

Application of the Neuro-Immune-Endocrine System Model to Evaluate the Role of Inflammation in Systemic Toxicity

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Assessment**

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Food and Drug Administration

LPS Potentiated Toxicity

- Benzyl alcohol (Cebula et al., 1984),
- Aflatoxin B1, (Barton et al., 2001),
- Aroclor 1248 (Brown et al., 1996),
- Mercury (Rubmeiha et al., 2000),
- Lead (Dyatlov et al., 1998),
- Kainic acid (Lee et al., 2000),
- Estrogen (Ikejima et al., 1998),
- Morphine (Roy et al., 1999), and
- Shiga toxin (Yuhas et al., 1995).
- Deoxynivalenol (Pestka et al., multiple)
- Ranitidine (Maddox et al., 2005)

Neuro-Immune-Endocrine System Model

- Psychoneuroimmunology (Friedman et al.,1969)
- Psychoendoneuroimmunology (Ray, 2004)
- Neuroimmunomodulation (Brook et al.,1982)
- Neuroendocrinimmunology
- Neuroimmunotoxicology (El-Fawal et al,1999; Lawrence and Kim, 2000)
- Integrative Biology and Medicine-(Besedovsky and del Ray, 2007)
- Future of Toxicology (Liebler, 2006)

TOP 10 CAUSES OF DEATH IN AMERICA

1860

- 1. Consumption**
- 2. Unknown**
- 3. Fever**
- 4. Pneumonia**
- 5. Scarlet Fever**
- 6. Violence**
- 7. Croup**
- 8. Dropsy**
- 9. Old age**
- 10. Dysentery**

1900

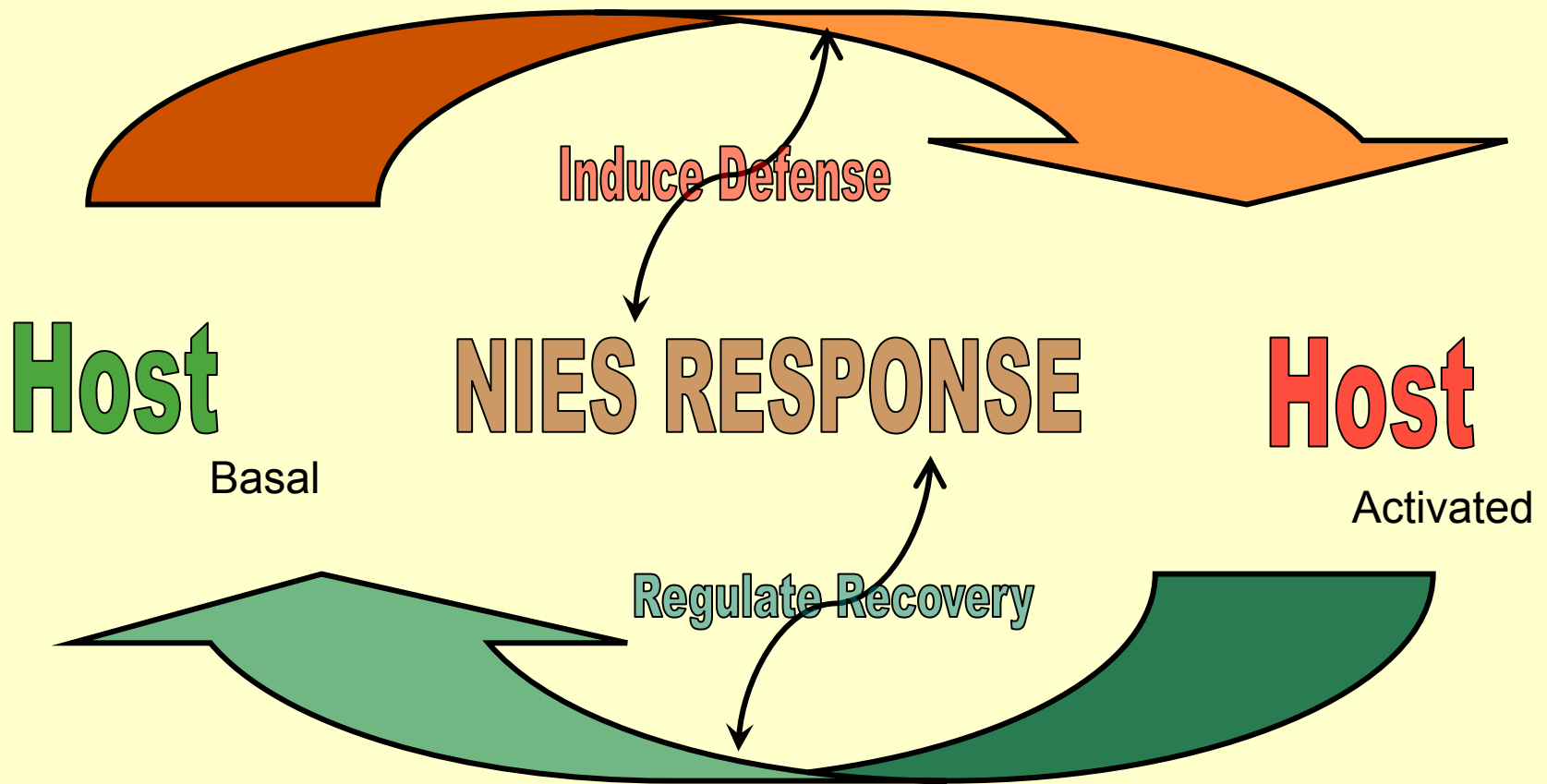
- Pneumonia, flu**
- Tuberculosis**
- Diarrhea, enteritis**
- Heart Disease**
- Stroke**
- Acute Kidney Disease**
- Accidents**
- Cancer**
- Senility**
- Diphtheria**

2001

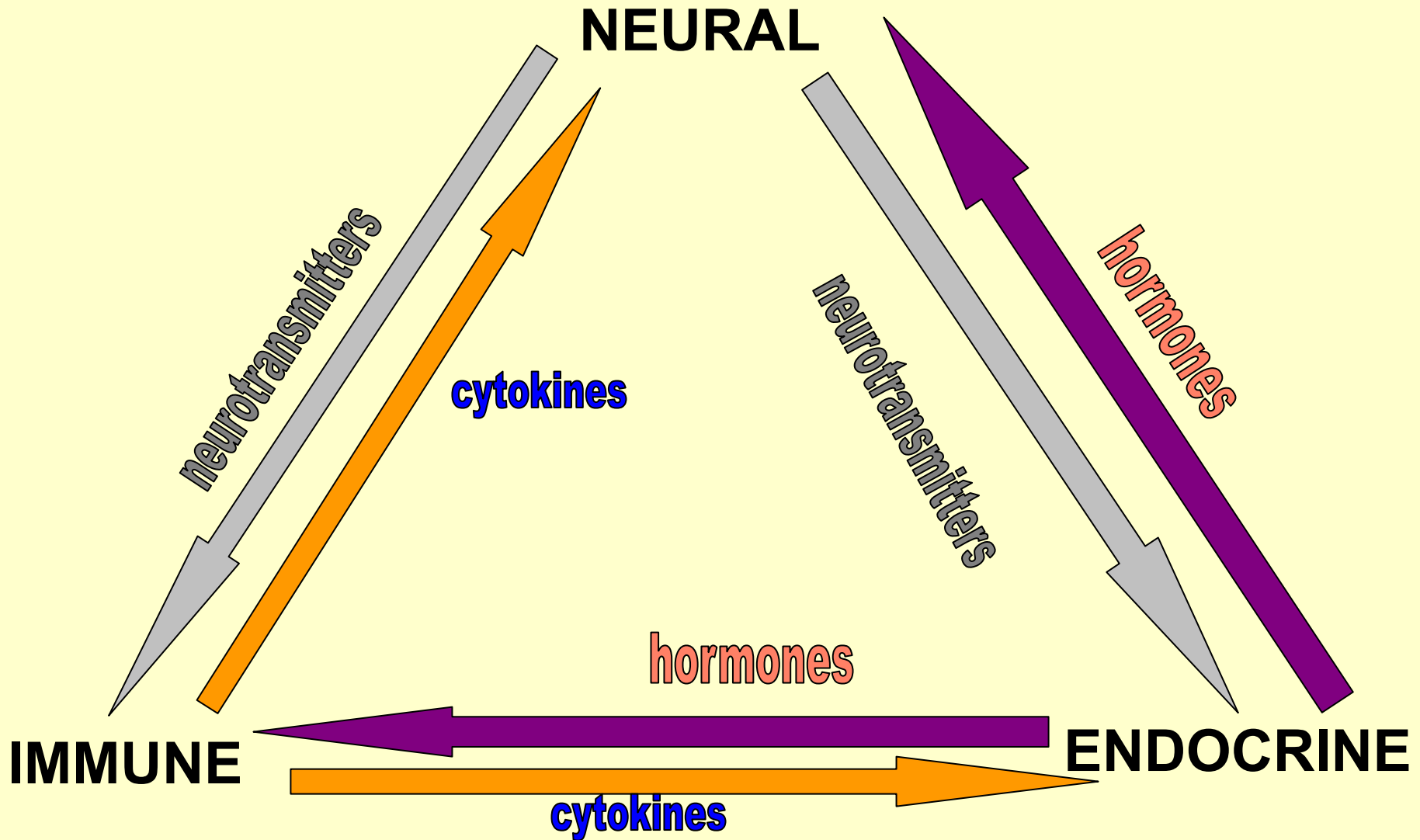
- Heart Disease**
- Cancer**
- Stroke**
- Pulmonary Disease**
- Accidents**
- Diabetes**
- Pneumonia**
- Alzheimer's Disease**
- Kidney Disease**
- Suicide**

**National Center for Health Statistics. In Ray (2004),
Psychoendoneuroimmunology, ANYAS, 1032, 35**

NIES: Integrated Regulatory Response



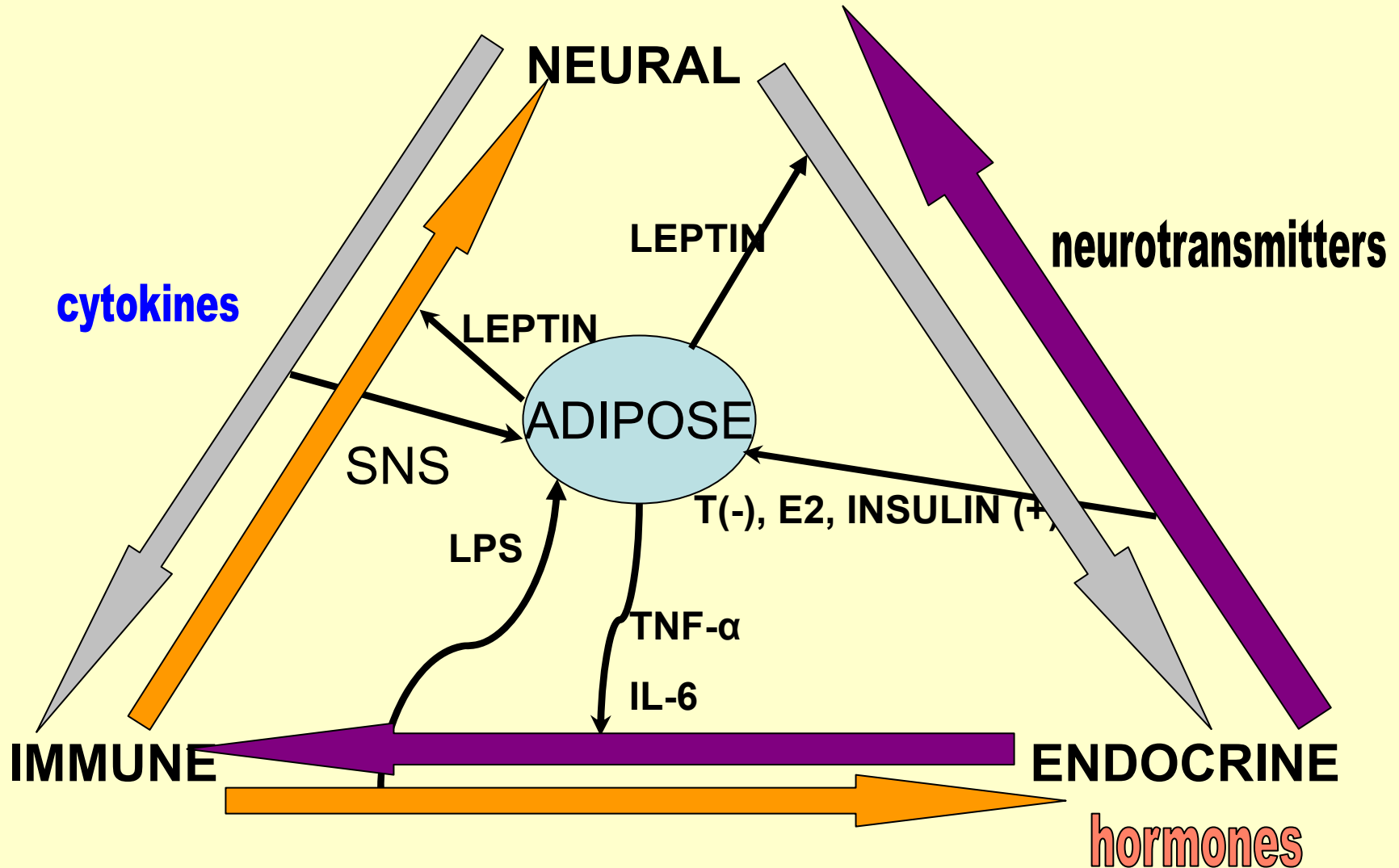
Neuro-Immune-Endocrine System

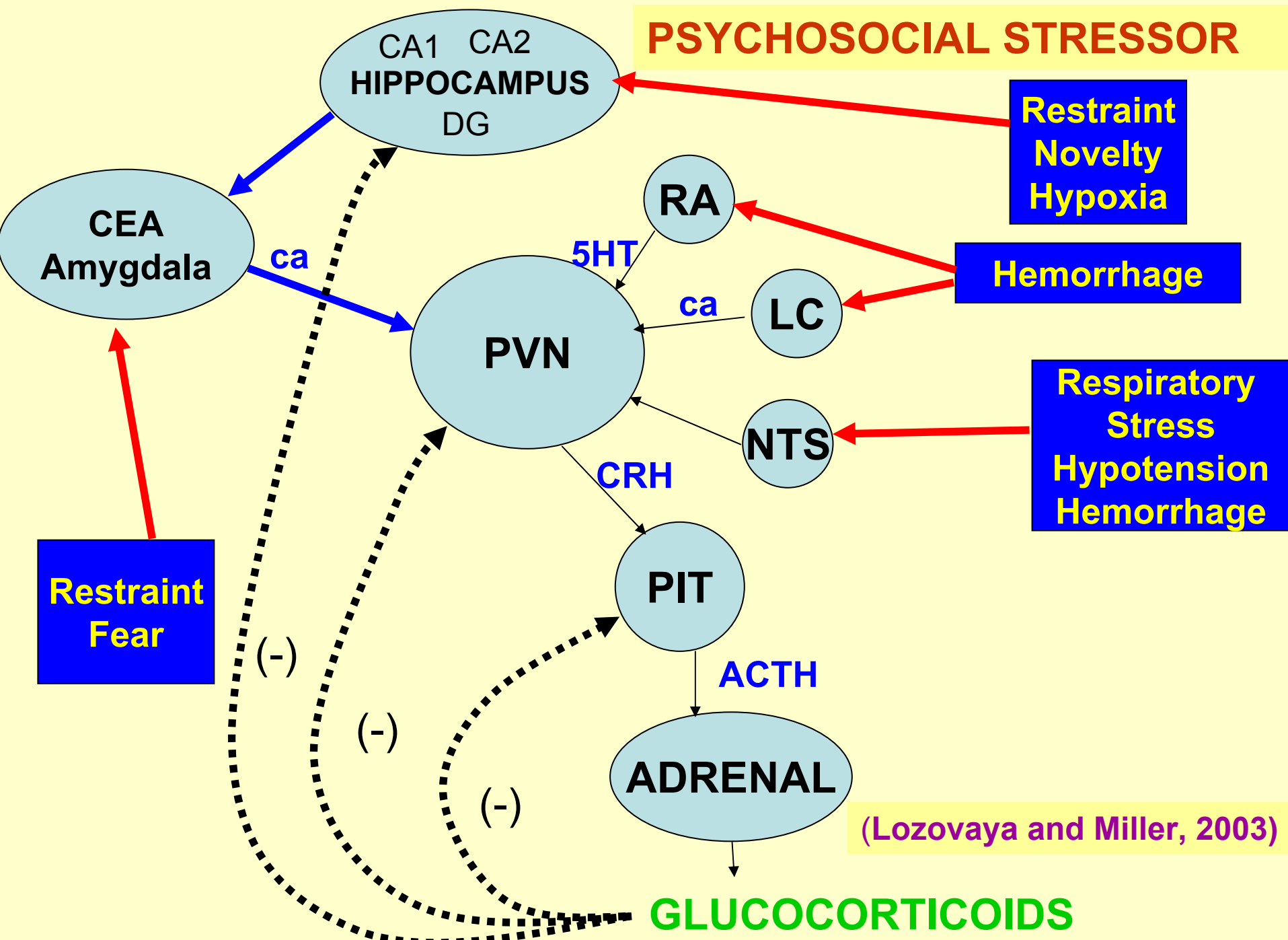


Stressors

- **Chemical**
 - Toxic contaminants, drugs (illicit and therapeutic), alcohol, smoking, ischemia, diet.
- **Biological**
 - Foodborne pathogens, genetic, ischemic, parasites, infectious stimuli, sleep apnea, eating disorders.
- **Physical**
 - Trauma (esp. TBI), surgery, overuse activities.
- **Psychosocial**
 - Anxiety, family/work problems, social issues, restraint stress.

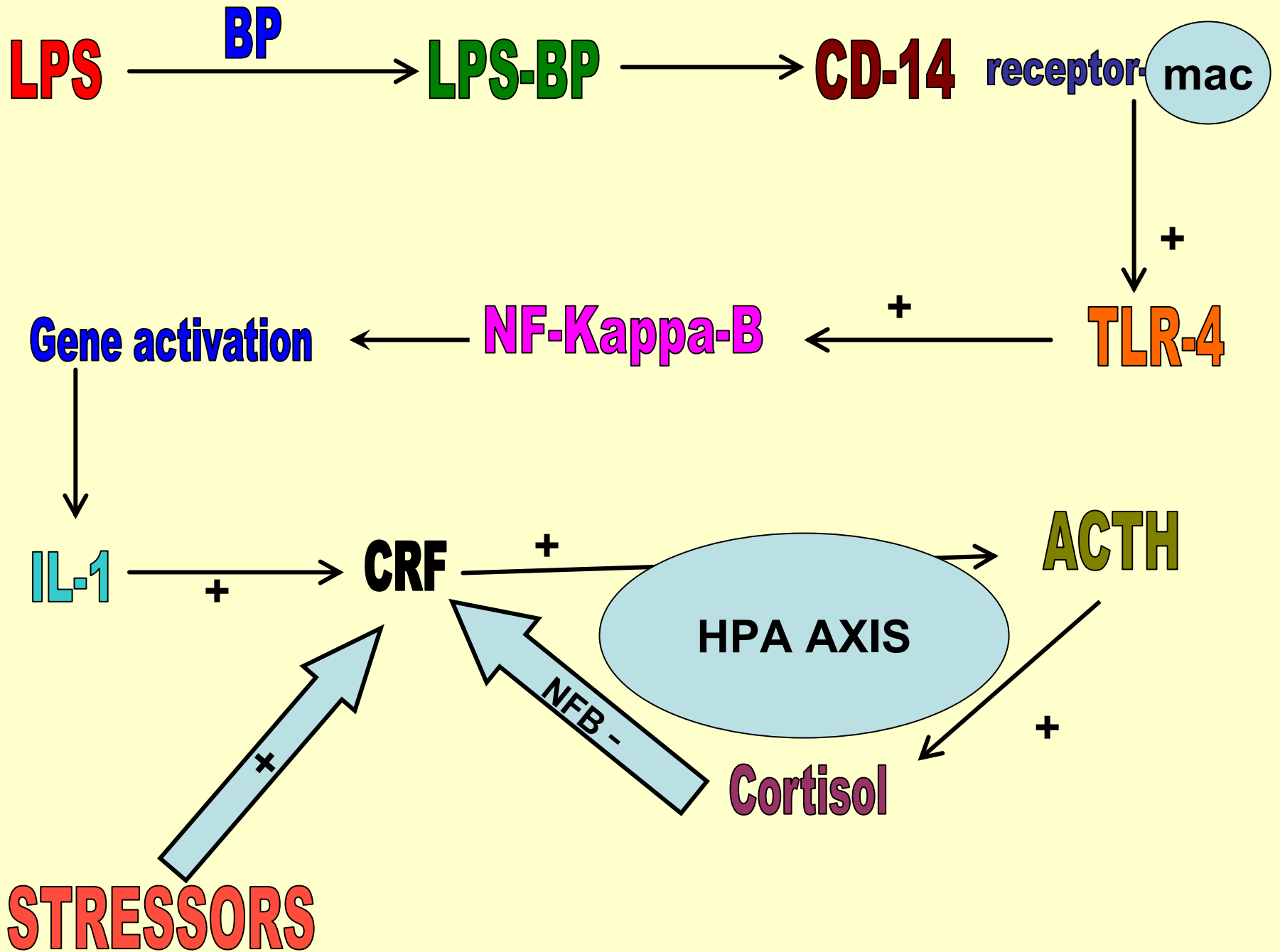
Role of Adipose Tissue in the Neuro-immune-endocrine System





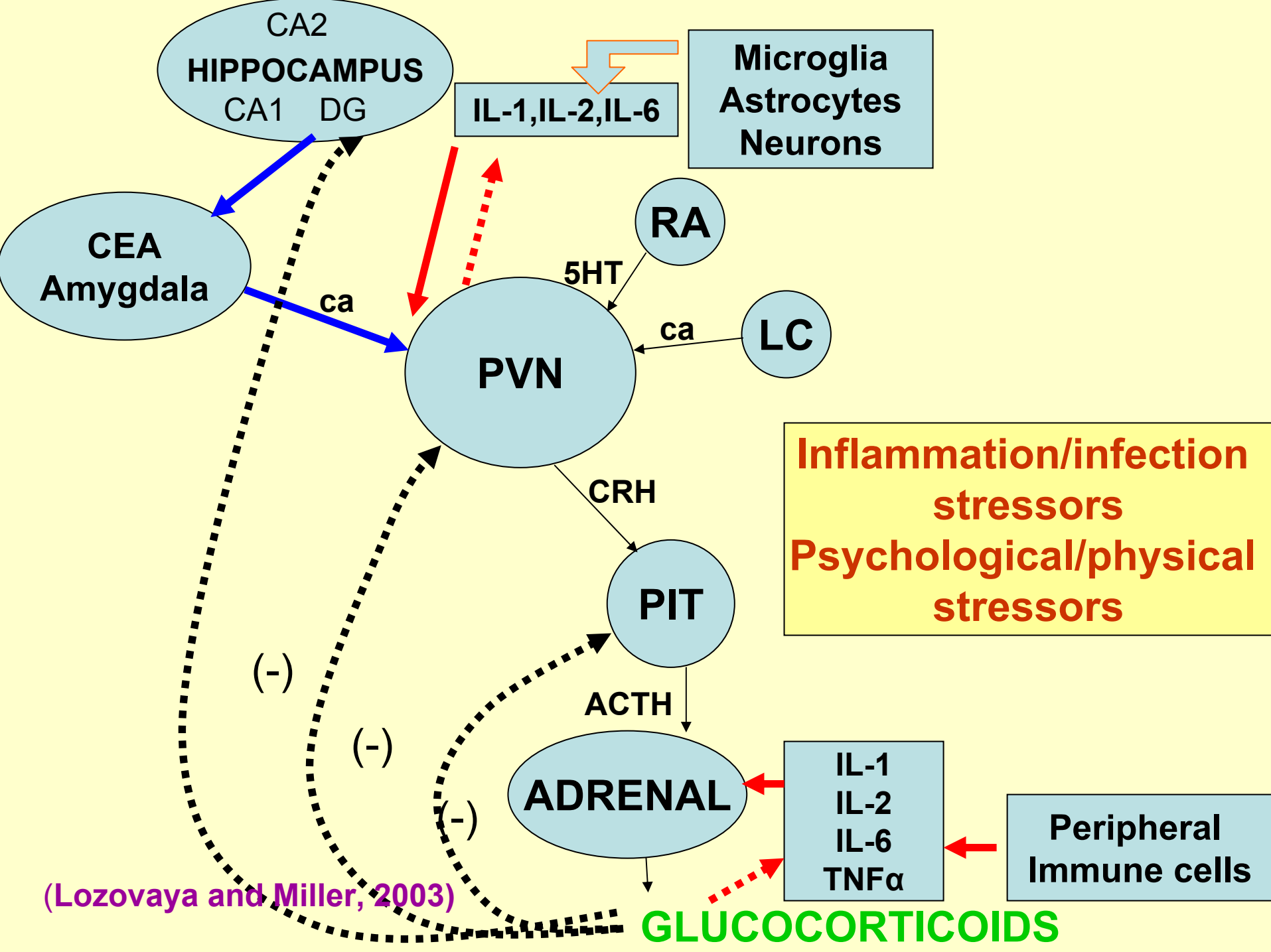
DISEASES AND PATHOLOGIES ASSOCIATED WITH DYSFUNCTION OF THE LIMBIC-HPA AXIS

- Clinical depression, anxiety disorders, other mental illnesses, hypertension, T2DM, infections, autoimmune disorders, obesity, Alzheimer's disease, AIDS dementia, anorexia nervosa, rheumatoid arthritis (Lozovaya and Miller, 2003)



ACUTE PHASE RESPONSE/ SICKNESS SYNDROME

- Liver, primarily, changes production profile: 30 acute phase reactants, increase or decrease within 90 min.
- Lethargy, fever, anorexia, adipsia, decreased libido, decreased social activity.



EXAMPLES OF CYTOKINE TOXICITY

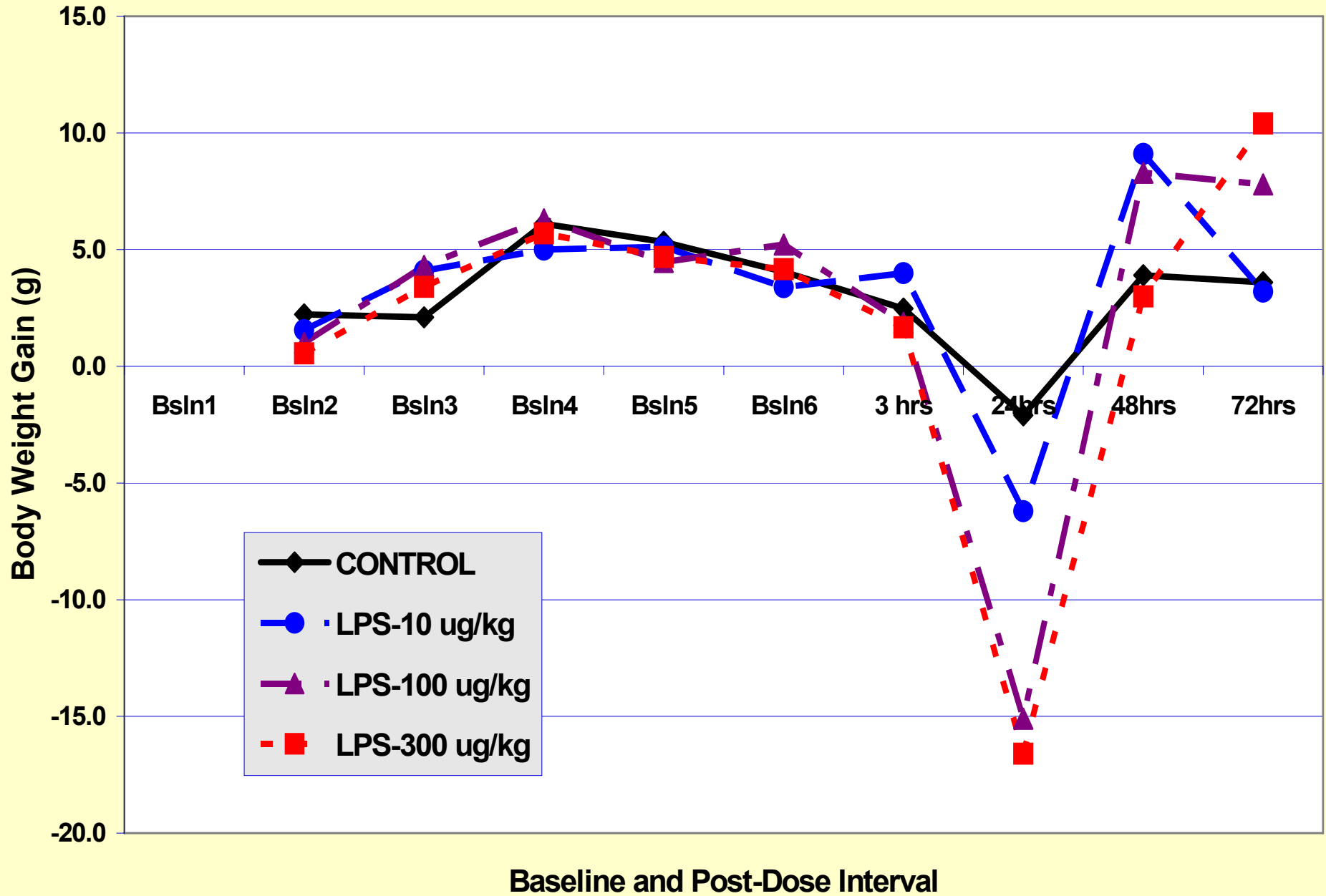
- **IL-1**: mediates many forms of **neurotoxicity** including enhancement of excitotoxic neuron loss (Patel et al.-2006).
- **Increased cytokines** in CNS from activated microglia from high cholesterol diet in mice produced elevated β -amyloid and decline in working memory (Bhat, 2006).
- Common Picornaviruses infect more than 1 billion people/yr. Infection of mice with a picornavirus produces **neuroinflammation**, memory deficits, and severe damage to the hippocampus. (Howe et al., 2006)
- **Prenatal** exposure to **cytokines** produced increased fat depots in both genders of rats as adults. Females were hyperandrogenic. (Dahhgren et al., 2001).
- **Transgenic mice** (Campbell et al., 2002)

POTENTIAL SYNERGY

- Shown multiple pathways to increase production of proinflammatory mediators and classic stress hormones.
- What happens when you activate 2 or more of these pathways simultaneously?
- Apply a biological immune/inflammatory stressor and a psychosocial stressor
- Biological immune/inflammatory stressor (LPS) and a xenobiotic chemical stressor with many similar effects (deoxynivalenol).

**LIPOPOLYSACCHARIDE
and
Deoxynivalenol (DON)
in
Young Adult Male SD Rats**

Effect of LPS on Body Weight Gain



Effect of DON on Body Weight Gain

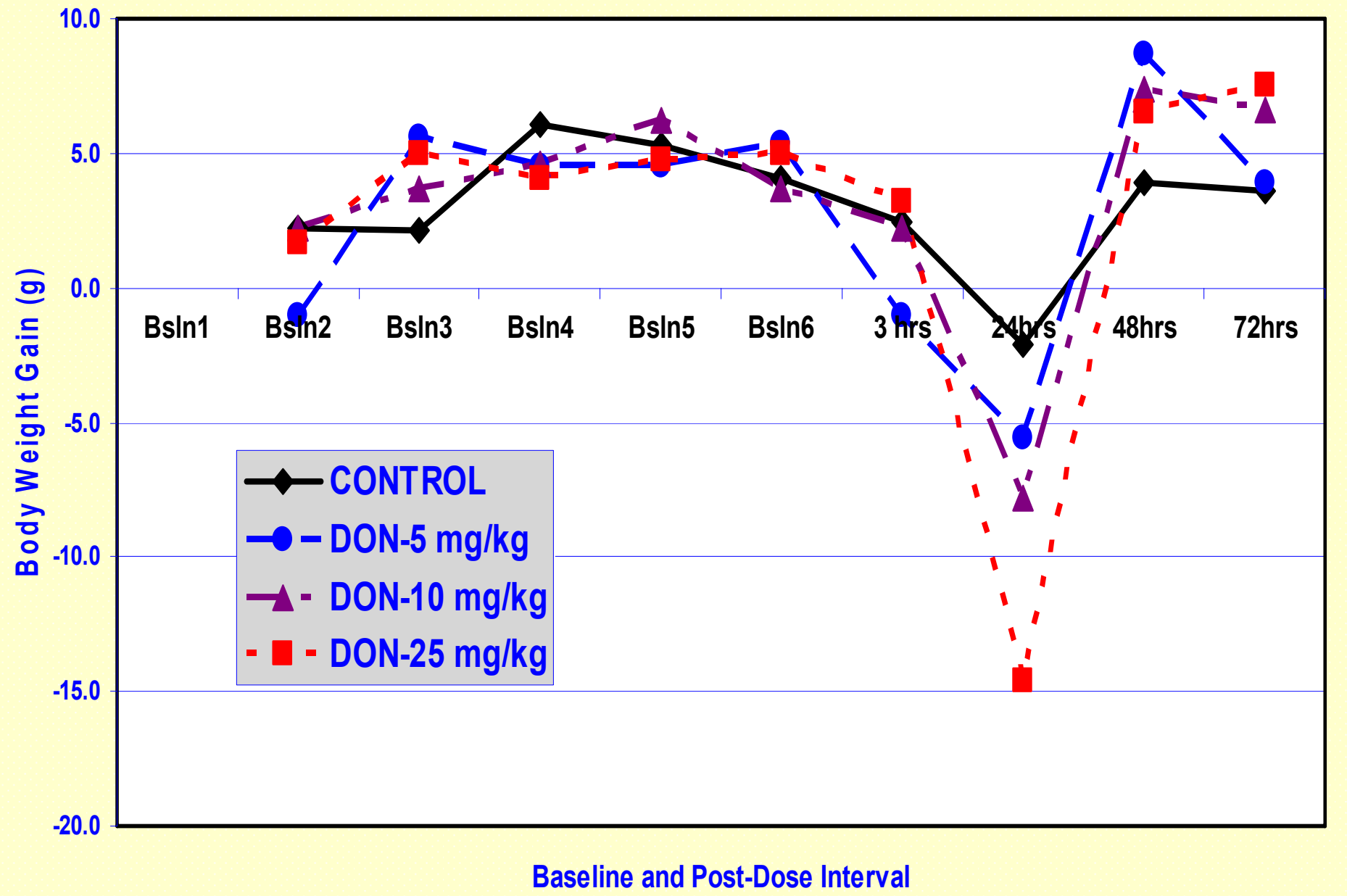


FIGURE # 3

Core Temperature Response to LPS or DON

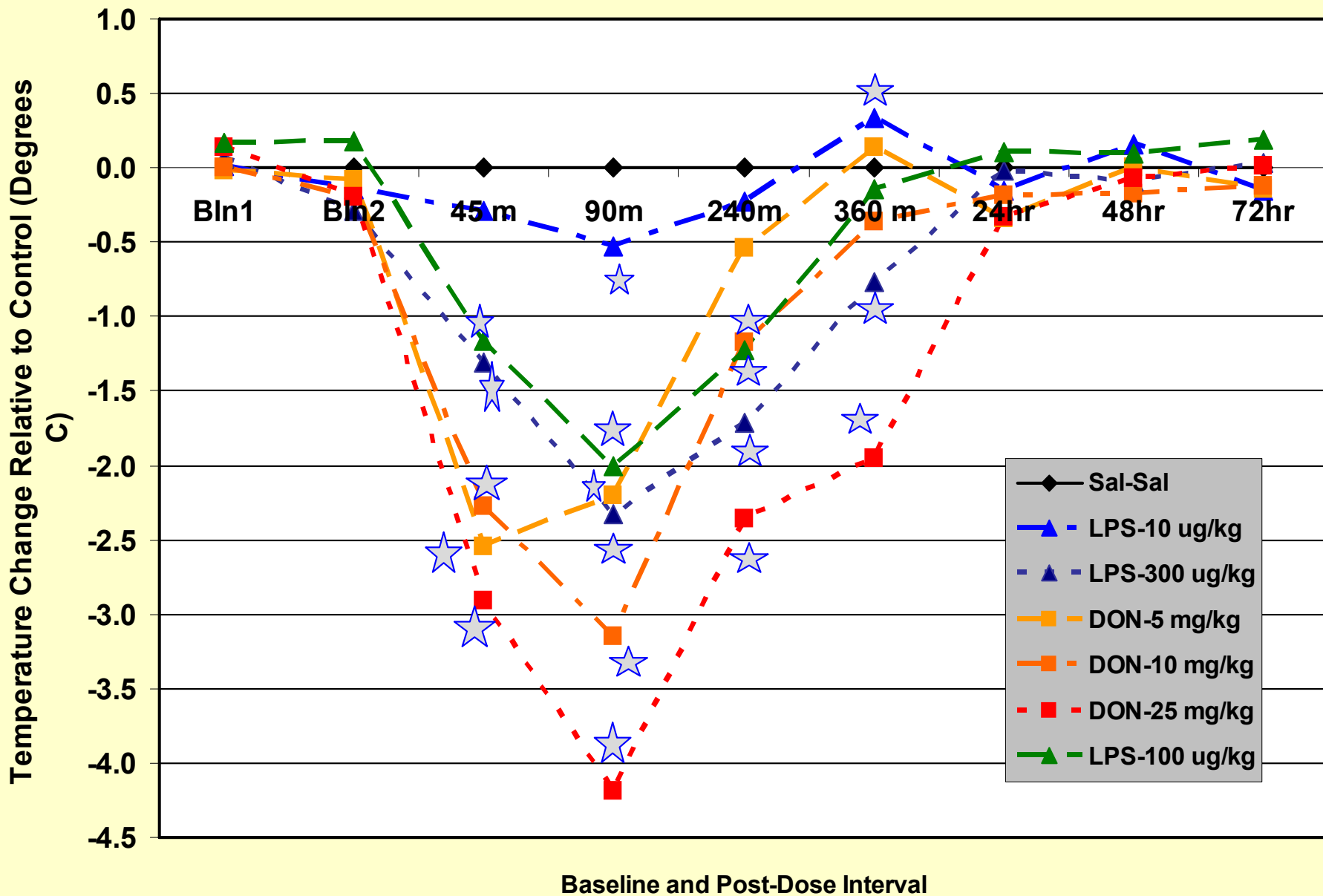


FIGURE # 12

**Exp 2: Food Intake
No Interdose Interval**

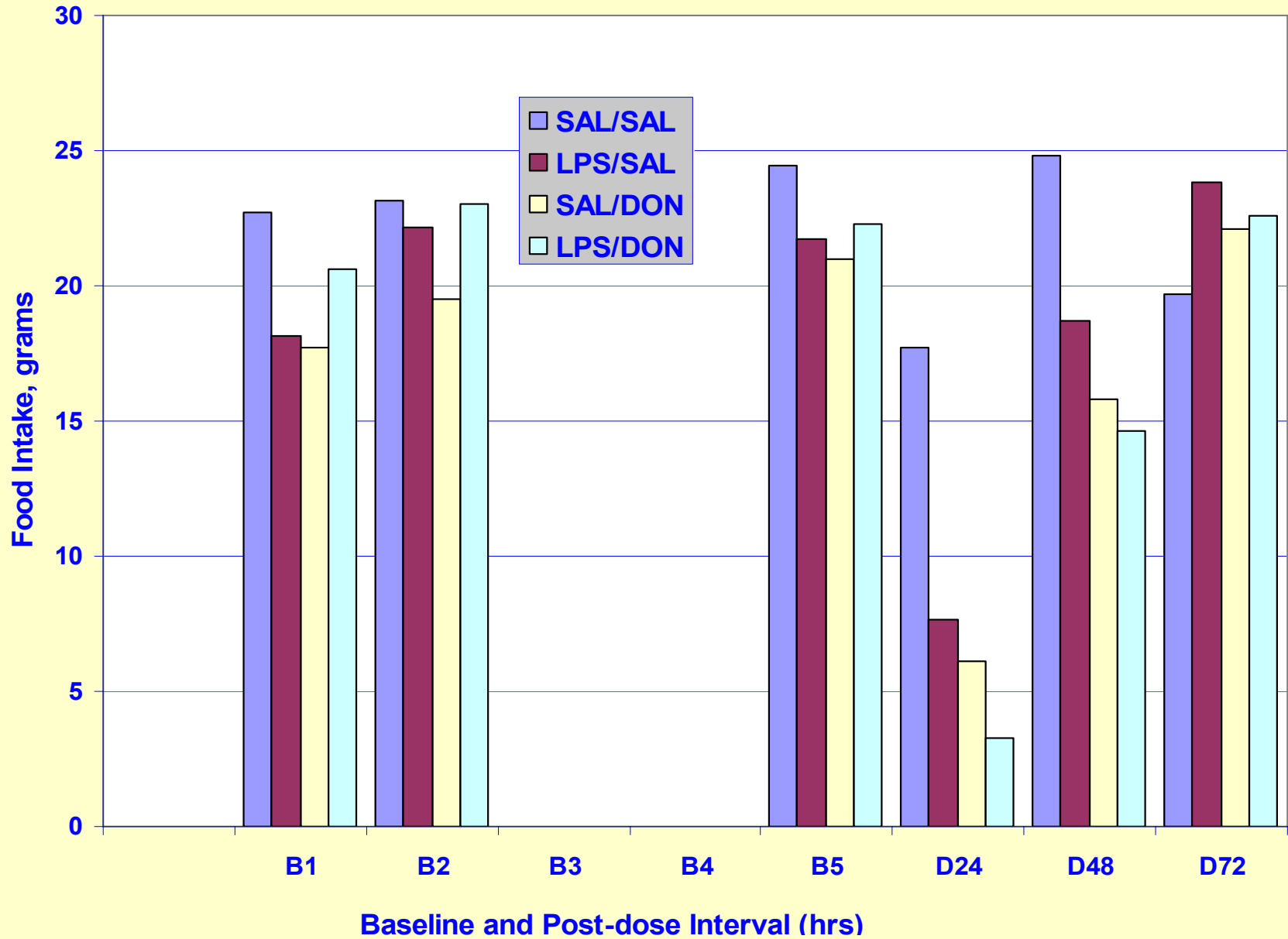
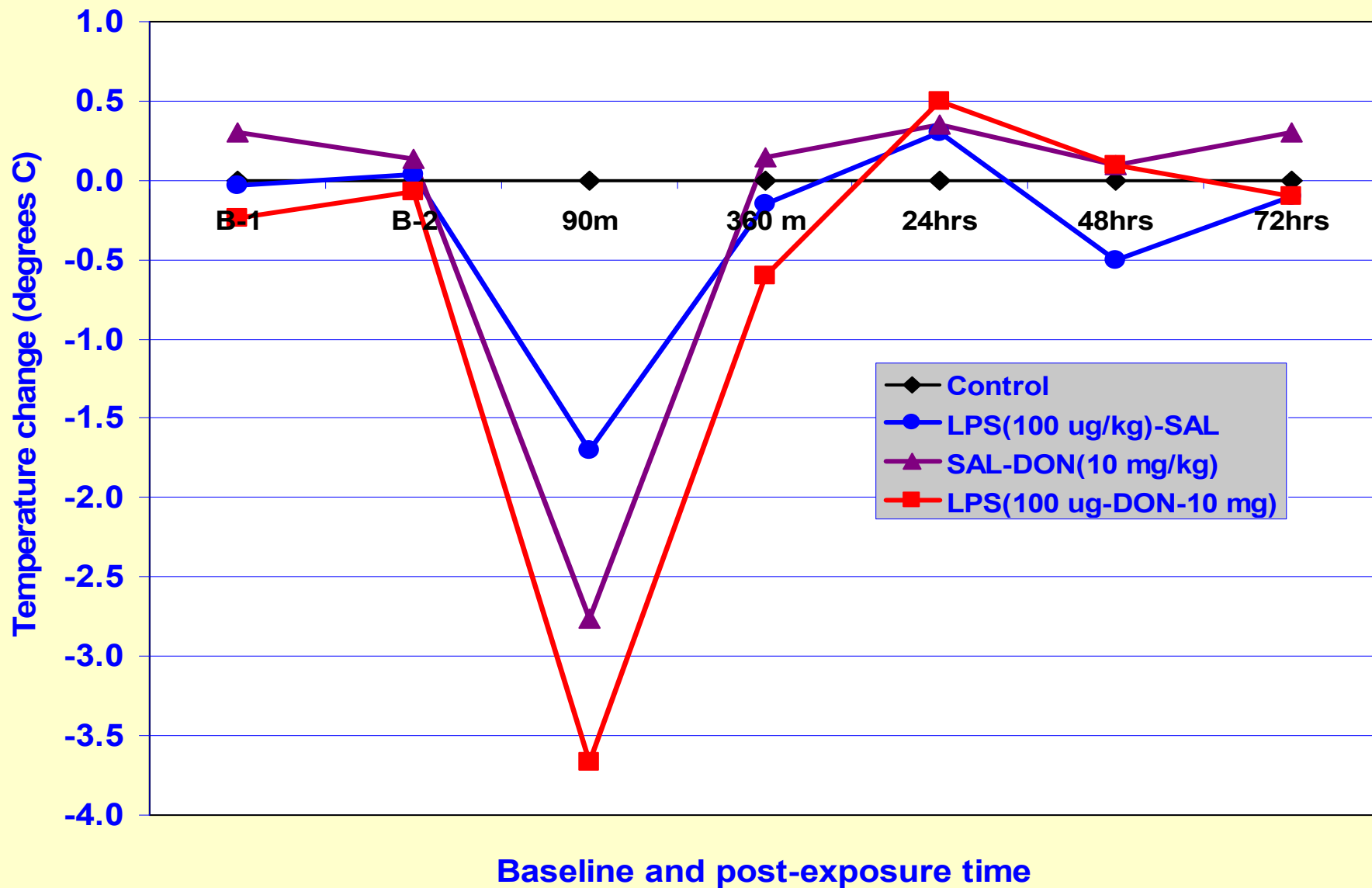


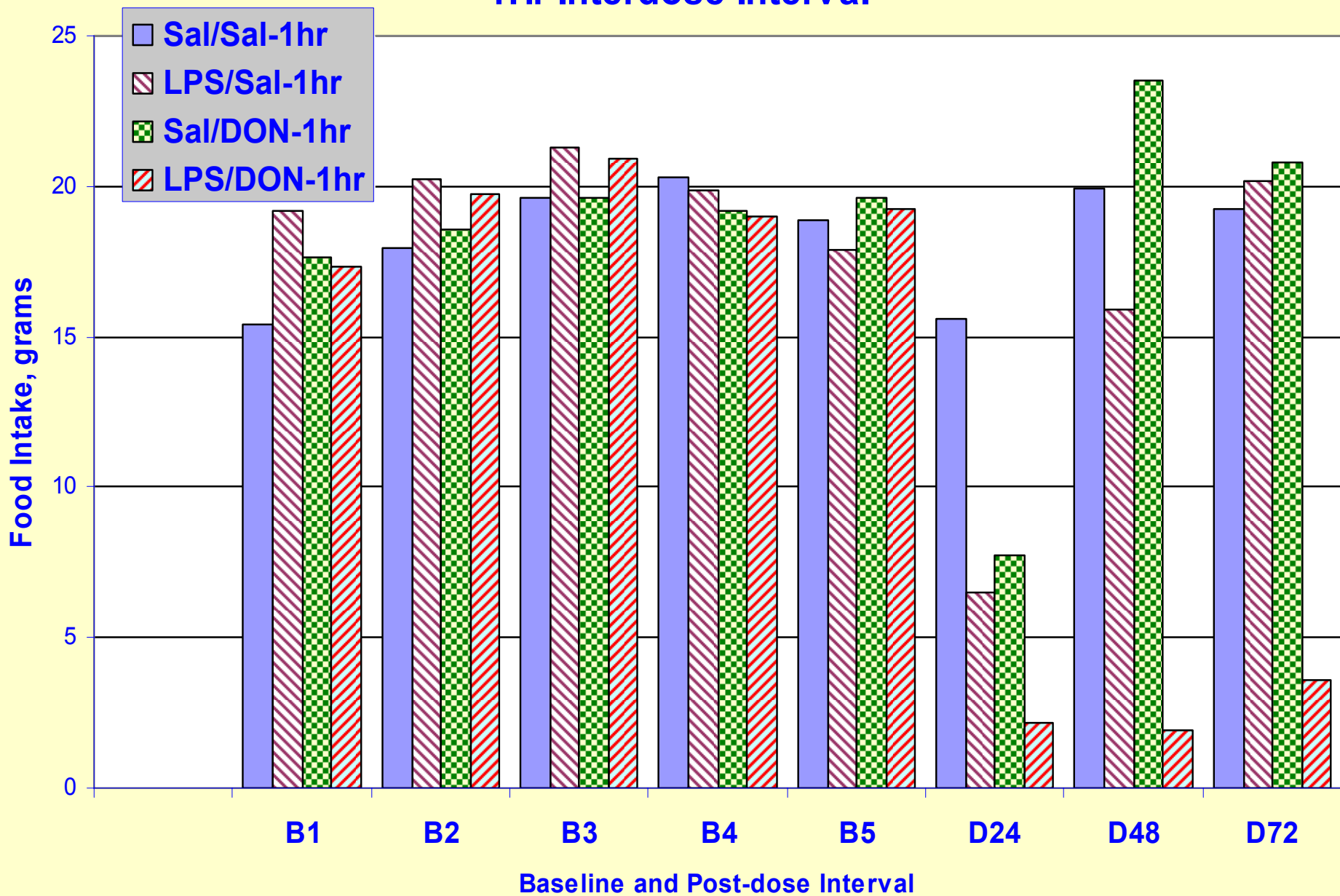
FIGURE # 13

Effect of LPS and DON on Body Temperature

Dose-interval time = zero

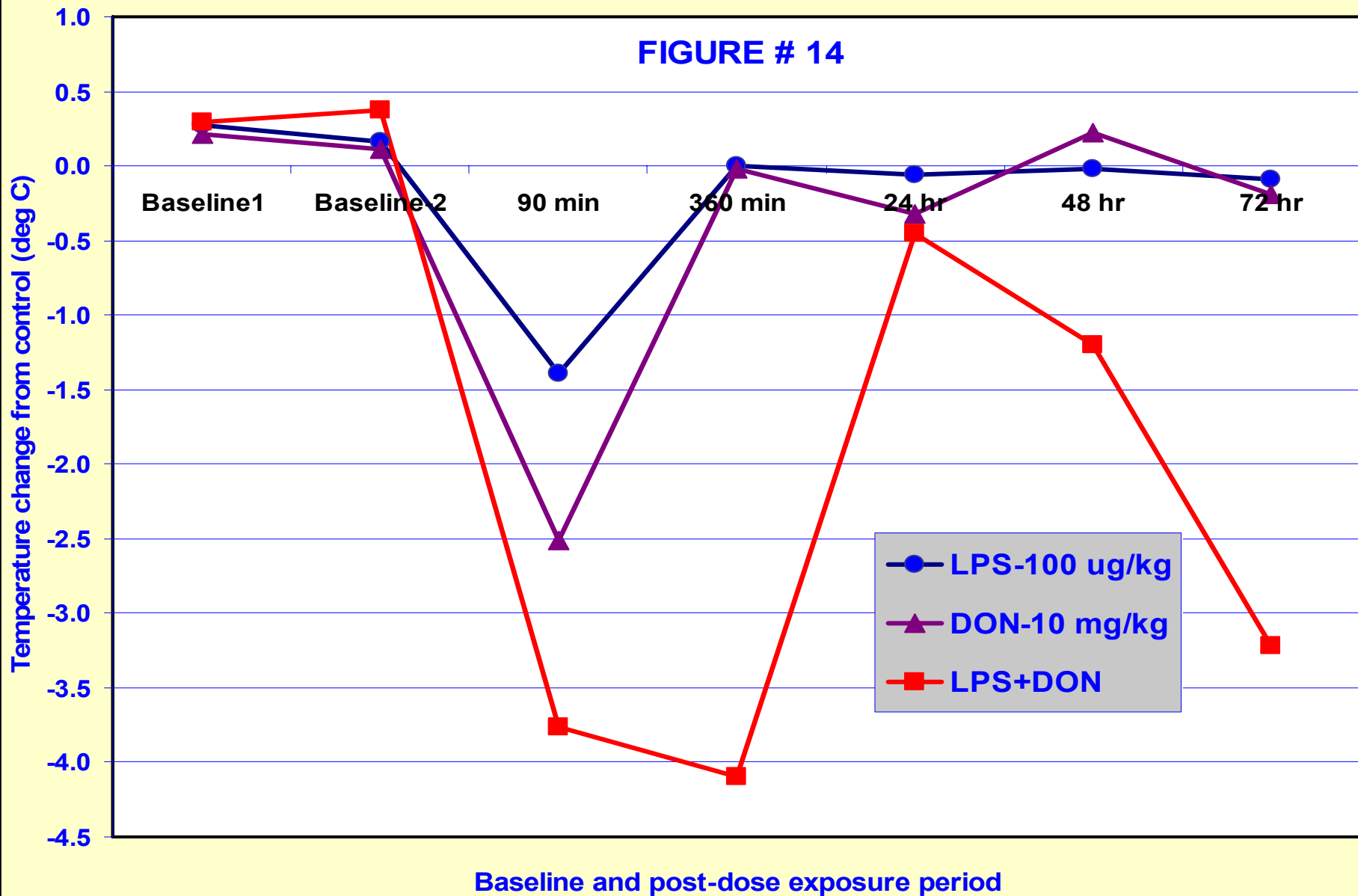


Exp 2: Food Intake 1Hr Interdose Interval

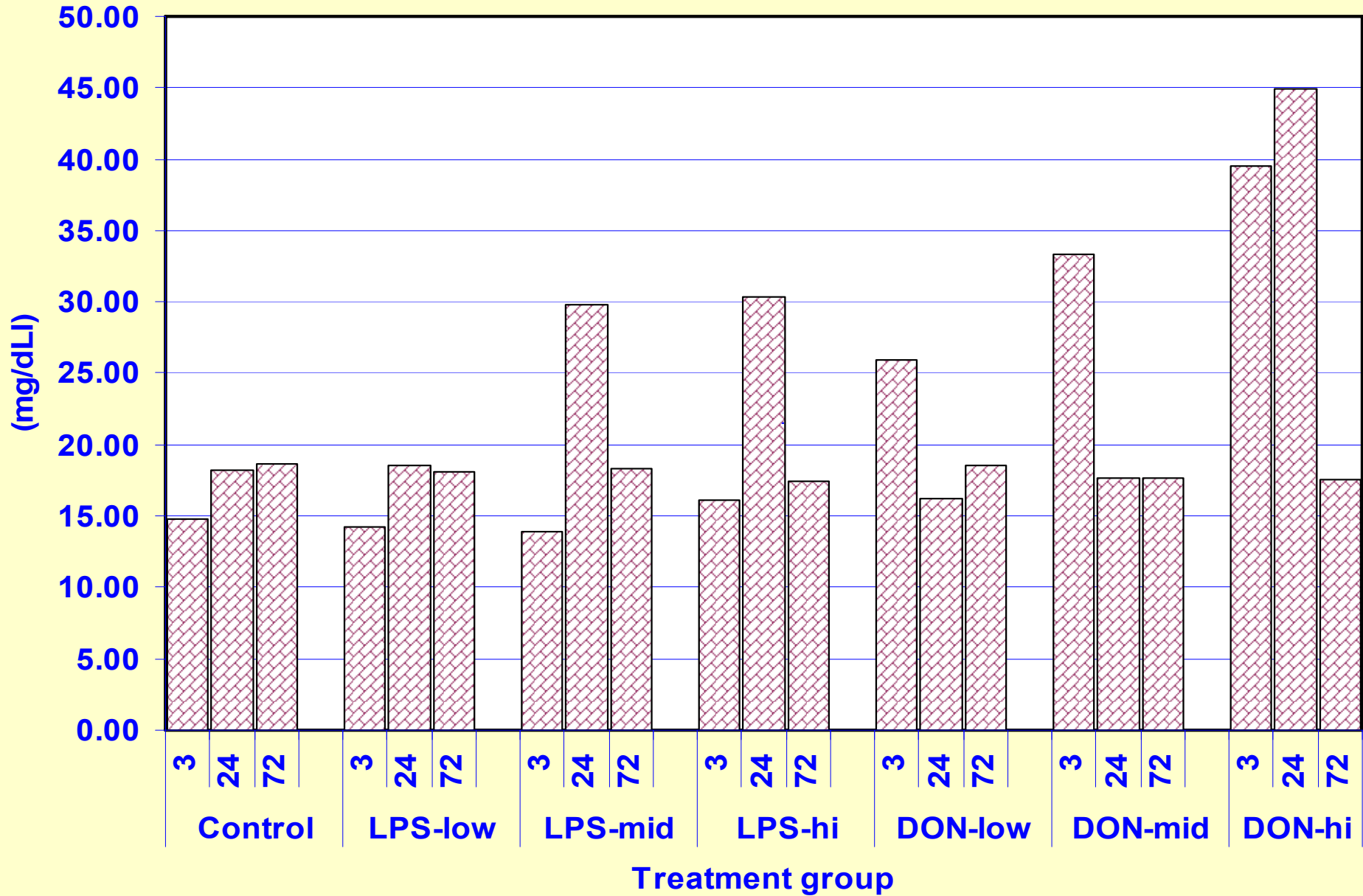


Effect of LPS and DON on Body Temperature-1 hr dose interval

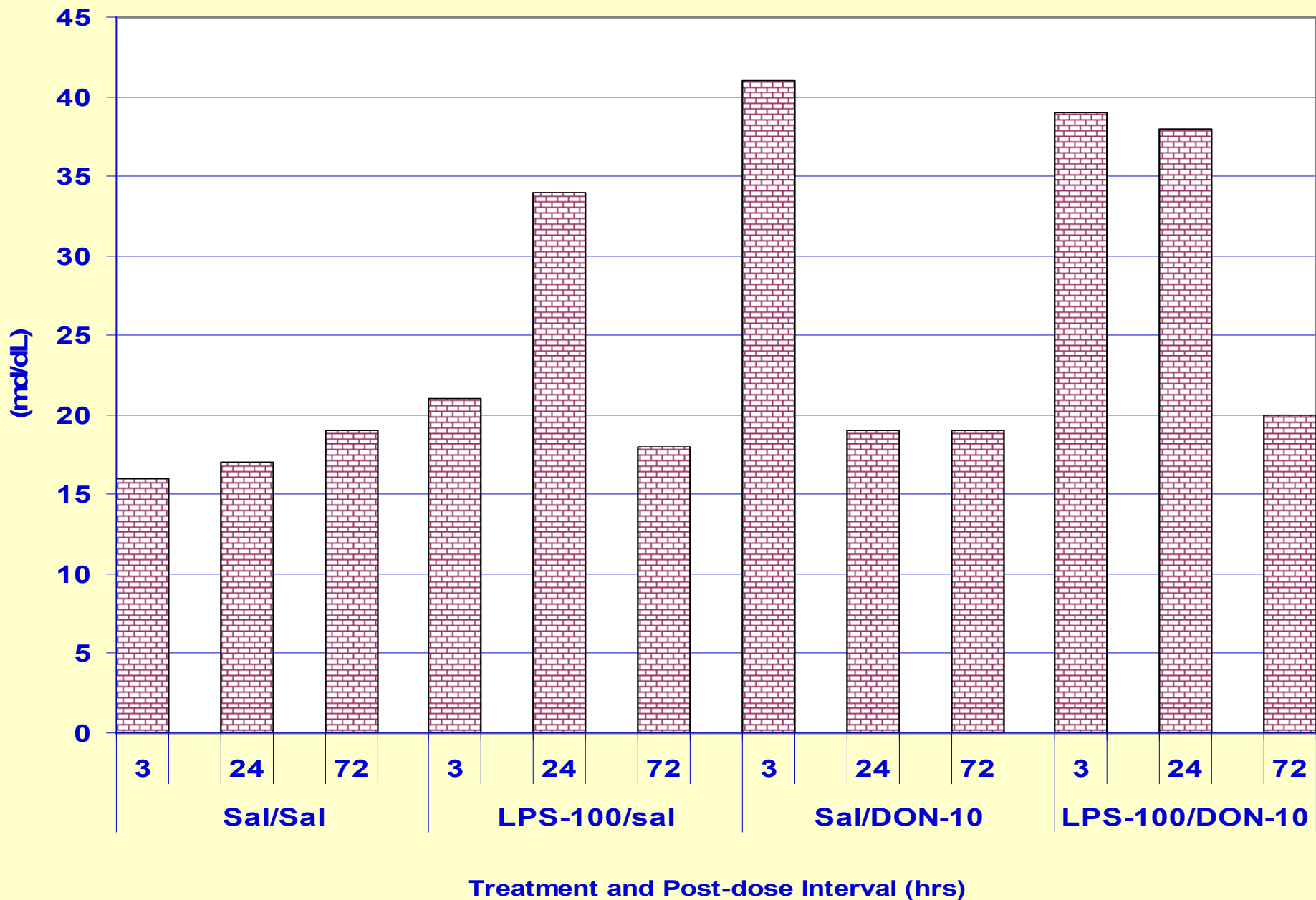
FIGURE # 14



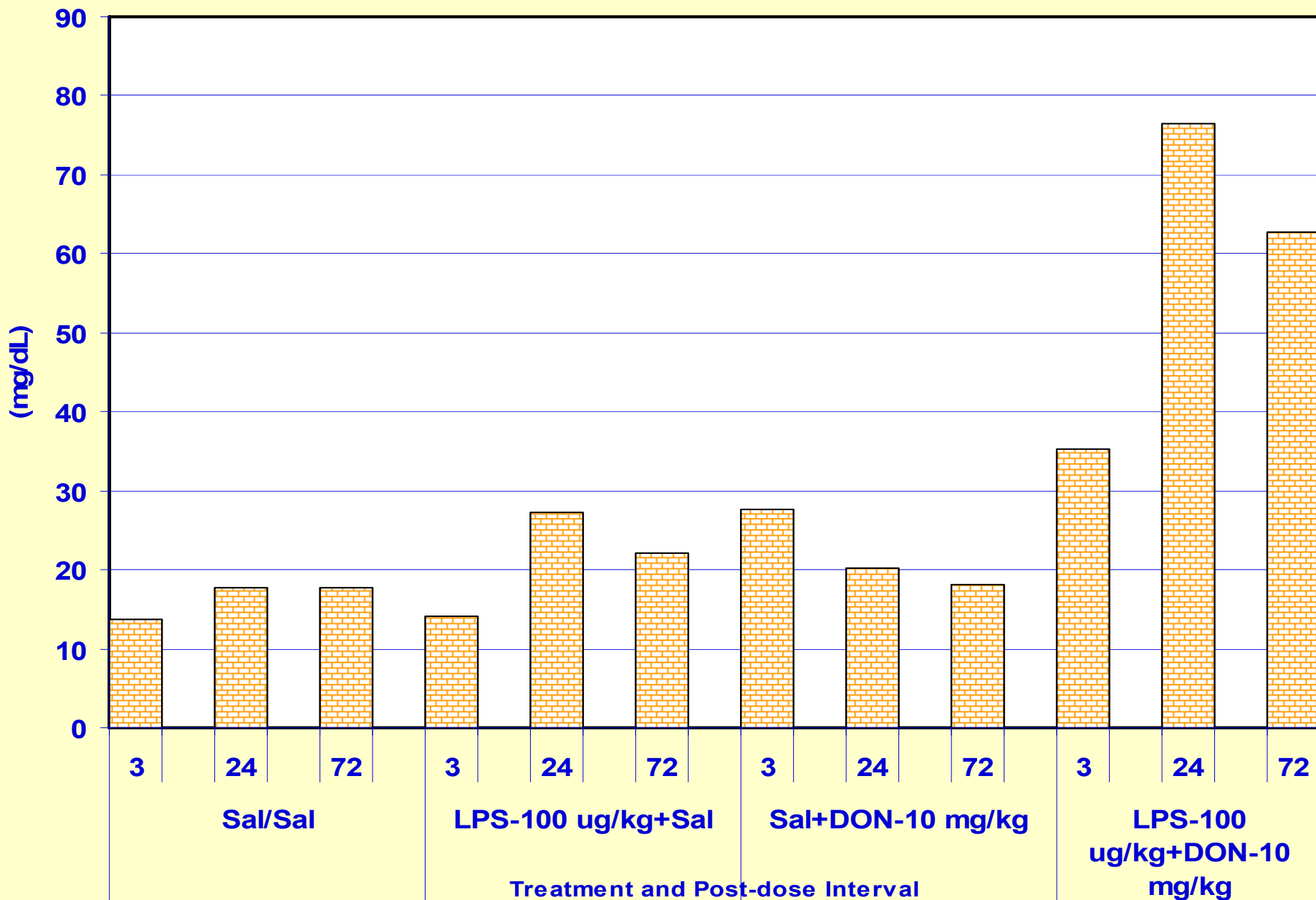
Effect of LPS or DON on Serum BUN



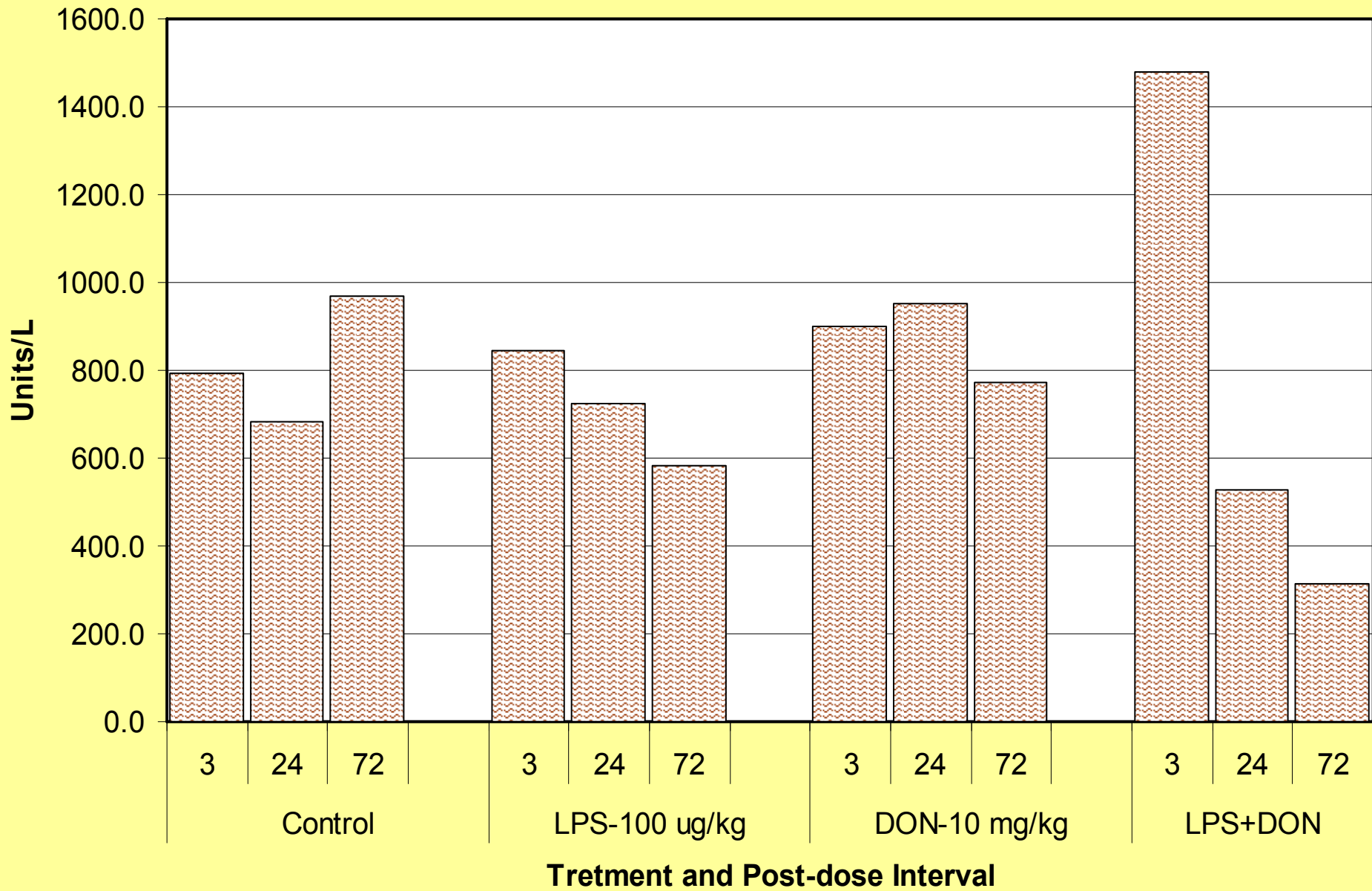
**Effect of LPS and DON on Serum Urea Nitrogen-
No Interdose Interval**



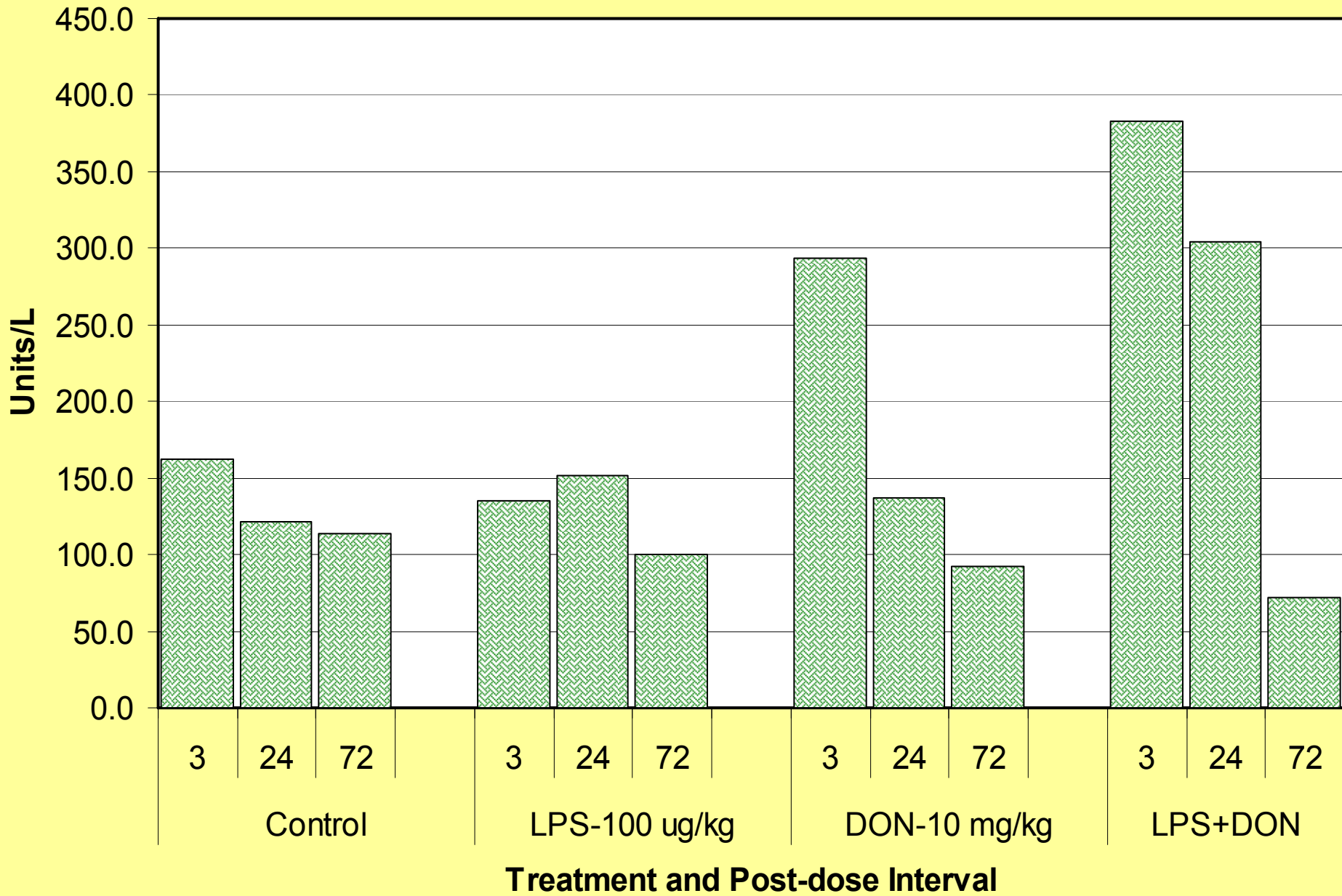
Effect of LPS and DON on Serum Urea Nitrogen- 1 hr Interdose Interval



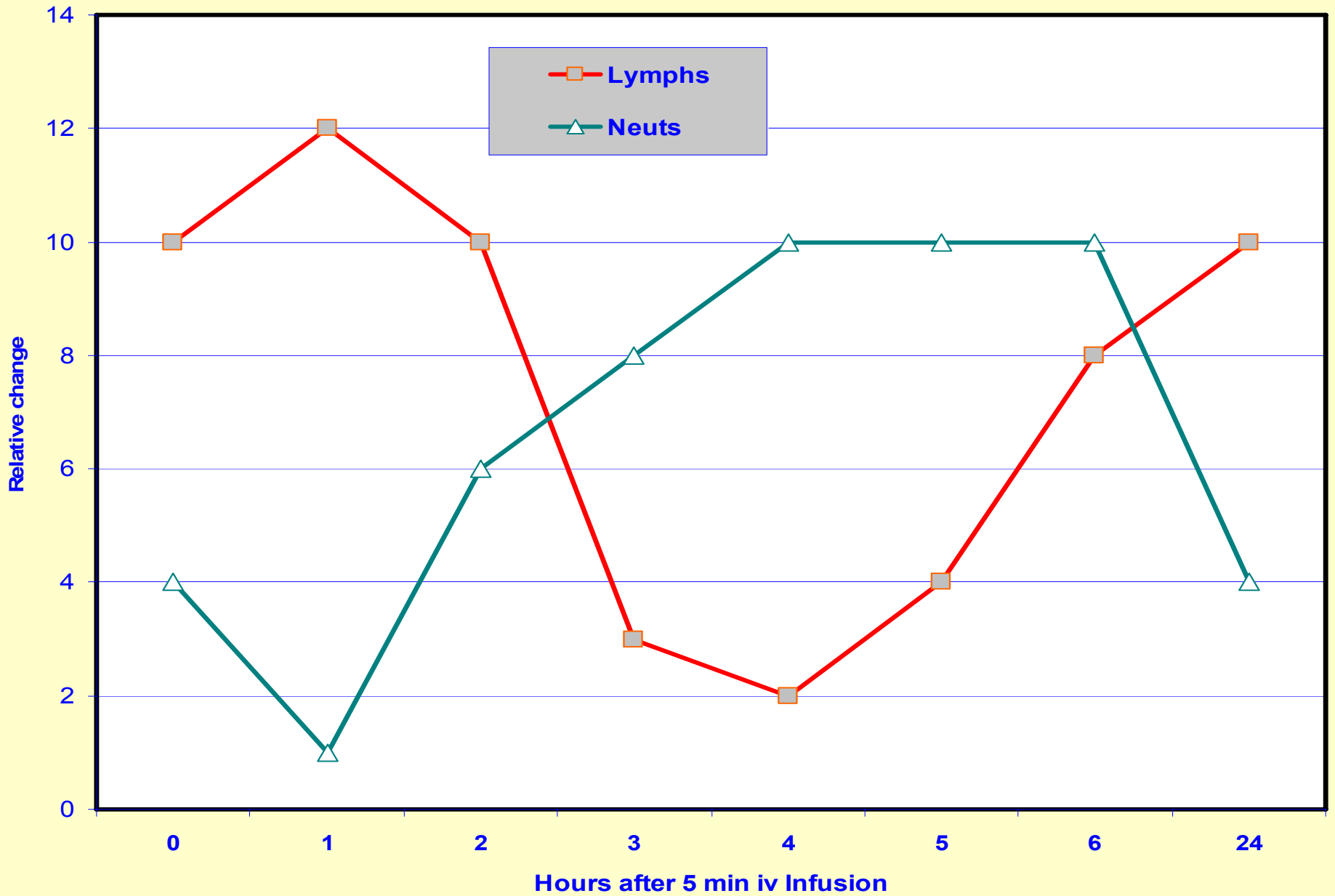
Effect of LPS and DON on Serum LDH activity



Effect of LPS and DON on Serum GOT Activity



Response of Total Lymphocytes and Neutrophils to LPS over Time



Monocytes-Effect of LPS and DON Coexposure

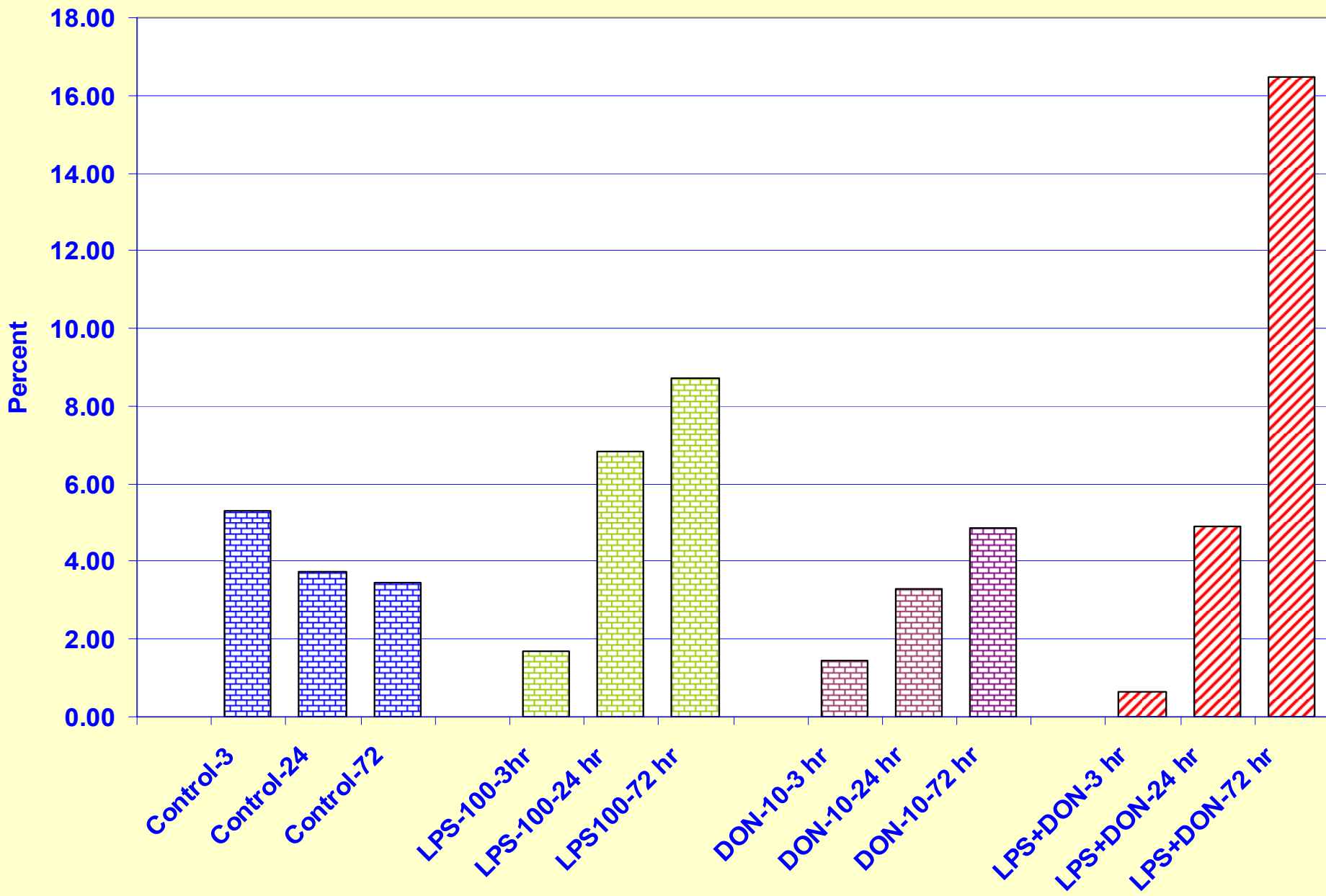


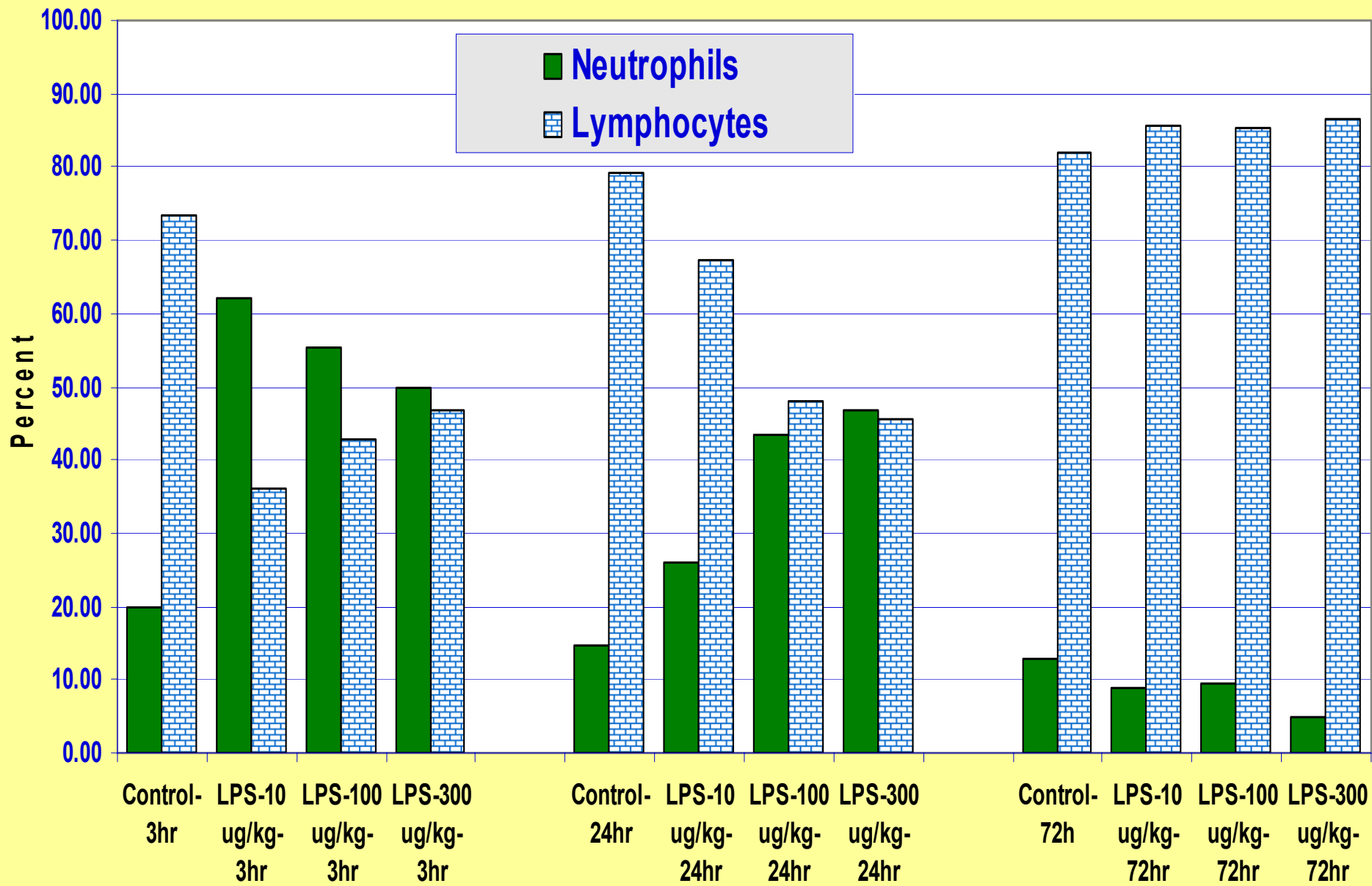
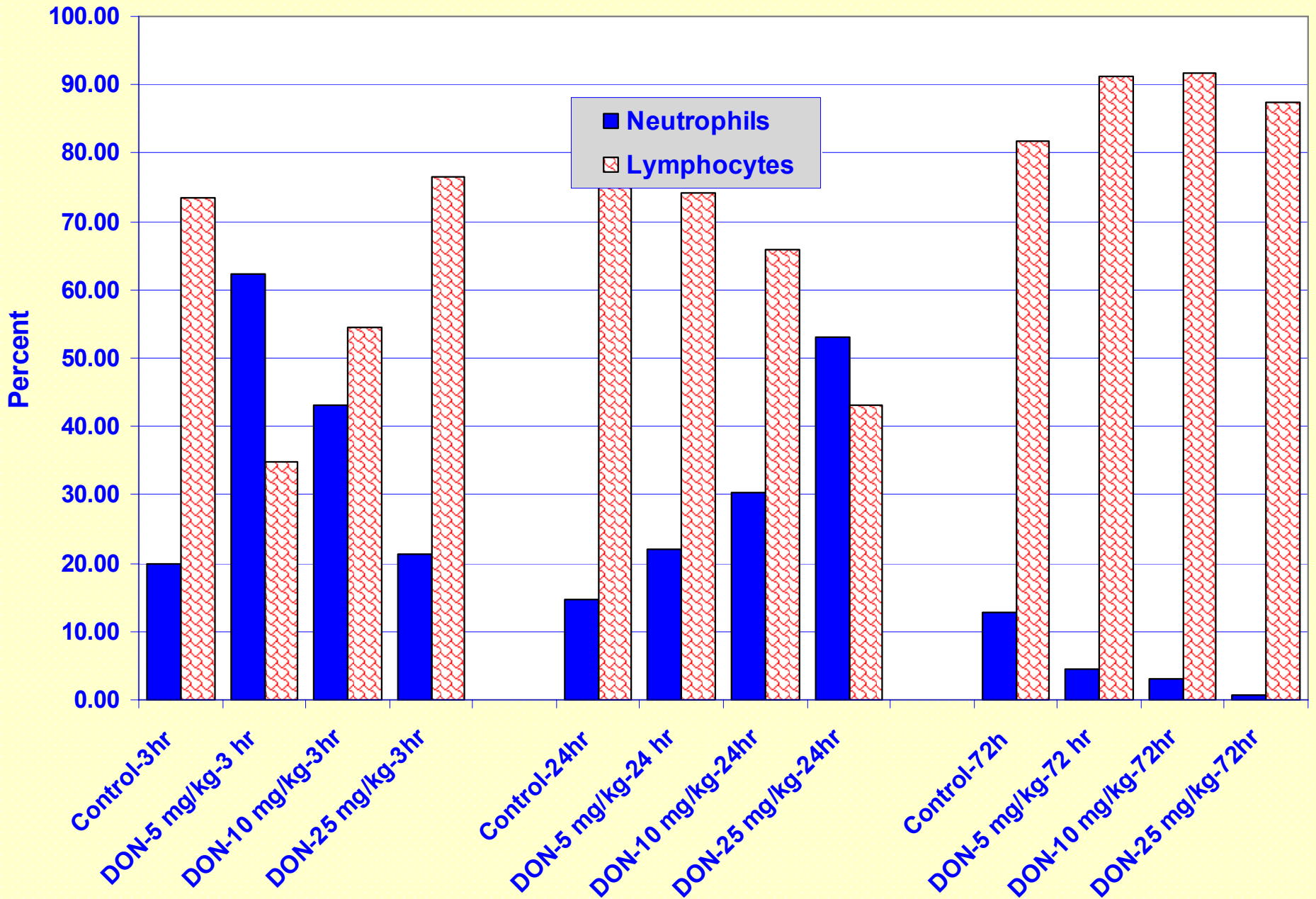
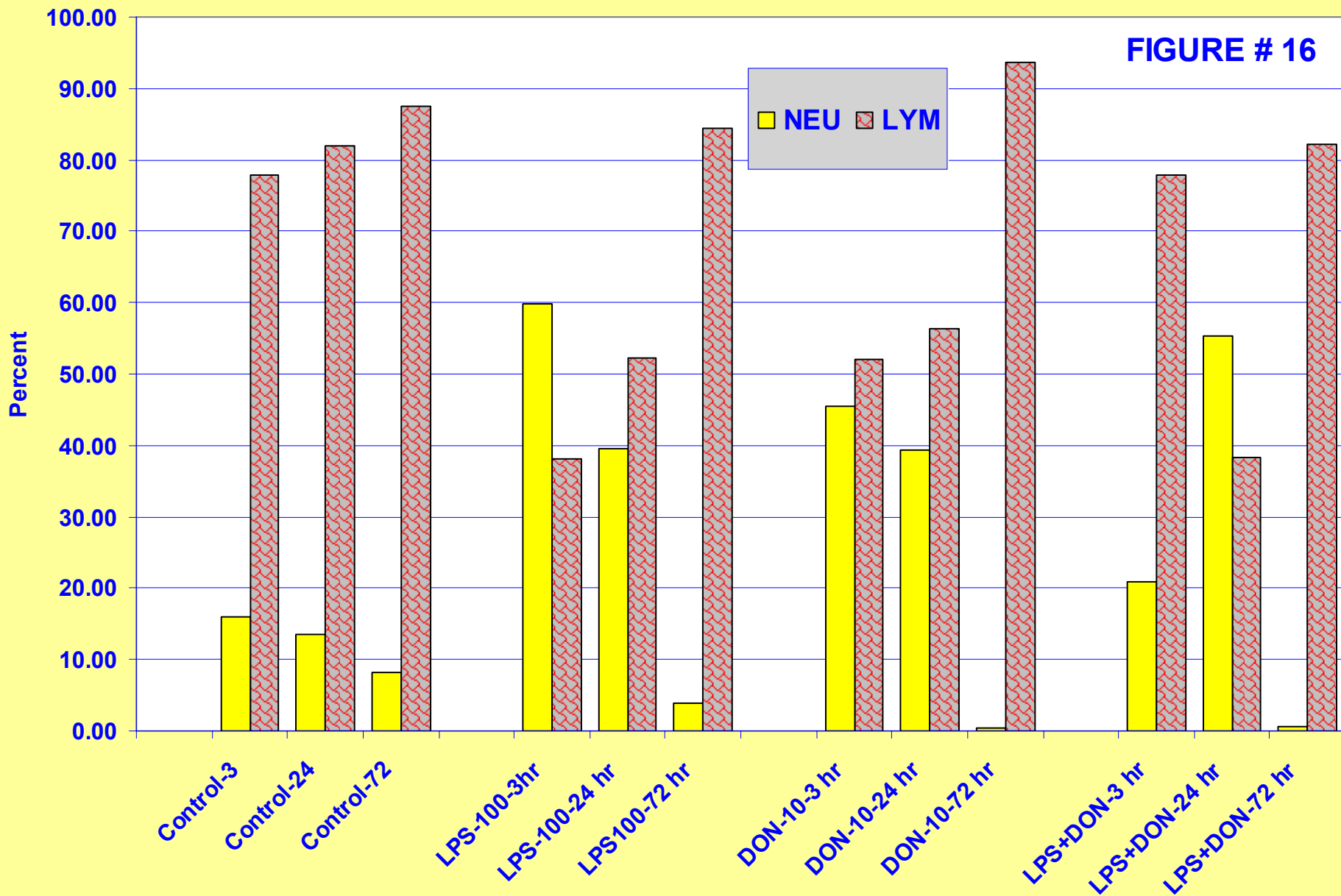
FIGURE # 7**Response of Neutrophils and Lymphocytes to LPS**

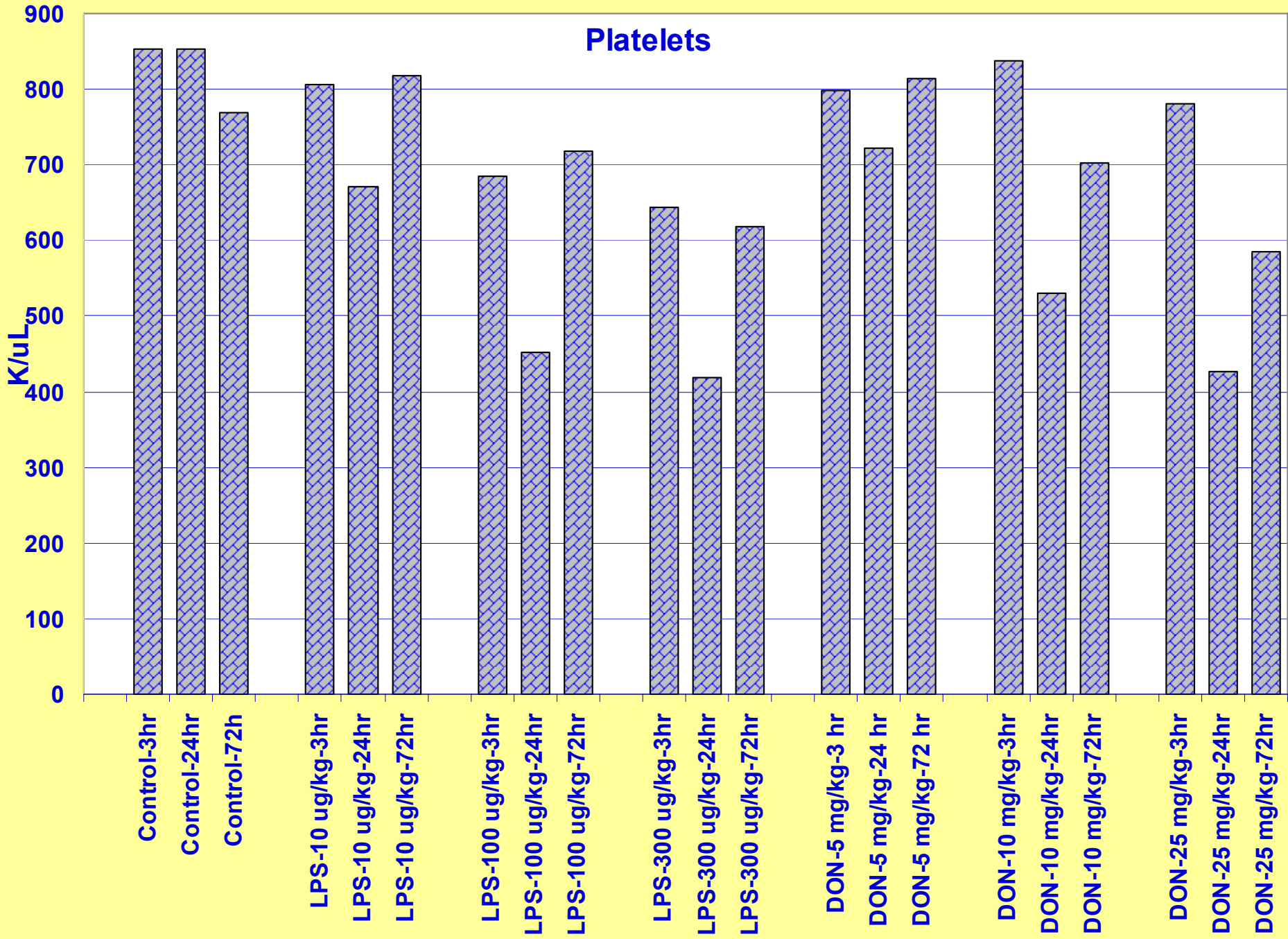
FIGURE # 8**Response Of Neutrophils and Lymphocytes to DON**

Neutrophils and Lymphocytes-Effect of LPS and DON

FIGURE # 16

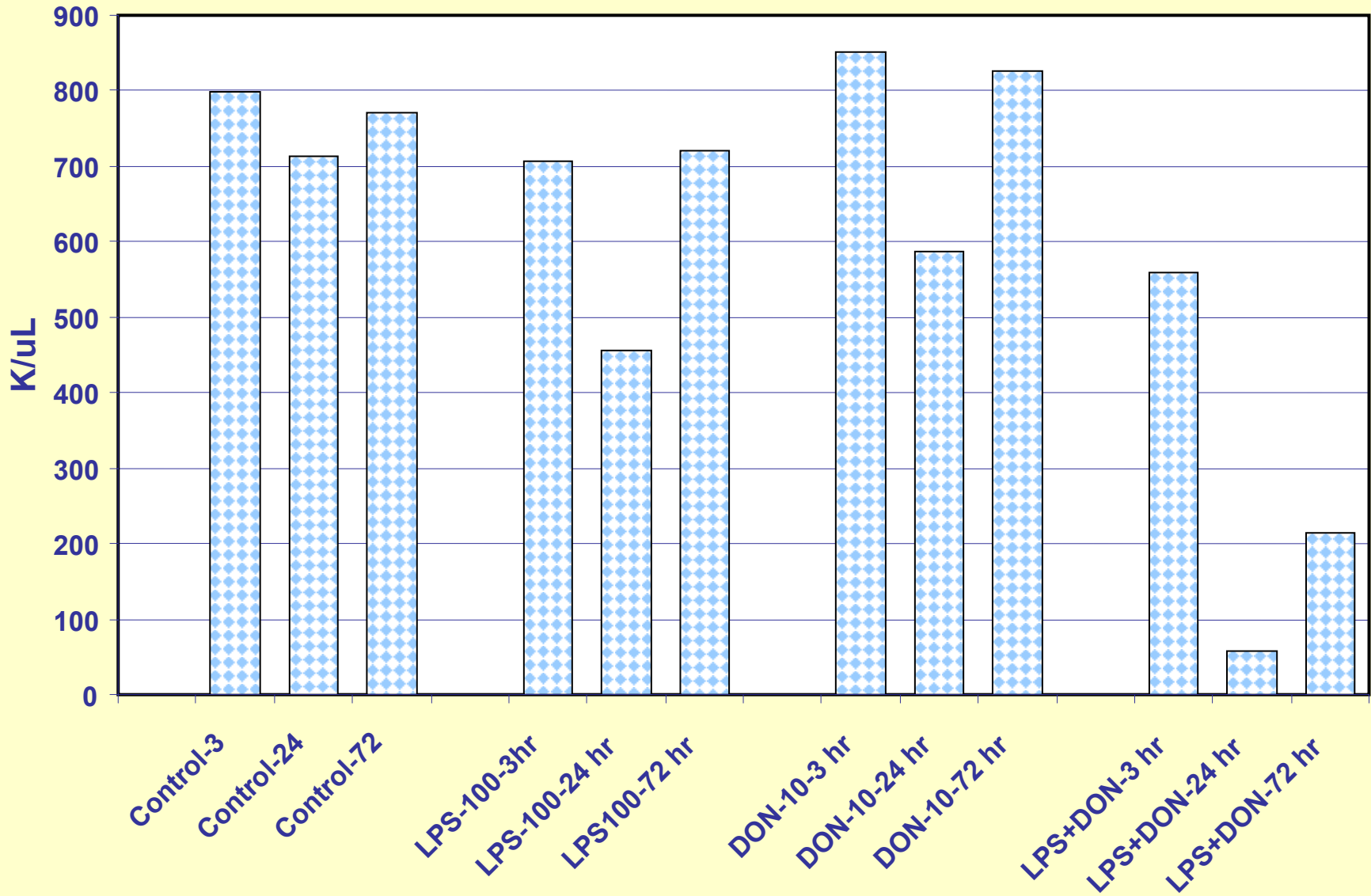


Platelets



Effect of LPS and DON on Platelets

FIGURE # 17



COLCHICINE

- Alkaloid extracted from *Colchicum Automnale* (autumn crocus and meadow saffron), Mol Wt 399
- Soluble in organic and aqueous solution
- Binds to tubulin
- **Disrupts microtubules**
- Promotes microtubule depolymerization:
Blocks mitosis.
- Disrupts axonal transport
- Stabilize neutrophils: **anti-inflammatory**

COLCHICINE in HUMANS

- Therapeutic doses of colchicine are usually 1-2 mg/day.
- Lethal acute human dose is 7-12 mg.
- Absorption is unpredictable.
- Half-life in humans is 9 - 31 hr.
- Undergoes P-450 mediated hepatic metabolism.

3 STAGES OF COLCHICINE TOXICITY in HUMANS

STAGE 1: GI symptoms - within 2 to 12 hr
Generalized sickness

**STAGE 2: Complications for virtually any
organ system (within 24 h)**

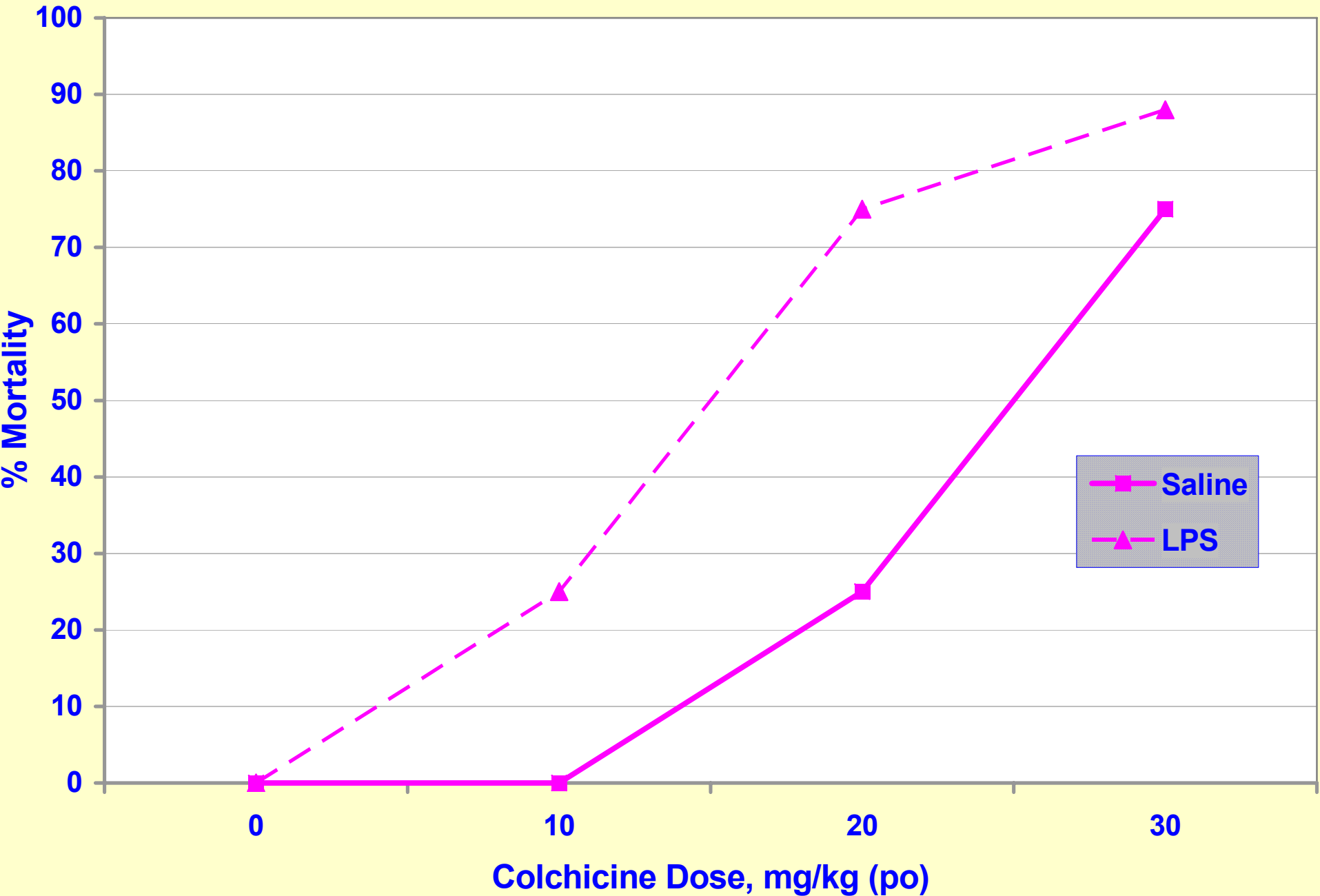
STAGE 3: Two Possibilities (within 3 -15 days).

DEATH from acute organ failure.

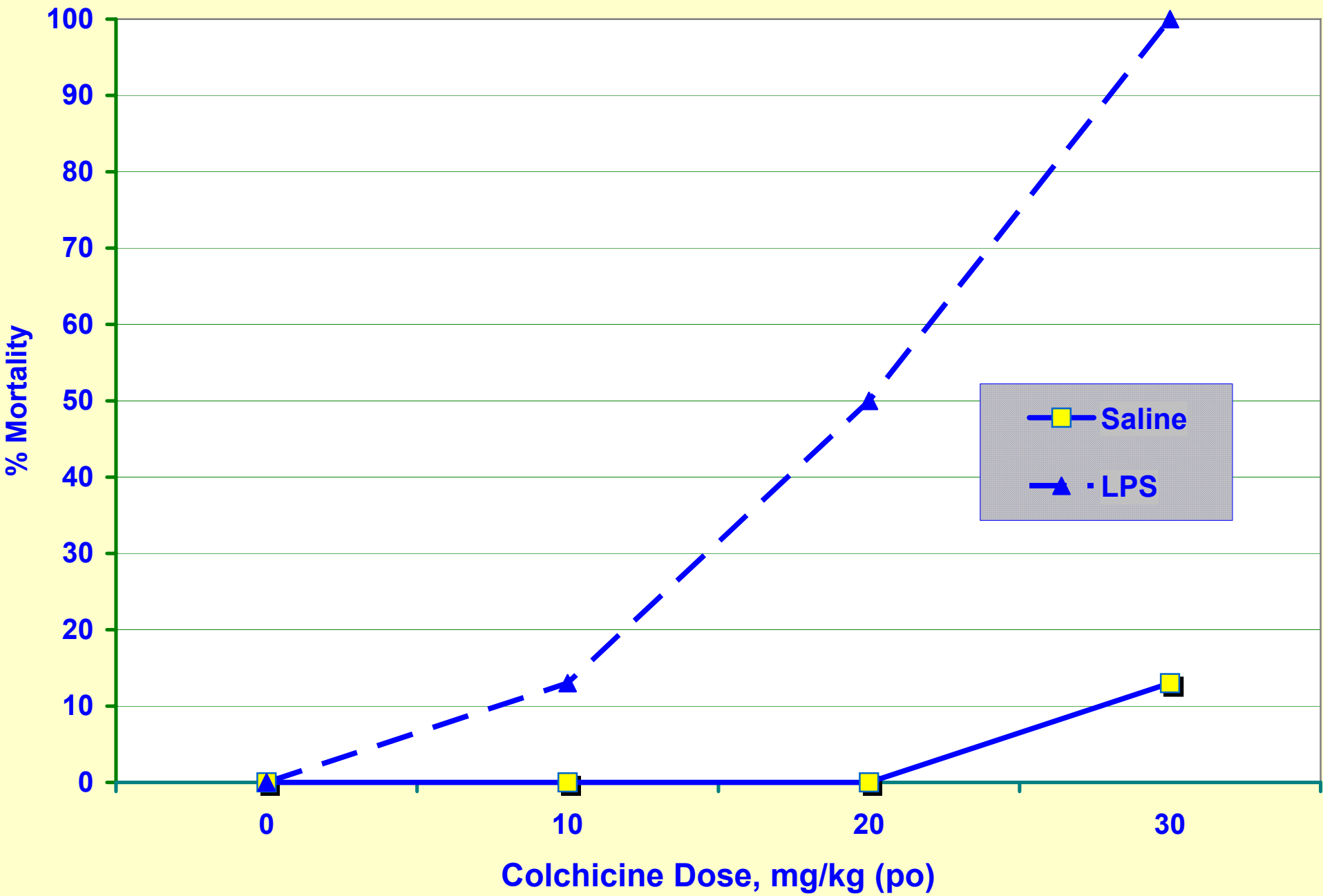
- **Multiple organ failure**
- **Cardiac arrest**
- **OR**
 - **RECOVERY**

**with transient alopecia, rebound leukocytosis,
and diarrhea**

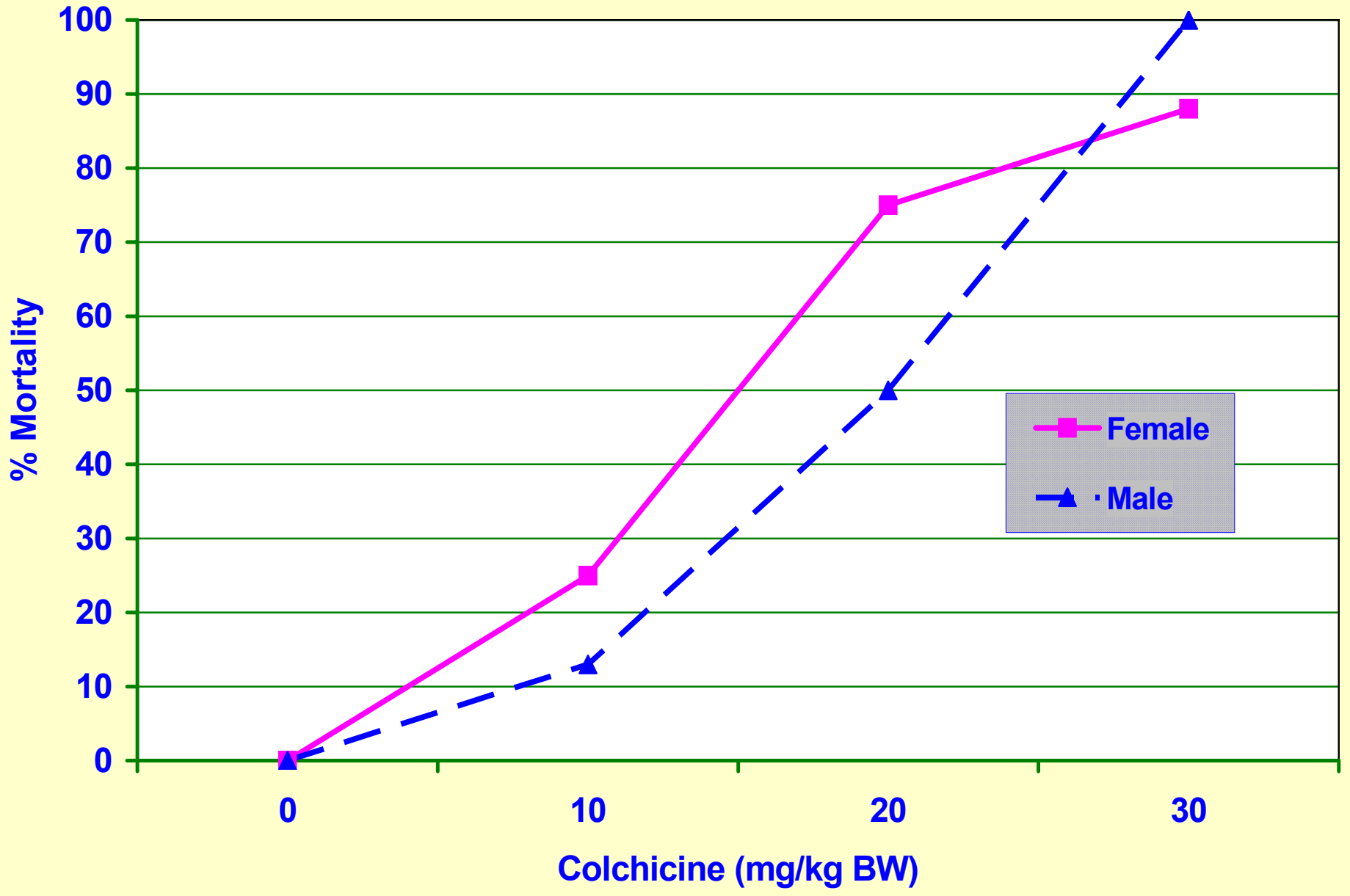
Colchicine Induced Mortality in Female Rats



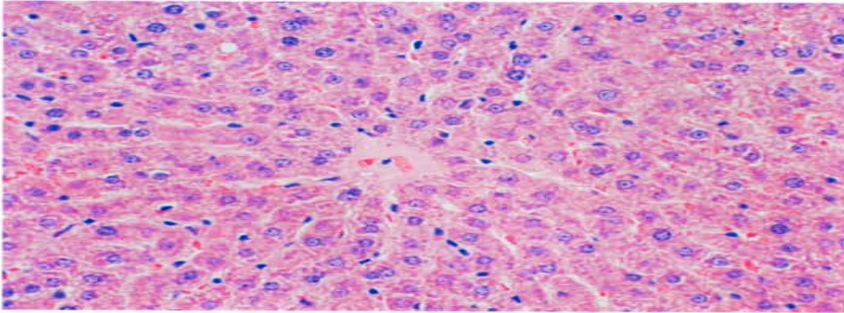
Colchicine Induced Mortality in Male Rats



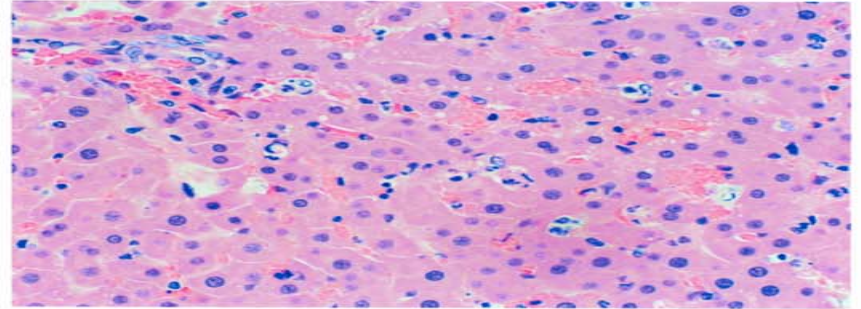
Gender Differences in Mortality in LPS and Colchicine Treated Rats



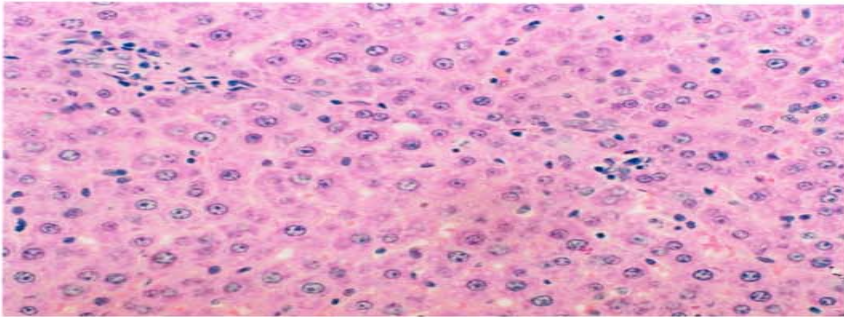
HISTOPATHOLOGY OF RAT LIVER



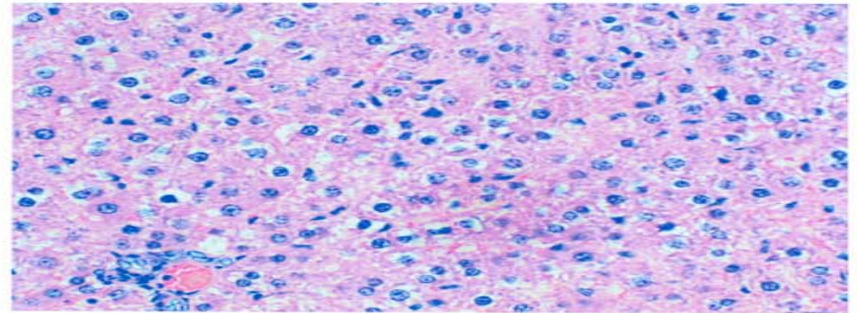
**Control Rat (388) @ 3 hrs. Liver - note the central vein in mid portion of the photo along with the cords of hepatocytes surrounding the central vein.
(H&E stain, Original Mag. 200X).**



**Rat (363) LPS83/DON10 @ 3hrs. Liver - note the random necrosis of individual hepatocytes along with an increase of cells in sinuses.
(H&E stain, Original Mag. 200X).**



**Rat (357) LPS83/DON10 @ 24 hrs. Liver - note the random necrosis of individual hepatocytes along with an increase of cells in sinuses
(H&E stain, Original Mag. 200X).**



**Rat (366) LPS83/DON10 @ 72 hrs. Liver - note the clear cytoplasmic vacuolation of hepatocytes.
(H&E stain, Original Mag. 200X).**

ADDITIONAL STUDY PERSONNEL

Jessica Suagee- UM Graduate School (ORISE)

Yvonne Quander

Stewart Long

Clides Kelly

Matthew Smith

Sun Mi Ahn-OARSA Clinical Chemist

Maria Lorenzo, DVM - Attending Veterinarian

Martin Robl, Ph.D., DVM - Veterinary Pathologist

Curtis Barton, Ph.D. – Statistician

Mona Calvo, Ph.D.

Thomas Sobotka, Ph.D.

Thomas Flynn, Ph.D.

Paddy Wiesenfeld, Ph.D.

Christine Anderson, Ph.D.-Microbiologist-CBER

Eugene Lewis - Animal Husbandry

Sue Artiss - Animal Husbandry

Jan Johannessen, Ph.D.