhotic rats

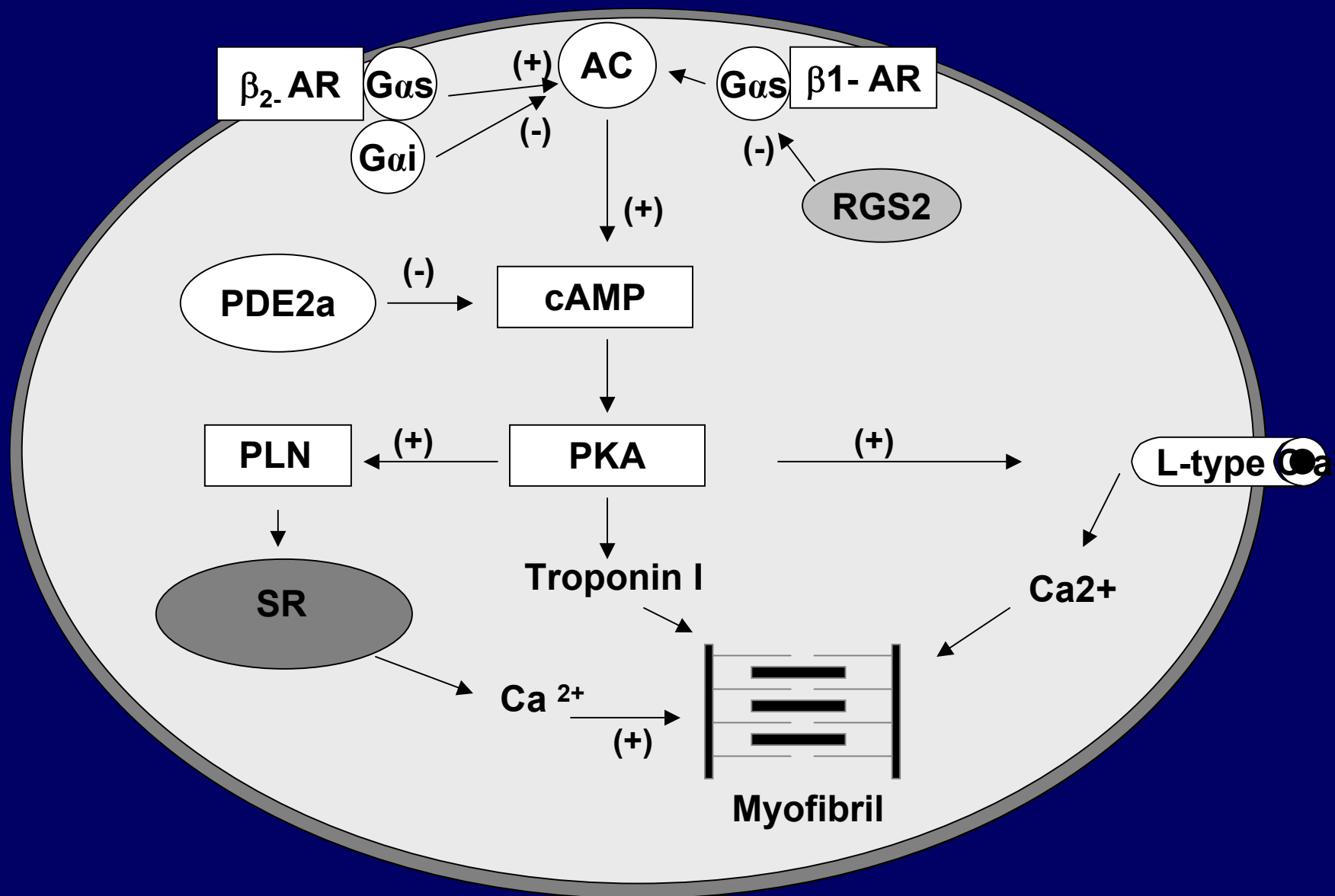


# ALBUMIN AND OXIDATIVE STRESS

## Albumin $\beta$ -adrenergic signaling in cardiac tissue



$\beta$ -adrenergic signaling in cardiac tissue



## ALBUMIN AND OXIDATIVE STRESS

### Effects of albumin on $\beta$ -adrenergic signaling in cardiac tissue

