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Naturally occurring hypothermia is more advantageous than fever in severe forms of lipopolysaccharide- and Escherichia coli-induced systemic inflammation

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1Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences, Albany, New York; 2Center for Immunology and Microbial Disease, Albany Medical College, Albany, New York; 3Thermoregulation Laboratory, Legacy Holladay Park Medical Center, Portland, Oregon; 4Therma Research, St. Joseph’s Hospital and Medical Center, Phoenix, Arizona; and 5Department of Microbiology and Immunology, New York Medical College, Valhalla, New York

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Liu E, Lewis K, Al-Saffar H, Krall CM, Singh A, Kulchitsky VA, Corrigan JJ, Simons CT, Petersen SR, Musteata FM, Bakshi CS, Romanovsky AA, Sellati TJ, Steiner AA. Naturally occurring hypothermia is more advantageous than fever in severe forms of lipopolysaccharide- and Escherichia coli-induced systemic inflammation. Am J Physiol Regul Integr Comp Physiol 302: R1372–R1383, 2012. First published April 18, 2012; doi:10.1152/ajpregu.00023.2012.—The natural switch from fever to hypothermia observed in the most severe cases of systemic inflammation is a phenomenon that continues to puzzle clinicians and scientists. The present study was the first to evaluate in direct experiments how the development of hypothermia vs. fever during severe forms of systemic inflammation impacts the pathophysiology of this malady and mortality rates in rats. Following administration of bacterial lipopolysaccharide (LPS; 5 or 18 mg/kg) or of a clinical Escherichia coli isolate (5 × 10^9 or 1 × 10^10 CFU/kg), hypothermia developed in rats exposed to a mildly cool environment, but not in rats exposed to a warm environment; only fever was revealed in the warm environment. Development of hypothermia instead of fever suppressed endotoxemia in Escherichia coli-infected rats, but not in LPS-injected rats. The infiltration of the lungs by neutrophils was similarly suppressed in Escherichia coli-infected rats of the hypothermic group. This exaggeration, however, occurred independently of changes in inflammatory cytokines and prostaglandins. Despite possible costs, development of hypothermia lessened abdominal organ dysfunction and reduced overall mortality rates in both the Escherichia coli and LPS models. By demonstrating that naturally occurring hypothermia is more advantageous than fever in severe forms of aseptic (LPS-induced) or septic (E. coli-induced) systemic inflammation, this study provides new grounds for the management of this deadly condition.

immunity; infection; sepsis; shock; temperature

THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)—TERMED “SEPSIS” WHEN IT RESULTS FROM INFECTION—is a deadly complication in critically ill patients. In SIRS, changes in leukocyte counts, respiratory rate, heart rate, and other bodily functions occur in the presence of an altered deep body temperature (Tb); for a review, see Ref. 27. An increase in Tb (fever) is the most common manifestation of SIRS and is believed to have an adaptive value for enhancing the ability of the immune system to eliminate invading microorganisms (19). However, as SIRS intensifies in severity, a switch from fever to hypothermia is often observed (49). It is presently unknown whether this switch in thermal state aids or harms subjects with severe forms of septic or aseptic SIRS.

The debate surrounding this fundamental matter is currently based on circumstantial evidence. The prevailing view is that naturally occurring hypothermia is detrimental in sepsis due to its association with poor prognosis (3, 8). Nevertheless, this association may purely reflect the fact that hypothermia is a consequence of severe sepsis and does not imply that hypothermia itself worsens the outcome. On the other side of the debate is the notion that a switch from fever to hypothermia represents an adaptive strategy of the host to cope with the deleterious effects of an overt inflammatory response (54). This notion stems from the observation that rats with severe forms of systemic inflammation “want” to be hypothermic, as they choose to stay in cooler environments that favor their ability to develop hypothermia naturally by suppressing thermogenesis (2, 53, 68). Indirect evidence in support of this notion is found in a study by Romanovsky et al. (52), in which bacterial LPS was used to induce SIRS in two independent experiments, one involving confined rats kept in a homogenous thermal environment and the other involving freely moving rats that could choose a preferred thermal environment. Compared with the confined rats, the freely moving rats responded to LPS with more pronounced hypothermia and exhibited a lower mortality rate. Although this association is interesting, Tb-unrelated factors, such as psychological stress, locomotor activity, feeding, and drinking, might have accounted for the observed differences in mortality rate. Another important consideration is that even if hypothermia happens to be beneficial when SIRS is induced aseptically by LPS, it might not be beneficial when SIRS is induced by an infection. Indeed, it is
not uncommon for interventions that improve the outcome of aseptic (LPS-induced) SIRS to worsen the outcome of septic SIRS (12, 47, 64).

The present study was the first to directly assess how naturally occurring hypothermia, as opposed to fever, impacts the pathophysiology of SIRS. This assessment was performed in rats injected with high, shock-inducing doses of bacterial LPS or *Escherichia coli*, because it is during the most severe cases of SIRS that a switch from fever to hypothermia occurs naturally (2). To either allow or prevent the development of hypothermia, we took advantage of the fact that this response is revealed when the environment is cool, but not when the environment is warm—a pattern consistent with hypothermia being brought about by inactivation of cold-defense effectors (53). In nonmortality experiments, four pathophysiological aspects of SIRS were evaluated: 1) endotoxemia and bacterial burden, which trigger the inflammatory response (11, 61); 2) lung inflammation, which is the primary component of the respiratory distress syndrome that compromises blood oxygenation and carbon dioxide elimination (34); 3) hypotensive shock, which compromises perfusion of vital organs (42); and 4) abdominal organ dysfunction, which frequently progresses to multiple organ failure (69). Mortality rates were evaluated in subsequent experiments.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats from Charles River Laboratories (Wilmington, MA) were studied in two centers under approval of the respective Animal Care and Use Committee. The nonmortality experiments and the *E. coli*-induced mortality experiment were conducted at the Albany College of Pharmacy and Health Sciences. The LPS-induced mortality experiment was conducted at the Legacy Holladay Park Medical Center. In both centers, the rats were caged with corn-cob bedding and had free access to tap water and standard rodent chow. The colony rooms were maintained under a 12:12-h light-dark cycle (lights on at 7 AM) and at a temperature of 23–26°C. The rats were housed in groups until they were subjected to the surgical procedures, at which time a single housing was necessary. The rats weighed 240–390 g at the time of the experiments. Each rat was used in an experiment once and, if pertinent (nonmortality experiments), euthanized with pentobarbital sodium (100 mg/kg ip) at the end of the experiment.

**SIRS-inducing agents.** LPS from *E. coli* 0111:B4 (Sigma-Aldrich, St. Louis, MO) was used to induce SIRS in the absence of an infection. LPS suspensions (2.5 and 9 mg/ml) were prepared, stored at 4°C, and used in an experiment within 2 wk. The suspensions were administered over a volume of 2 ml/kg to deliver doses of 5 mg/kg (nonmortality experiments) and 18 mg/kg (mortality experiment). A clinically relevant strain of *E. coli* was used to induce SIRS in the presence of an infection (sepsis). The strain was originally isolated from the blood of a septic patient by the Clinical Microbiology Department at John Dempsey Hospital (University of Connecticut Health Sciences Center, Farmington, CT) as part of routine clinical care. The strain was provided by the clinical laboratory as a deidentified specimen for use in research. For preparation of bacterial inoculums, *E. coli* were grown in LB medium at 37°C. At the active mid-log phase of growth, the medium was aliquoted, snap frozen under liquid nitrogen, and stored at −80°C. Bacterial viability and concentration in the frozen medium were determined by serial dilution of a thawed aliquot followed by plating on LB agar. On the day of an experiment, an aliquot was thawed, centrifuged (6,000 g, 15 min, 4°C), and the resulting pellet was suspended in saline to achieve bacterial concentrations of 2.5 × 10^6 and 5.0 × 10^8 CFU/ml. The bacterial suspensions were administered over a volume of 2 ml/kg to deliver doses of 5 × 10^8 CFU/kg (nonmortality experiments) and 1 × 10^10 CFU/kg (mortality experiment).

**Surgery.** Seven to four days prior to an experiment, a rat was chronically implanted with a transponder (G2 E-Mitter; Mini Mitter, Bend, OR), an arterial catheter and/or a venous catheter, as necessary (see *Experimental setups and in vivo measurements*). The procedures were performed aseptically under anesthesia (ketamine-xylazine-acetpromazine, 80:8:1 mg/kg ip) and antibiotic protection (enrofloxac in, 5 mg/kg sc), while the rat was maintained on an operating board warmed to 37°C.

The temperature-measuring transponder was implanted via a midline laparotomy and secured to the internal side of the dorsolateral abdominal wall with silk thread. For the arterial catheterization, the left carotid artery was isolated and temporarily clamped. The tip of a 3-Fr polyurethane catheter filled with heparinized saline was inserted into the jugular vein and advanced toward the superior vena cava. The arterial and venous catheters were secured in place with ligatures. The distal end of each catheter was closed with a stainless-steel plug, tunneled under the skin, and exteriorized at the nape. After the surgical wounds were closed, the time taken for a rat to regain consciousness was typically 2–3 h.

The rats were monitored daily following surgery, and their catheters were flushed with heparinized saline. In general, it took less than 2 days for a rat to display weight gain, as well as normal grooming, locomotor, and exploratory behaviors. The few rats that lost ≥20% of body mass, or that showed signs of behavioral impairment, were not used in the experiments; they were euthanized with pentobarbital sodium (100 mg/kg ip).

**Experimental setups and in vivo measurements.** The nonmortality experiments involving LPS and *E. coli*, as well as the *E. coli*-induced mortality experiment, were conducted at the Albany College of Pharmacy and Health Sciences in freely moving rats preimplanted with abdominal temperature transponders and arterial catheters. At different time points after the day of the experiment, rats housed singly in their home cages were placed inside an environmental chamber (model NQ1; Environmental Growh Chambers, Chagrin Falls, OH). Under each cage was an ER-4000 receiver (Mini Mitter), which captured the radio frequency of the abdominal temperature transponder and conveyed it to a computer, where the signal was processed and recorded by the Vital View software. Each arterial catheter was extended with polyethylene (PE)-50 tubing filled with saline and passed to the exterior of the chamber via a swivel (Instech Laboratories, Plymouth Meeting, PA). An infusion harness worn by the rat and a spring coil protected the extension from bites and scratches. The arterial line was employed for measurements of arterial pressure and heart rate using the Datamax system (Columbus Instruments, Columbus, OH). The arterial line was also employed for administration of LPS, *E. coli*, or saline. These agents were injected in bolus at 11 AM. In nonmortality experiments, the doses of LPS and *E. coli* were 5 mg/kg and 5 × 10^6 CFU/kg, respectively. In the *E. coli*-induced mortality experiment, the bacterial inoculums were injected at a dose of 1 × 10^10 CFU/kg. Even though these doses differ in their ability to cause mortality, they do not differ in their ability to induce hypothermia in rats exposed to a cool environment (2, 7, 65). Several groups of rats in the nonmortality experiments were designated for tissue harvesting. At the time point of interest (80, 240, or 360 min), rats in these groups were quickly euthanized with anesthetic (ketamine-xylazine: 8:1 mg/kg) via the arterial line; then samples were collected and processed as described below (see *Ex vivo measurements*).
ical Center. Because this setup was not used for measurement of arterial pressure, the rats did not need to be implanted with arterial catheters. Venous catheters were employed for LPS administration in this setup. At ~7 AM on the day of the experiment, each rat had its venous catheter extended with PE-50 tubing filled with saline and was placed inside a conical confiner to which it had been extensively adapted. The rat was equipped with a colonic thermocouple connected to a Digi-Sense thermometer (Cole-Parmer, Vernon Hills, IL), which conveyed Tb data to a computer. The instrumented rat was then transferred to an environmental chamber (model 3940; Forma Scientific, Marietta, OH). At 11 AM, LPS was injected in bolus at a dose of 18 mg/kg. This dose corresponds to the LD50 for LPS in many rat experiments (14, 39). Tb measurements in this setup were interrupted at 7 PM, when the rats were returned to their home cages. From then on, the rats were visually monitored for the presence of respiratory and locomotor activities.

**Ex vivo measurements.** For determination of endotoxin levels, blood was collected from the inferior vena cava, allowed to clot for 20 min at room temperature, and centrifuged (13,000 g, 8 min, 4°C). The resulting serum was stored at ~80°C until assay. Endotoxin concentration was determined by a kinetic limulus amebocyte lysate assay (Endochrome-K; Charles River Endosafe, Charleston, SC). The limulus amebocyte lysate was reacted with endotoxin standards or samples at 37°C for 60 min, and the course of the reaction was monitored by absorbance readings at 405 nm using a Synergy 2 microplate reader and the Gen 5 software (Biotek, Winoski, VT). Endotoxin concentration was determined on the basis of the Vmax of the reaction, the kinetic parameter that most strongly correlated (r2 = 0.986, four parameter fitting) with endotoxin concentrations over the range of 0.0064 to 100 EU/ml. All samples and standards were assayed in triplicate. If necessary, samples were diluted with endotoxin-free water prior to assay.

For determination of bacterial burden, blood (1 ml) was collected from the inferior vena cava and mixed with heparin (5 IU). The central lobe of the liver and the right lung were excised, weighed, and then homogenized in PBS using a Mimi-Bead Beater-8 (BioSpec Products, Bartlesville, OK). The tissue homogenates were centrifuged at 1,000 g for 2 min to pellet tissue debris but not bacterial cells. The heparinized blood and the tissue homogenate supernatants were serially diluted and plated on LB agar. After 24 h of incubation at 37°C, plates that displayed isolated colonies were subjected to colony counting for CFU determination.

For histopathological assessment of the lungs, rats were subjected to transcardial perfusion with saline (30 ml) via the right ventricle. Their lungs were then excised and fixed by immersion in 10% buffered formalin at 4°C for at least 48 h, after which the lung specimens were cryoprotected in 20% sucrose for another 48 h at 4°C. The specimens were then frozen, cryosectioned (6-μm thickness), and stained with hematoxylin-and-eosin. The stained sections were examined blind-folded using a histopathological scoring system, according to which sections were graded on a scale of 0 (least severe) to 4 (most severe) with regard to the following parameters: neutrophil infiltration, perivascular edema, interstitial edema, peribronchiolar inflammation, and hemorrhagic foci (details in Table 1). The scoring system was developed for this study based on the main histopathological characteristics of lungs during the respiratory distress syndrome (34).

**Table 1. Scoring system used to assess lung inflammation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Severity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil infiltration</td>
<td>0</td>
</tr>
<tr>
<td>Perivascular edema</td>
<td>None</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>None</td>
</tr>
<tr>
<td>Peribronchiolar inflammation (% of bronchioles affected)</td>
<td>None</td>
</tr>
<tr>
<td>Hemorrhagic foci</td>
<td>None</td>
</tr>
</tbody>
</table>

For determination of inflammatory mediators, blood from the inferior vena cava was collected and placed into EDTA-coated Eppendorf tubes. The tubes were centrifuged (6,000 g, 10 min, 4°C), and the resulting plasma was stored at ~80°C until assay. The plasma levels of TNF-α, IL-1β, and IL-6 were determined by sandwich ELISA using kits from Thermo Scientific (manufactured by Pierce Biotechnology, Rockford, IL) and a Synergy 2 microplate reader (Biotek). Plasma samples were assayed at a dilution of 1:200 in the TNF-α assay, 1:50 in the IL-6 assay, and 1:20 in the IL-1β assay. Assay ranges were 25.6–2,500 pg/ml for TNF-α and IL-1β, and 31–2,000 pg/ml for IL-6. All samples were run simultaneously, in duplicate.

The plasma samples were also assayed for PGE2, PGD2, PGF2α, and 6-keto-PGF1α (a stable PGI2 metabolite) by LC-MS/MS. Each sample (900 μl) was acidified to pH 3 with HCl, spiked with an internal standard (PGE1, a synthetic prostaglandin analog), and subjected to solid-phase extraction in a C18 Sep-Pak column (Waters, Milford, MA) via sequential washings with water (2 ml), 10% methanol in water (2 ml), and methyl formate (2 ml). The methyl formate fraction was dried under a stream of nitrogen, and the residue was reconstituted in 20 μl of methanol. Each sample (10 μl) was introduced into a Waters ACQUITY system (Waters, Milford, MA). Separation was performed on a 2.1 × 50 mm C18 column packed with 1.7-μm particles (BEH C18, Waters), which was eluted (0.1 ml/min) with acetonitrile and water containing 0.1% formic acid, according to a gradient program: the acetonitrile-water ratio was kept at 0.61 for 2.2 min, increased linearly to 19 for 5.3 min, and then decreased linearly back to 0.61 over 2.5 min. Detection was done by negative ion electrospray ionization tandem mass spectrometry using the following parameters: source temperature of 150°C, desolvation temperature of 400°C, capillary voltage of ~3.6 kV, cone voltage of ~30 V, and argon gas collision pressure and flow rate of 11 psi and 0.3 ml/min, respectively. The analytes and the internal standard were detected by selected reaction monitoring using the following m/z transitions: 351.3 → 271.3 for PGE2 and PGD2, 353.4 → 193.2 for PGF2α, 369.4 → 163.1 for 6-keto-PGF1α, and 353.4 → 317.3 for PGE1. Quantification was linear (r2 = 0.9992) for concentrations ranging from 0.5 ng/ml to 200 ng/ml.

To assess markers of abdominal organ dysfunction, blood collected from the inferior vena cava was allowed to clot at room temperature for 20 min, and then centrifuged (13,000 g, 8 min, 4°C). The resulting serum was stored at 4°C for 24–48 h until analysis. Alanine transaminase, total bilirubin, creatinine, blood urea nitrogen, and lipase were measured by a certified clinical laboratory (IDEXX, North Grafton, MA).

**Statistical analyses.** The analyses were performed using Statistica Advanced 8.0 (StatSoft, Tulsa, OK) with the level of significance set at P < 0.05. The time courses of Tb, arterial pressure, and heart rate responses were compared by repeated-measures ANOVA followed by the Fisher least significant difference (LSD) test. Linear regression was performed to quantitatively evaluate the interactions of Tb with arterial pressure and heart rate. The levels of endotoxin, viable bacterial cells, inflammatory mediators and organ dysfunction mark-
Thermal responses in nonmortality experiments. LPS (5 mg/kg), *E. coli* (5 × 10⁹ CFU/kg) or their vehicle (saline) was administered via extensions of preimplanted arterial catheters to freely moving rats maintained inside an environmental chamber. The environmental chamber maintained an ambient temperature (Ta) of either 22°C or 28°C. This 6°C difference in Ta had no influence on the Tb of healthy rats, either before or after administration of saline (Fig. 1). However, this same difference in Ta had a major impact on the ability of rats to develop hypothermia in response to the high doses of LPS or *E. coli*. At a Ta of 22°C, both LPS- and *E. coli*-injected rats mounted hypothermic responses (P < 0.001 for LPS or *E. coli* vs. saline). The hypothermic response to LPS was monophasic and characterized by having an early onset (~20 min), a magnitude of ~2°C (at 70–90 min), and a duration of ~180 min. The hypothermic response to *E. coli* was biphasic, with its first phase corresponding in time to the monophasic hypothermia induced by LPS. At a Ta of 28°C, however, neither LPS- nor *E. coli*-injected rats mounted hypothermic responses. On the contrary, they developed febrile responses with maximal Tb rises of 1.5–2.0°C (P < 0.001 for LPS or *E. coli* vs. saline). The data from *E. coli*-infected rats are not shown beyond 300 min because of two (out of fifteen) unintended instances of mortality shortly after this time point.

Endotoxin levels and bacterial burden in nonmortality experiments. Endotoxemia was evaluated at two stages of SIRS: an “early” stage that corresponds to the first wave of inflammatory and physiological responses [80 min after LPS (53, 63) or *E. coli* (7)], and a “late” stage in which organ dysfunction becomes evident [360 min after LPS (56); 240 min after *E. coli* (present study)]. These stages were defined for the purpose of the present study and do not necessarily reflect a definition in clinical SIRS. As shown in Fig. 2, following a single bolus injection of LPS, considerable amounts of endotoxin were found in the blood serum, not only during the early stage, but also during the late stage. The early endotoxemia in this model likely reflects detection of the injected LPS itself. The late (lasting) endotoxemia has been reported previously (16), but the origin of endotoxin in this case (exocytosis by phagocytic cells, disruption of intestinal barrier, etc.) is less certain.
Regardless of origin, endotoxin levels were not affected by the thermal state of the LPS-injected rats. On the other hand, endotoxin levels were dependent on the thermal state in rats with an actual *E. coli* infection. Such a dependence was evident during the early stage of *E. coli*-induced SIRS, when endotoxin levels were significantly lower (*P* = 0.007) in those rats that were allowed to develop hypothermia (T_a of 22°C) than in those rats that were not (T_a of 28°C). This difference was no longer present during the late stage of *E. coli*-induced SIRS, when most of the circulating endotoxin had been cleared from the blood in this model. No endotoxin was detected in the serum of the saline-injected controls.

Regarding bacterial burden, viable bacteria were detected in blood, liver, and lungs of rats infected with *E. coli*, but not in tissues from rats injected with LPS or saline. During both the early and late stages of *E. coli*-induced SIRS, bacterial burden was highest in the liver, intermediary in the lungs, and lowest in the blood (Fig. 2B). The thermal state of the rats did not have any impact on bacterial burden during the early stage of SIRS, nor did it have widespread effects on bacterial burden during the late stage of SIRS. However, a selective increase in the liver bacterial burden was observed during the late stage of SIRS in rats that developed hypothermia at a T_a of 22°C, compared with rats that developed fever at a T_a of 28°C (*P* < 0.001).

**Lung inflammation in nonmortality experiments.** Lung sections from saline-injected rats presented the tissue architecture of uninjured lungs, i.e., thin-walled alveoli surrounded by capillaries (Fig. 3). At 360 min post-LPS and 240 min post-*E. coli* administration, lung inflammation was verified by the presence of infiltrating neutrophils, edema, and hemorrhagic foci (Fig. 3). A quantitative analysis of severity scores revealed that lung inflammation was influenced by the thermal state in the *E. coli* model, but not in the LPS model (Table 2). Specifically, the neutrophil infiltration scores were significantly lower (*P* = 0.016) in the *E. coli*-infected rats that developed hypothermia than in their febrile counterparts (Table 2). This effect was confirmed by neutrophil counts in three random high-magnification (×1,000) fields per lung section: 8.5 ± 0.5 neutrophils/field were present in the hypothermic group, whereas 14.0 ± 1.0 neutrophils/field were present in the febrile group (*P* < 0.001). The attenuated neutrophil infiltration was associated with a strong tendency (*P* = 0.070) for reduction of perivascular edema (Table 2). Interstitial edema, peribronchiolar inflammation, and hemorrhages did not differ between the thermally distinct groups at the time point studied (240 min post-*E. coli*).

**Hypotensive shock in nonmortality experiments.** Mean arterial pressure fell transiently following injection of LPS or *E. coli*, but not following injection of saline (Fig. 4). These hypotensive responses had an onset at 30–40 min and reached a maximum at 70–80 min. The magnitude and duration of the hypotensive responses were significantly augmented in rats of the hypothermic group compared with rats of the febrile group [P ≤ 0.045 for the LPS model (70–160 min); P ≤ 0.049 for the *E. coli* model (60–300 min)]. Heart rate also changed following LPS or *E. coli* administration (Fig. 4). These changes consisted of tachycardic responses that began prior to the hypotensive responses and outlasted them. The tachycardic responses were less pronounced in rats of the hypothermic group than in those of the febrile group. Suppression of tachycardia in the hypothermic group occurred over two distinct time windows, an early window from 30 to 80 min (*P* ≤ 0.011 for the LPS model; *P* ≤ 0.045 for the *E. coli* model) and a late window from ~170 min to the end of the experiment (*P* ≤ 0.030 for the LPS and *E. coli* models).

Linear regression analysis was performed to identify the time points at which the arterial pressure-T_b and the heart rate-T_b correlations were at their strongest and to estimate the rates of change in arterial pressure and heart rate as a function of T_b at each of those time points. The analysis included data from LPS- and *E. coli*-injected rats of both the hypothermic and febrile groups. The strongest arterial pressure-T_b correlations were asynchronous with the strongest heart rate-T_b correlations (Fig. 5A). At 80 min, when the arterial pressure-T_b correlations were strong and statistically significant, arterial pressure changed as a function of T_b, but not in the LPS model. At 280 min, the arterial pressure-T_b correlations were strong and statistically significant, the rate of change was ~8 mmHg/°C, which, over the T_b range of 35–38°C, corresponded to Q_{10} values of 2.6 and 2.3 for the LPS and *E. coli* models, respectively (Fig. 5B). At 280 min, when the heart rate-T_b correlations were strong and statistically significant, the rate of change was ~33 bpm/°C, which, over the T_b range of 35–38°C, corresponded to Q_{10} values of 2.5 and 2.0 for the LPS and *E. coli* models, respectively (Fig. 5C).
We also examined whether augmentation of hypotension during hypothermia might be associated with an exaggerated inflammatory response. We measured proinflammatory cytokines (TNF-α and IL-1β) known to mediate hypotensive shock (55), as well as a cytokine (IL-6) that reliably indicates the overall proinflammatory status (15). In addition, we measured the active products of three major prostaglandin pathways (PGE_{2}, PGD_{2}, and PGF_{2α}) and the metabolite of another major prostaglandin pathway (6-keto-PGF₁α, aPGI_{2} metabolite); at least one of these products is likely involved in the mediation of hypotensive shock by cyclooxygenase enzymes (63). Measurements were made in plasma collected at 80 min, when hypotension was augmented in association with development of hypothermia in both the LPS and E. coli models. The cytokines and prostaglandins measured were barely detectable or undetectable in saline-injected rats, but they were all detectable and elevated in LPS- and E. coli-injected rats (Table 3).

The thermal state of the rats affected neither the LPS-induced nor the E. coli-induced changes in the levels of the inflammatory mediators measured.

Abdominal organ dysfunction in nonmortality experiments.

The levels of organ dysfunction markers are depicted in Fig. 6. Alanine transaminase and bilirubin are markers of hepatic dysfunction (37). The levels of these markers did not differ statistically between LPS- and saline-injected rats, but they were significantly elevated in E. coli-infected rats (P < 0.001)

Table 2. Histopathological scores for lungs from rats injected with saline, LPS, or E. coli at a Ta of 22°C (hypothermia in SIRS) or 28°C (fever in SIRS)

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Neutrophil Infiltration</th>
<th>Perivascular Edema</th>
<th>Interstitial Edema</th>
<th>Peribronchial Inflammation</th>
<th>Hemorrhages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>LPS at Ta of 28°C</td>
<td>3.0 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>2.9 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>(fever)</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
</tr>
<tr>
<td>LPS at Ta of 22°C</td>
<td>2.8 ± 0.6</td>
<td>2.2 ± 0.6</td>
<td>2.4 ± 0.5</td>
<td>3.0 ± 0.7</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>(hypothermia)</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
</tr>
<tr>
<td>E. coli at Ta of 28°C</td>
<td>3.6 ± 0.3</td>
<td>3.2 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>3.1 ± 0.5</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>(fever)</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
</tr>
<tr>
<td>E. coli at Ta of 22°C</td>
<td>2.5 ± 0.4#</td>
<td>2.2 ± 0.4†</td>
<td>2.9 ± 0.3</td>
<td>2.7 ± 0.4</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>(hypothermia)</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
</tbody>
</table>

Lungs were harvested 360 min following LPS administration (5 mg/kg), 240 min following E. coli administration (5 × 10⁵ CFU/kg), and 240–360 min following saline administration. The number of animals (n) is indicated. #Significant difference (P < 0.05) in the hypothermic group compared to the corresponding febrile group. †Strong tendency towards a difference (P = 0.07) in the hypothermic group compared to the corresponding febrile group. SIRS, systemic inflammatory response syndrome.
for both markers). Whereas the elevation in bilirubin was not dependent on the thermal state, the elevation in alanine transaminase was. Specifically, alanine transaminase levels in the *E. coli* model were 55% lower in rats that developed hypothermia at a Ta of 22°C than in rats that developed fever at a Ta of 28°C (P = 0.017).

Creatinine and urea are markers of renal dysfunction (46); urea levels may also reflect muscle wasting (30). Compared with saline administration, these markers were elevated by administration of both LPS (P < 0.001) and *E. coli* (P ≤ 0.022). Creatinine levels were dependent on the thermal state in both the LPS and *E. coli* models of SIRS, with rats of the hypothermic group exhibiting lower levels (P = 0.021 for the LPS model; P = 0.035 for the *E. coli* model). Urea levels, on the other hand, were not affected by the thermal state.

Circulating lipase is a marker of pancreatic dysfunction (5). Compared with saline, LPS and *E. coli* significantly (P ≤ 0.039) elevated lipase levels in rats of the febrile group, but they exerted no effect on this marker in rats of the hypothermic group.

**Mortality experiment.** This experiment involved higher doses of LPS (18 mg/kg) and *E. coli* (1 × 10^{10} CFU/kg). The setup used in the *E. coli* mortality experiment was identical to that used in nonmortality experiments, in which rats were freely moving, insulated by bedding, and exposed to a Ta of 22°C or 28°C. The setup employed in the LPS mortality experiment involved confiners to which the rats had been extensively adapted. Because the confiners offered a lower level of thermal insulation (compared to bedding), higher ambient temperatures had to be used in the LPS mortality experiment (25.5°C and 31.5°C). The magnitude of the difference in Ta between setups was chosen on the basis of direct physiological assessments of the thermoneutral zone using the tail-vasodilation method (23, 50). Injection of LPS or *E. coli* under these conditions led to development of thermoregulatory responses that resembled those observed in nonmortality experiments.

The development of hypothermia (vs. fever) exerted a pronounced influence on survival rates. In the LPS model, 74% of the rats in the hypothermic group survived until the end of the experiment, but only 54% of the rats in the febrile group

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**Table 3.** Plasma levels of inflammatory mediators 80 min after administration of saline, LPS, or *E. coli* in rats exposed to a Ta of 22°C (hypothermia in SIRS) or 28°C (lack of hypothermia in SIRS)

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>TNF-α (ng/ml)</th>
<th>IL-1β (ng/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>PGE₂ (ng/ml)</th>
<th>PGD₂ (ng/ml)</th>
<th>6k-PGF₁α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.12 ± 0.03</td>
<td>n.d.</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>LPS at Ta of 28°C</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 11</td>
<td>n = 9</td>
<td>n = 11</td>
</tr>
<tr>
<td>(fever)</td>
<td>145 ± 29</td>
<td>1.9 ± 0.5</td>
<td>60 ± 13</td>
<td>1.10 ± 0.15</td>
<td>0.92 ± 0.06</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>LPS at Ta of 22°C</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>(hypothermia)</td>
<td>108 ± 27</td>
<td>2.1 ± 0.6</td>
<td>52 ± 15</td>
<td>1.10 ± 0.24</td>
<td>0.98 ± 0.20</td>
<td>0.36 ± 0.11</td>
</tr>
<tr>
<td><em>E. coli</em> at Ta of 28°C</td>
<td>n = 6</td>
<td>n = 5</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>(fever)</td>
<td>89 ± 8</td>
<td>10.7 ± 1.1</td>
<td>37 ± 7</td>
<td>0.19 ± 0.04</td>
<td>0.39 ± 0.06</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td><em>E. coli</em> at Ta of 22°C</td>
<td>n = 6</td>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>(hypothermia)</td>
<td>78 ± 12</td>
<td>10.2 ± 1.0</td>
<td>33 ± 3</td>
<td>0.25 ± 0.04</td>
<td>0.47 ± 0.03</td>
<td>0.13 ± 0.03</td>
</tr>
</tbody>
</table>

LPS was administered at 5 mg/kg, while *E. coli* was administered at 5 × 10⁶ CFU/kg. n.d., nondetectable. The number of animals (n) is indicated.
survived. Survival rates in these groups were statistically different ($P = 0.007$) from 4 to 48 h post-LPS (Fig. 7A). However, at earlier time points, the survival rate tended ($P = 0.100$) to be higher in the febrile group. This intriguing observation was related to a difference in the time to death of the rats that succumbed, which was significantly longer ($P = 0.007$) in the febrile group than in the hypothermic group (Fig. 7B).

**DISCUSSION**

The present study breaks new ground for providing the first evaluation of how development of hypothermia vs. fever during severe forms of SIRS impacts the pathophysiology of this malady and mortality rates in rats. Such an evaluation relates to an unanswered, fundamental clinical question: Should those septic patients who develop hypothermia be allowed to stay hypothermic or should they be rewarmed? Although it is not uncommon for septic patients who become hypothermic to be rewarmed with heating blankets (6, 22), the value of this therapeutic strategy has not undergone careful scrutiny. From a basic science perspective, a switch from fever to hypothermia appears to be a deliberate reaction of rats to systemic inflammation of increasing severity (2) and an evolutionarily conserved phenomenon in vertebrates (36, 40). It is, therefore, conceivable to propose that it may have some biological values. Here, potential benefits, and costs, of a fever-hypothermia switch were evaluated in two rat models of severe SIRS: an aseptic model in which SIRS was induced by a high dose of LPS, and a septic model in which SIRS was induced by a high dose of *E. coli*. Rats with either form of SIRS were exposed to a cool environment that allowed them to develop hypothermia naturally, or they were exposed to a warm environment that did not allow them to develop hypothermia, thereby favoring the manifestation of fever. This approach differs considerably from previous studies involving forceful cooling of septic subjects under anesthesia, which yielded contradictory results; see, for example, L’Her et al. (25) vs. Torossian et al. (67).

Development of hypothermia suppressed the early surge in circulating endotoxin in the *E. coli* model of SIRS. This suppression was probably unrelated to changes in the rate of endotoxin clearance because it was not observed in rats injected with purified endotoxin (LPS). Most likely, $T_b$ impacted endotoxemia by influencing bacteriolysis, bacterial shedding, or both. The importance of bacteriolysis for the endotoxin surge in Gram-negative sepsis has been demonstrated and seems to reflect extracellular bacterial killing by complement and neutrophils (10, 26). Whereas the effects of temperature on complement activity are still uncertain (35, 59), a decrease in temperature has been reported to inhibit neutrophil cytotoxicity (1, 70). With regard to bacterial shedding, Mackowiak (32) has demonstrated that, at least in non-heat-adapted *E. coli*, temperature-dependent changes in the shedding rate parallel changes in the growth rate, both being lower at hypothermic temperatures.

Given that endotoxin plays upstream roles in the pathogenesis of Gram-negative sepsis (11, 61), suppression of endotoxemia during hypothermia may favorably impact downstream events. This may be the case for neutrophil infiltration in lungs. Like endotoxemia, neutrophil infiltration was attenuated in hypothermic rats of the *E. coli* model, but not in those of the LPS model. This model selectivity cannot be explained by a general effect of $T_b$ on neutrophil infiltration. In fact, not all
Another important finding of the present study is that development of hypothermia instead of fever exaggerated the fall in arterial pressure (hypotension) induced by either LPS or \textit{E. coli}. This exaggeration occurred in the absence of systemic changes in inflammatory mediators. The existence of multiple opposing effects of temperature on cellular and molecular inflammatory events \cite{13, 31, 33, 71} may explain the lack of an overall change in inflammatory status, at least early in SIRS. The hypothermia-related exaggeration of hypotension may reflect the direct effects of \( T_b \) on the cardiovascular system. In line with this idea, we found that development of hypothermia not only lowered arterial pressure during hypotension, but also lowered heart rate during tachycardic responses to LPS and \textit{E. coli}. The rates of change in arterial pressure and heart rate as a function of \( T_b \) (Q10 of 2.0–2.6) were consistent with the rates reported for the direct influence of temperature on cellular events related to cardiac, vascular, and autonomic functions \cite{9, 18, 43, 44}. Also of importance is the finding that \( T_b \) impacted arterial pressure and heart rate in an asynchronous manner, which suggests a loss of reciprocity between arterial pressure and heart rate, as well as the involvement of distinct \( T_b \)-dependent mechanisms. Loss of reciprocity may reflect an impairment of the cardiac component of the baroreceptor reflex due to inflammation \cite{45, 58}, hypothermia \cite{57}, or a combination of the two. The distinct \( T_b \)-dependent mechanisms involved have yet to be identified, but it is worth noting that sympathetic outflow to blood vessels and the heart are typically changed independently and in opposite directions during SIRS \cite{45, 58}. This independence might allow \( T_b \) to impact sympathetic outflow to vessels and the heart with a different time course.

By exaggerating hypotension (lower perfusion pressure) and suppressing tachycardia (lower cardiac output), hypothermia may exacerbate the drop in tissue perfusion during SIRS. If this exacerbation is not completely matched by the metabolic inhibition that accompanies regulated hypothermia \cite{54, 62}, the severity of tissue hypoxia in SIRS will be increased. On the other hand, if tissue perfusion falls less than metabolic rate during hypothermia, tissue hypoxia will be alleviated. Future studies will be necessary to draw cost-benefit relationships for the interplay among metabolic rate, \( T_b \), and cardiovascular function in SIRS.

Perhaps most remarkable is the fact that development of hypothermia instead of fever lessened abdominal organ dysfunction and reduced overall mortality rates, thus indicating that the overall benefits of a fever-hypothermia switch outweigh the costs. Renal and pancreatic dysfunctions were similar in the LPS and \textit{E. coli} models and were similarly affected by the thermal state in both models. Liver dysfunction was more pronounced in the \textit{E. coli} model than in the LPS model, as was the ability of hypothermia to protect this organ. The
mechanisms by which hypothermia protects abdominal organs in the most severe forms of SIRS have yet to be established, but some speculation is possible. First, it should be considered that a hypothermia-associated increase in bacterial burden, within certain limits, does not appear to adversely impact organ function, as was the case for the liver in the present study. Additionally, it should be considered that suppression of endotoxemia and alleviation of lung inflammation (with improved blood oxygenation) might contribute to organ protection in the *E. coli* model. However, the same mechanisms cannot explain alleviation of renal and pancreatic dysfunctions in the LPS model. It is likely that *Tb*-dependent cellular processes contribute to organ protection during hypothermia. In this context, inhibition of metabolic demands in multiple organs during regulated hypothermia (54, 62) may contribute to maintain, at least partly, aerobic metabolism when tissue perfusion decreases during hypotensive shock. Furthermore, hypothermia has been shown to suppress oxidative stress during tissue hypoperfusion (21, 48). Via attenuation of oxidative stress or via other mechanisms (e.g., “heat-shock proteins”), hypothermia may ultimately preserve mitochondrial integrity and calcium homeostasis in hypoperfused tissues (41, 48). The relevance of these mechanisms to SIRS remains to be determined.

It is intriguing that some *E. coli*-infected rats of the hypothermic group died earlier than their febrile counterparts, despite the fact that the overall mortality rate was lower in the hypothermic group. Because this phenomenon was not observed in the LPS-injected rats, it might be related to the impact of the thermal state on bacterial burden. Even though a hypothermia-associated accumulation of bacteria in the liver did not adversely impact liver function, it might have triggered mortality prematurely via other mechanisms. A mechanism to consider is disseminated intravascular coagulation, which is activated not only by LPS but also by other *E. coli*-associated molecular patterns (60), and which may be amplified at a lower *Tb* (28). If that were the case, early mortality could be eliminated by adjunct anticoagulant therapy.

In conclusion, the present study demonstrates that, in the most severe cases of SIRS, natural development of hypothermia may be more advantageous than development of fever, not only when SIRS is induced aseptically by LPS, but also when it is induced septicly by *E. coli*. The advantages of hypothermia over fever include, but are not limited to, suppression of endotoxemia and of the lung infiltration by neutrophils. These advantages are likely to come with costs related to an increase in the liver bacterial burden or an exaggeration of hypotensive shock. However, regardless of potential costs, development of hypothermia instead of fever alleviates multiple organ dysfunction and reduces overall mortality rates. These findings challenge the view that naturally occurring hypothermia is detrimental in severe sepsis.

**Perspectives and Significance**

Since the seminal study by Kluger and colleagues (20), the notion that fever is an adaptive response to infection has gained considerable support. We do not dispute that notion. Rather, we work with the hypothesis that fever and hypothermia represent two distinct adaptive strategies in the face of an infection, each beneficial under different conditions. Originally proposed by Romanovsky and colleagues (51, 54), this hypothesis lacked strong experimental support until now. The present study was the first to clearly demonstrate that naturally occurring hypothermia aids the infected host better than fever in the most severe forms of the systemic inflammatory response. In overwhelming inflammation, the need for elimination of invading microorganisms seems to give way to the need for preservation of vital bodily functions threatened by the injurious consequences of the inflammatory response itself. We and others have also suggested that comorbidities, such as preexisting pathology and malnutrition (23, 51), and even physiological conditions, such as pregnancy (68), may play decisive roles with respect to the value of the fever-hypothermia switch. More animal studies are necessary to put these suggestions to the test. Ultimately, clinical trials are required to evaluate whether human lives could be actually saved by not rewarming those patients who naturally become hypothermic during sepsis or during aseptic forms of SIRS (e.g., trauma, disease).

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