

# LPS dose-dependently induces body temperature change in rabbits

Brie Culp<sup>1</sup>; Ioan Petrescu<sup>1</sup>; Peter Szczerba<sup>1</sup>; Ser Mien Chia<sup>3</sup>; Xiaoli Ping<sup>1</sup>; Liming Yang<sup>2</sup>; Christian Nunes<sup>1</sup>; James Destefano<sup>1</sup>; Todd Schnauer<sup>1</sup>; Marc Washington<sup>1</sup>; Mark Ault<sup>1</sup>; Loise Gichuru<sup>1</sup>; Larry Handt<sup>1</sup>; Nina Li<sup>2</sup>; Sherri Motzel<sup>1</sup>; Xiaolan Shen<sup>1</sup>

Departments of <sup>1</sup>Laboratory Animal Resources (West Point, PA), <sup>2</sup>Cardio-Metabolic Disease (South San Francisco, CA), Merck & Co., Inc., Kenilworth, NJ, USA; <sup>3</sup>Genome and Biomarker Sciences, MSD, Singapore

## Abstract

Lipopolysaccharide (LPS), a component of the Gram-negative bacteria outer membrane, has been generally used for experimental modeling of inflammation in different species. In rabbits, the LPS causes a reversible inflammatory status that clinically appears as lethargy, ruffled fur, shivering, and body temperature (BT) and hematologic changes. In this poster, the effects of LPS (*Escherichia coli* O55:B5,  $3 \times 10^4$  endotoxin units [EU]/ $\mu\text{g}$ , Sigma Chemical Company, St. Louis, MO) on body temperature were investigated in New Zealand rabbits. BT telemetric transponders (UCT-2112, UID Identification Solutions, Lake Villa, IL) were implanted subcutaneously at the base of the animal's ear. LPS was injected intravenously at doses of 1 and 3  $\mu\text{g}/\text{kg}$  ( $3 \times 10^4$  and  $9 \times 10^4$  EU/kg accordingly) and animal BTs were monitored via telemetric transponders in intervals as short as 5 minutes. As a result, hyperthermia ( $+1.9^\circ\text{C} \pm 0.3^\circ\text{C}$  at 2 hours) was observed in the 1  $\mu\text{g}/\text{kg}$  group for up to 6 hours. Meanwhile, hypothermia ( $-1.5^\circ\text{C} \pm 0.31^\circ\text{C}$  at 2 hours) was observed in the 3  $\mu\text{g}/\text{kg}$  group for up to 8 hours and was back to normal in 24 hours; the hypothermic rabbits were provided with external temperature support for 6-7 hours post-LPS inoculation. Recorded data suggests that the endotoxin appeared to have a dose-dependent effect on the body temperature of the rabbits. Blood chemistry and hematology testing were also conducted in the 3  $\mu\text{g}/\text{kg}$  group. Decreasing white blood cell, platelet, neutrophil, and lymphocyte counts were confirmed. In conclusion, the different concentrations of endotoxin could be a critical factor causing either hyperthermia or hypothermia in rabbits.

## Material and methods

- All animal study protocols were reviewed and approved by Merck's Institutional Animal Care and Use Committee (IACUC)
- New Zealand white male rabbits (*Oryctolagus cuniculus*; weight, 2.4 to 3.2 kg) were acquired from Envigo Global Services Inc. Rabbits were housed in individual cages and acclimated to a 12:12-hour light:dark cycle in a temperature- and humidity-controlled environment. Rabbits were provided with food and water ad libitum. All rabbits were acclimated to the facility for at least 7 days before any procedures
- Body temperature telemetric transponders (UCT-2112, UID Identification Solutions, Lake Villa, IL) were implanted subcutaneously in conscious animals at the base of the ear
- LPS preparation and administration
  - LPS (*Escherichia coli* O55:B5;  $3 \times 10^4$  endotoxin units [EU]/ $\mu\text{g}$ ; Sigma Chemical Company, St. Louis, MO) was diluted in sterile, nonpyrogenic saline (0.9% sodium chloride; Baxter, Deerfield, IL)
  - 1 or 3  $\mu\text{g}/\text{kg}$  (0.5 mL/kg, 2 or 6  $\mu\text{g}/\text{mL}$ ) of fresh prepared LPS or equivalent volume of saline was administered intravenously to rabbits
  - Rabbits were divided into 3 groups: vehicle, 1  $\mu\text{g}/\text{kg}$  LPS, and 3  $\mu\text{g}/\text{kg}$  LPS (N=5-7 per group)
- BT and blood collection time points
  - BTs were monitored via telemetric transponders in intervals as short as 5 minutes. Blood was taken at 30, 60, 90, and 120 minutes and 2, 4, and 8 hours after LPS exposure and once daily thereafter
  - If animal temperatures dropped below  $36^\circ\text{C}$ , thermal support was provided via warm gel packs placed with the animal in the cage until a stable temperature was obtained
- Food intake was measured daily for up to 8 days

## Results

Figure 1. Effect of LPS IV on body temperature in NZW rabbits

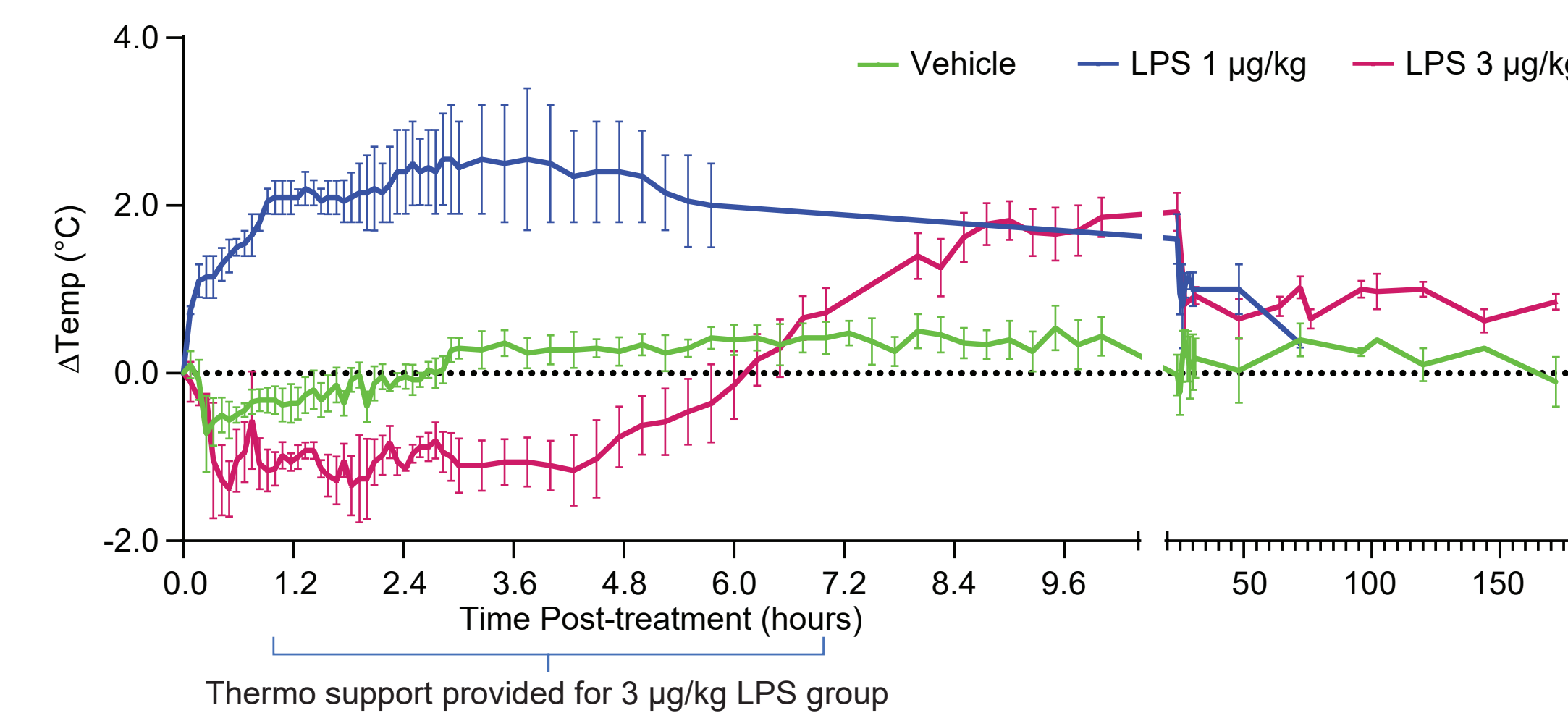


Figure 2. Temperature change from baseline in the first 2 hours post-treatment

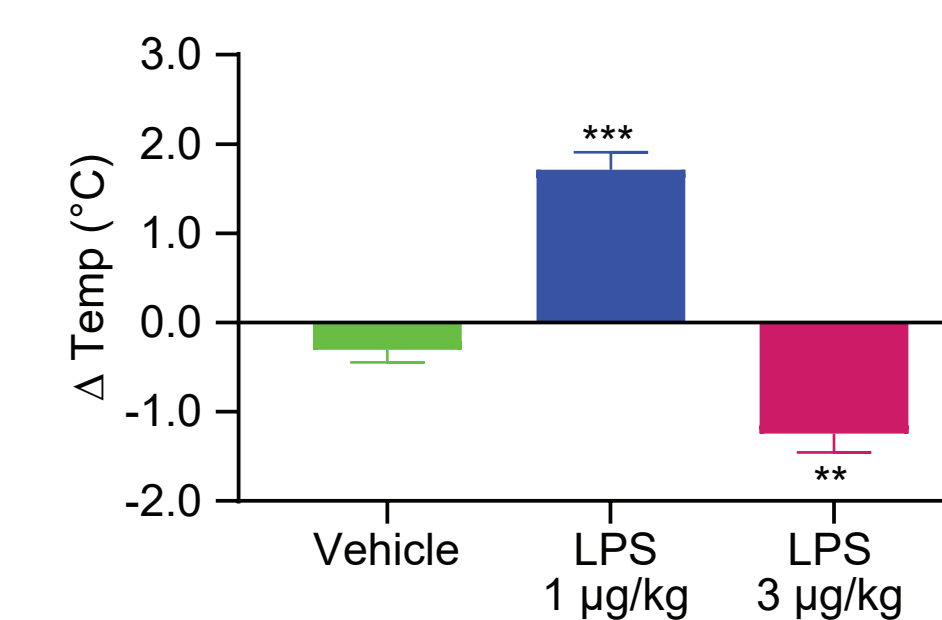
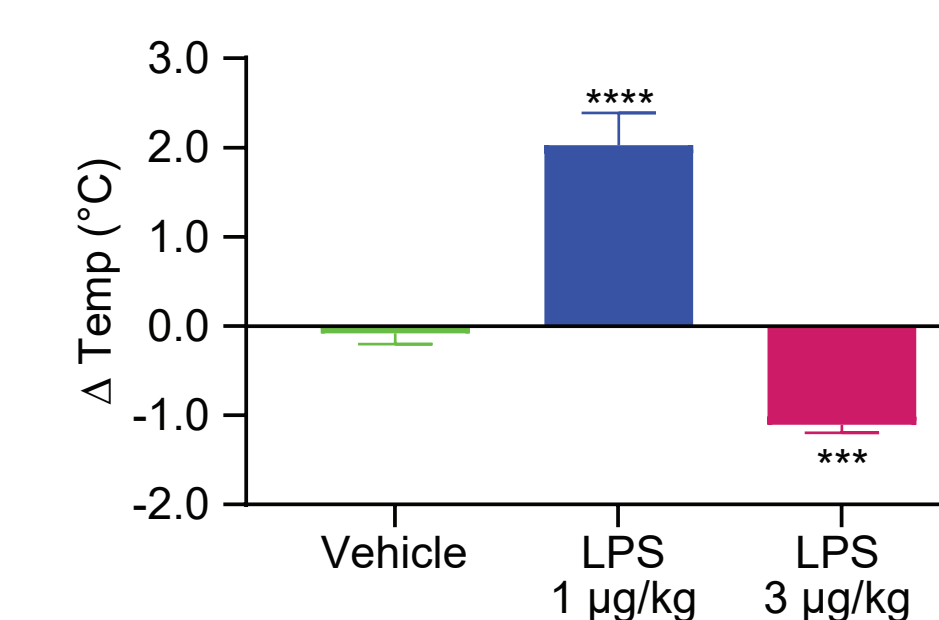
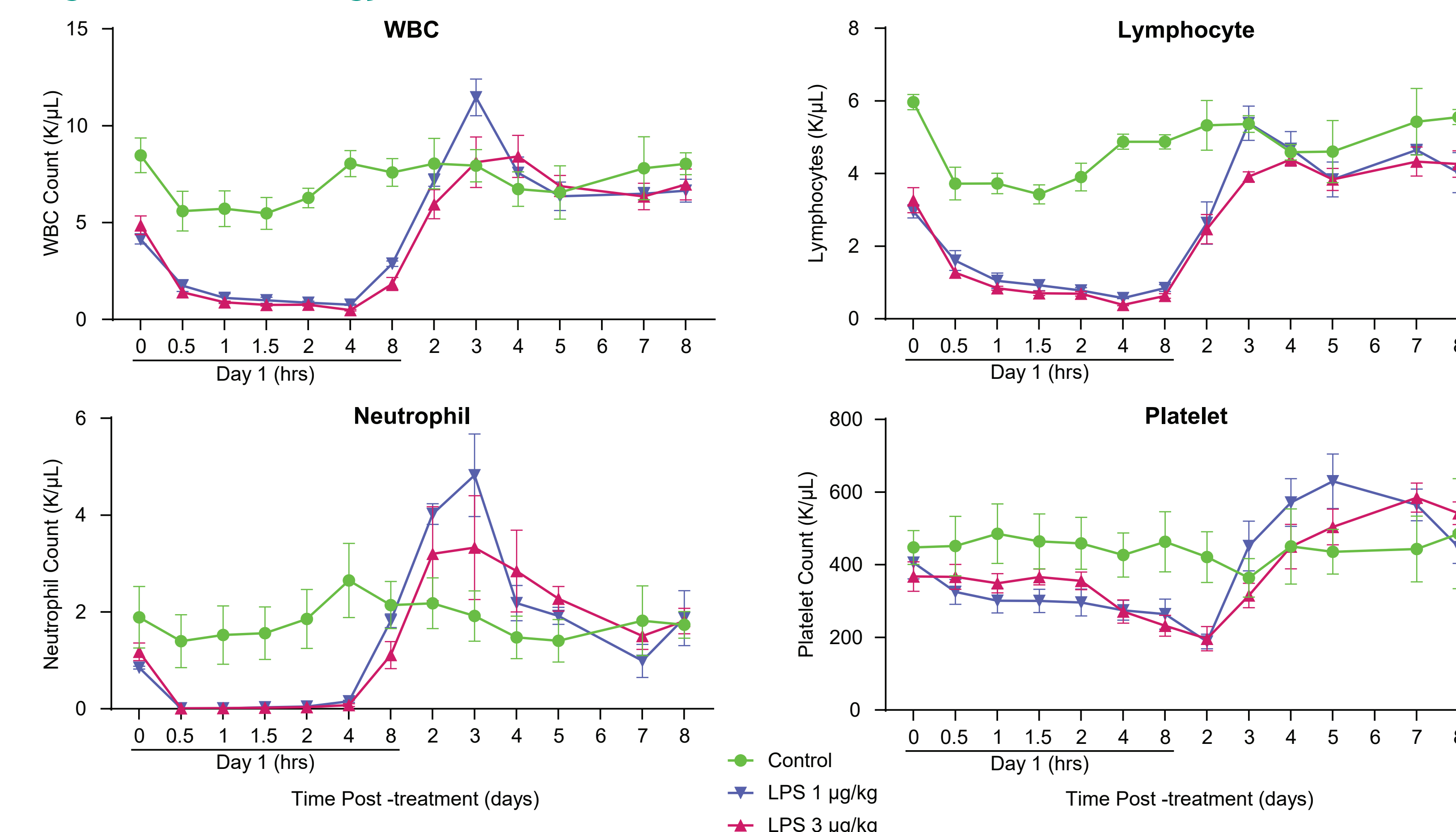


Figure 3. Temperature change from baseline in the first 6 hours post-treatment



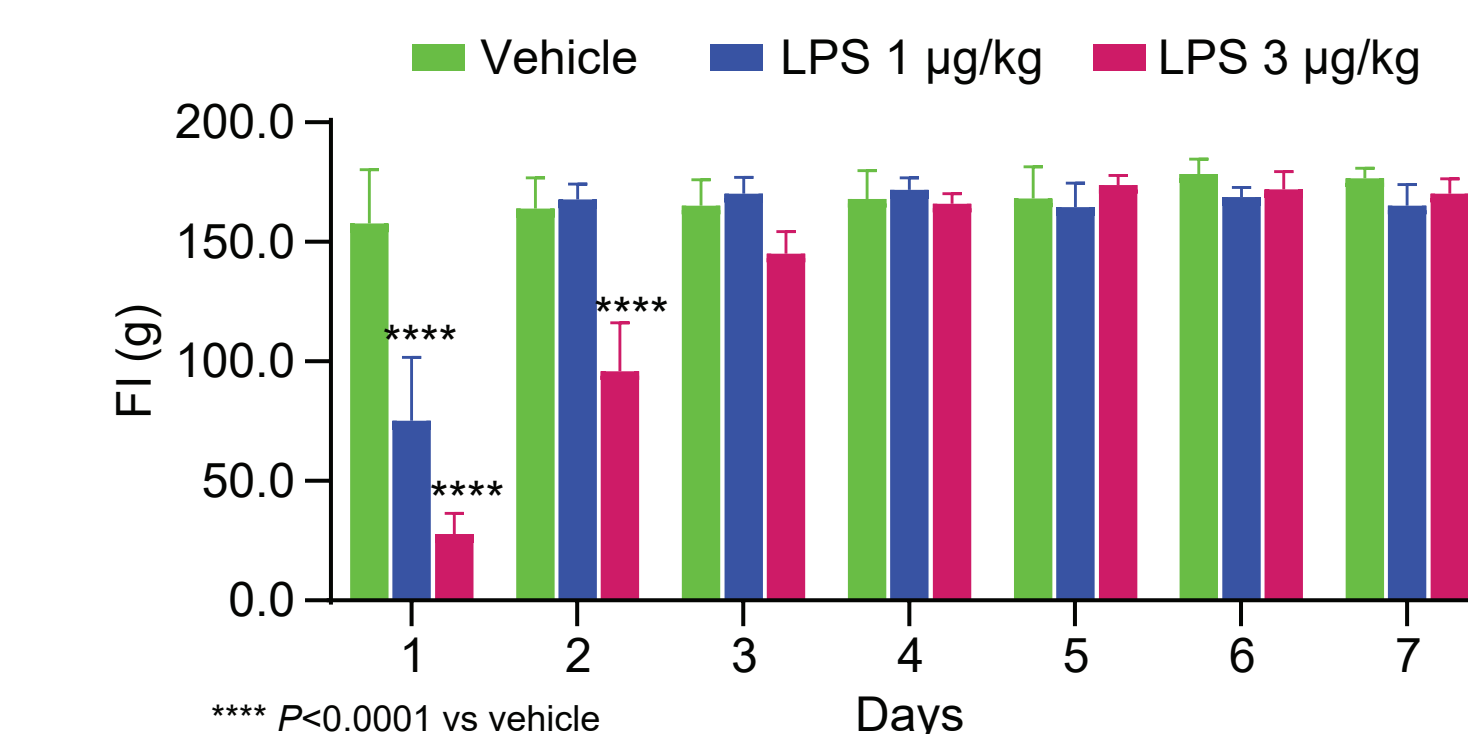
- Hyperthermia was observed in the 1  $\mu\text{g}/\text{kg}$  group for up to 6 hours
- Hypothermia was observed in the 3  $\mu\text{g}/\text{kg}$  group for up to 8 hours and was back to normal in 24 hours

Figure 4. Hematology results



- WBC, lymphocyte, and neutrophil counts were significantly changed from normal values less than 1 hour after LPS exposure. Those parameters were back to normal in 1-2 days.

Figure 5. Food intake



- Food consumption was significantly reduced in both LPS groups and back to normal 2 days after LPS exposure.

## Conclusion and discussion

- The variable changes that LPS induces in the body temperature of rabbits have been observed here. This variability is apparently related to the concentration of LPS. LPS is a component of the Gram-negative bacteria outer membrane and is composed of a complex glycolipid consisting of lipid A, a core polysaccharide, and peripheral O-antigenic chains. Lipid A is thought to be responsible for virtually all of the biological activities induced by LPS. Lipid A injections produce a hyperthermic response in cats and rabbits. This data suggests that hypothermia can be seen at relatively higher doses of LPS
- The phenomenon of hyperthermia causing low doses of LPS could not be repeated in our lab. This may be due to the animals becoming stressed by activities such as overhandling during the study and unfamiliar noises. This can be explained by the elevated creatine kinase levels seen in the animals during study, including the control group (data is not included)
- The inflammatory response to LPS was also observed in the hematology results. WBC, lymphocyte, and neutrophil counts were significantly changed from normal values less than 1 hour after LPS exposure. Those parameters returned to normal levels in 1-2 days
- Interpretation of WBC data from rabbits is different from other domestic species, including dogs, cats, and birds. With rabbits, although leukocytosis can be identified in cases that have been diagnosed with lymphosarcoma, it is not the usual response to inflammation. Laboratory investigations have shown no increase in the total number of circulating leukocytes in rabbits injected with bacteria or yeast, but fevers and lymphopenia could be observed. Rabbits with sepsis can show a variety of responses, including neutropenia and lymphopenia
- There are no significant blood chemistry changes in this study (data is not included). It suggests that although the inflammatory response has been observed, no further organ damage has occurred in these animals. The BT quickly returning to baseline can also correlate this observation

## References

- Melillo A. *J Exot Pet Med.* 2007;16(3):135-145.
- Yee SB, et al. *Comp Med.* 2013;63(3):252-261.
- Dogan MD, et al. *Life Sci.* 2000;67(19):2319-2329.
- Feldberg W, et al. *J Physiol.* 1975;249(3):601-615.
- Dey PK, et al. *J Physiol.* 1975;253(1):103-119.
- Kenedi E, et al. *J Physiol.* 1982;328:361-370.