Endotoxin depresses heart rate variability in mice: cytokine and steroid effects

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Submitted 6 March 2009; accepted in final form 28 July 2009

Fairchild KD, Saucerman JJ, Raynor LL, Sivak JA, Xiao Y, Lake DE, Moorman JR. Endotoxin depresses heart rate variability in mice: cytokine and steroid effects. Am J Physiol Regul Integr Comp Physiol 297: R1019–R1027, 2009. First published August 5, 2009; doi:10.1152/ajpregu.00132.2009.—Heart rate variability (HRV) falls in humans with sepsis, but the mechanism is not well understood. We utilized a mouse model of endotoxemia to test the hypothesis that cytokines play a role in abnormal HRV during sepsis. Adult male C57BL/6 mice underwent surgical implantation of probes to continuously monitor electrocardiogram and temperature or blood pressure via radiotelemetry. Administration of high-dose LPS (Escherichia coli LPS, 10 mg/kg, n = 10) caused a biphasic response characterized by an early decrease in temperature and heart rate at 1 h in some mice, followed by a prolonged period of depressed HRV in all mice. Further studies showed that LPS doses as low as 0.01 mg/kg evoked a significant decrease in HRV. With high-dose LPS, the initial drops in temperature and HR were temporally correlated with peak expression of TNFα 1 h post-LPS, whereas maximal depression in HRV coincided with peak levels of multiple other cytokines 3–9 h post-LPS. Neither hypotension nor hypothermia explained the HRV response. Pretreatment with dexamethasone prior to LPS significantly blunted expression of 7 of the 10 cytokines studied and shortened the duration of depressed HRV by about half. Interestingly, dexamethasone treatment alone caused a dramatic increase in both low- and high-frequency HRV. Administration of recombinant TNFα caused a biphasic response in HR and HRV similar to that caused by LPS. Understanding the role of cytokines in abnormal HRV during sepsis could lead to improved strategies for detecting life-threatening nosocomial infections in intensive care unit patients.

IN THE HEALTHY STATE, HEART RATE (HR) has beat-to-beat variability, regulated in large part by input from the autonomic nervous system (27, 31). Efferent signals from sympathetic and parasympathetic nerves converge on specialized sinoatrial node pacemaker cells, resulting in accelerations and decelerations of beating rate. Heart rate variability (HRV) is commonly quantified using time-domain analysis, such as the standard deviation of normal R-R intervals (SDNN). These data can also be transformed to detect variability within different frequency spectra that are attributed to sympathetic and parasympathetic branches of the autonomic nervous system and other physiological processes affecting HR, including respiration, blood pressure, and temperature.

Depression of HRV is associated with adverse outcomes in a number of chronic and acute pathological states, including myocardial infarction (17), congestive heart failure (3), diabetes (19), fetal asphyxia (18), and sepsis (6). In all of these conditions, the assumption has been that there is abnormal signaling from or response to the autonomic nervous system, though mechanistic studies are few. In addition, limited clinical studies suggest an association between a systemic inflammatory response and decreased HRV (15). In the case of chronic illness, an inverse correlation has been reported between plasma TNFα levels and HRV in adults with congestive heart failure (20) and between plasma IL-6 levels and HRV in adults with diabetes (9). In acute illness, elevated plasma IL-6 has also been linked to depressed HRV in adults with sepsis (32). In experimental sepsis in both animal models and healthy human volunteers, administration of lipopolysaccharide (LPS, endotoxin from gram-negative bacteria) decreases HRV (2, 7, 8, 21). While these data point to an association between the systemic inflammatory response and changes in HRV, causal links between the two have not been established.

Our group has previously shown that abnormal HR characteristics (HRC) comprising depressed HRV and repetitive brief HR decelerations often precede clinical signs of sepsis by as much as 24 h in neonatal intensive care unit patients (12, 15–17, 19, 20, 26). Other conditions and interventions have also been associated with abnormal HRC in neonatal intensive care unit patients without sepsis. Further characterization of abnormal HRC during sepsis would add to the diagnostic utility of HRC monitoring, and, to this end, we developed a mouse model of continuous radiotelemetric monitoring of ECG during exposure to bacterial toxins. We show that LPS leads to increased levels of multiple cytokines and to depression of HRV in both parasympathetic and sympathetic frequency bands, suggesting a balanced effect on the autonomic nervous system (ANS), or a non-ANS mechanism. TNFα administration was sufficient to induce similar changes, while cytokine-suppressing glucocorticoids reduced the duration of LPS-induced HRV depression and, interestingly, profoundly increased HRV when given alone. The changes in HRV were not obviously due to systemic effects of LPS or cytokines, such as hypotension or hypothermia. We propose that cytokines play a mechanistic role in reduced HRV during sepsis.

METHODS

Mouse ECG telemetry. Male C57BL/6 mice (Charles River) ages 8–14 wk were used for all experiments. Animals were housed singly and had free access to food and water throughout the experiments. The telemetry facility was maintained at ambient temperature 20–22°C and a 12:12-h light-dark cycle. All experiments were begun in the morning to control for diurnal variations. Experimental protocols were approved by the University of Virginia Animal Care and Use Committee and conform to guidelines established in the American Physiology Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Telemetry probes (ETA-F20, Data Sciences International) were surgically implanted for continuous measurement of ECG, tempera-
ture, and activity. Mice were anesthetized with 2% isoflurane, and the abdominal skin was cleaned with povidone-iodine and alcohol. A 2-cm vertical incision was made in the skin and peritoneum, and the telemetry battery was placed in the peritoneal cavity. The ECG leads were tunneled under the skin with the tips guided to the right and left axillary regions. The peritoneum and the abdominal skin were then closed with sutures. Buprenorphine was given for pain on the day of surgery and the following day.

Experiments were conducted at least 6 days after surgery to allow recovery of baseline HRV. Experiments were begun in the morning to account for diurnal variations and recordings were made during both active and resting periods. Mice were placed in their standard cage on a telemetry receiver connected via a data exchange matrix to a PC. The ECG signal was sampled at 5,000 Hz using Data Sciences International hardware and Dataquest ART Gold 3.1 software. Digitized signals were transferred to MATLAB (MathWorks) for QRS detection and HRV and temperature analysis, using custom programs. The R-R intervals were extracted using a detection algorithm based on peak-picking and correlations with QRS waveform templates. We excluded extreme values of the R-R intervals (<70 ms, >500 ms) and other outliers on the basis of the mean and SD of nearby R-R values. The remaining R-R intervals are considered normal-to-normal (NN) intervals.

Time and frequency domain measures were calculated for each 2-min segment of normal R-R interval data and summarized for each time period relative to injection events by their median value within a window of 60 min. Heart rate, temperature, and activity data recorded by the Data Sciences International system were also collected at rate of at least every 5 min.

In the time domain, the mean R-R interval (R-Ri) and SDNN were calculated for each segment of data. HR was calculated as the reciprocal of the mean R-Ri measured in beats per minute (bpm). The pNN6 is the proportion of R-R intervals differing from preceding R-Ri by 6 ms or more. The coefficient of variance (CV), defined as the ratio SDNN/mean RRi, was also calculated. The normalized SDNN was calculated by dividing the SDNN by the median value of all measurements made in the 24 h before the injection.

In the frequency domain, the power spectral density of the R-R interval time series was computed using a Lomb periodogram (5) computed at a resolution of 0.005 Hz. Two different frequency-domain measures of HRV were calculated, low-frequency range (LF) 0.4–1.5 Hz and high-frequency range (HF) 1.5–4 Hz, with data expressed as arbitrary units showing the difference in pretreatment and posttreatment HRV. Short (2 min) segments of data were analyzed and medians of 30-min segments were then calculated to account for nonstationarity of heart rate. Parameters for low- and

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**Fig. 1.** Effect of vehicle treatment (A, B) and a high dose LPS (C, D) on heart rate. Continuous ECG, temperature, and activity data were acquired via radiotelemetry in 10 mice treated at time 0 with vehicle (sterile NS ip) or high-dose LPS (10 mg/kg ip). A: representative heart rate (HR; bpm) from a single mouse 9 h pre- to 24 h postvehicle administration. Mean HR for each 2-min interval for the 33-h period is shown. B: recording of activity (top, counts/min), HR (middle, bpm) and temperature (bottom, °C) for mouse shown in A from 0 to 12 h after vehicle treatment. C and D: representative heart rate from a single mouse before and after administration of LPS 10 mg/kg ip. Median HR of all mice before and after administration of 10 mg/kg LPS (n = 10) or vehicle (sterile NS, n = 4).
high-frequency HRV (0.4–1.5 and 1.5–4 Hz, respectively) and for percentage of sequential R-R intervals differing by >6 ms (pNN6) were chosen on the basis of review of our data, as well as the mouse HRV literature (3, 8, 33, 34, 36).

**Mouse blood pressure telemetry.** PA-C10 telemetry probes (Data Sciences International) were surgically implanted for continuous measurement of blood pressure and heart rate. Mice were anesthetized with 2% isoﬂurane, and the cervical area skin cleaned with povidone-iodine and alcohol. A 1-cm vertical incision was made in the left neck, and the left carotid artery was cannulated. The catheter was advanced 7 mm and sutured in place. A subcutaneous tunnel was created, and the telemetry battery was placed in the subcutaneous space in the left axillary region, and the skin was closed with sutures. Buprenorphine was given on the day of surgery and the following day.

**Cytokine assay.** To avoid effects of blood drawing and handling on HRV analysis, cytokine measurements were performed on mice not undergoing telemetry. Under light inhalational anesthesia with methoxyﬂurane, blood was obtained from the retroorbital venous plexus in a heparinized capillary tube. After centrifugation, plasma was collected and stored at 80°C. Cytokines were measured using Luminex MAP technology (Bio-Rad). Plasma samples diluted 1:4 with mouse serum diluent (Bio-Rad) and recombinant cytokine standards were incubated with ﬂuorescent antibody-tagged microspheres, then with a biotin-labeled detection antibody followed by streptavidin-phycocerythrin. Microspheres were analyzed in a Bio-Plex 200 dual laser ﬂuorometer (Bio-Rad), and cytokine concentrations were calculated from the standard curve with Bio-Plex Manager 4.0 software. Samples were analyzed in duplicate when sufﬁcient plasma was available. Lower limits of detection ranged from 2 to 20 pg/ml.

**Statistical analysis.** Proﬁles of 23 cytokines (Bio-Plex Mouse 23-plex, Bio-Rad) in plasma of mice treated with LPS or LPS + dexamethasone were grouped using hierarchical clustering analysis with the MATLAB Bioinformatics Toolbox (MathWorks). On the basis of this analysis, 10 cytokines were selected for subsequent studies. Cytokine levels are presented as picograms per milliliter and for statistical analysis were log10 transformed to assess time- and dexamethasone-dependent differences by two-way ANOVA followed by Bonferroni post hoc tests.

**RESULTS**

**Effects of high-dose LPS on HR, HRV, and temperature.** Figure 1A shows HR (median bpm for each 2-min period) from 9 h before to 24 h after intraperitoneal injection of vehicle (sterile normal saline) in a representative mouse. Fluctuations in baseline HR consistently coincide with a level of activity and core temperature, as shown in Fig. 1B. Administration of high-dose LPS (10 mg/kg) caused a prolonged period of increased HR (Fig. 1, C and D) with significant decrease in HRV occurring in all mice (Figs. 3, 4, and 6). These effects, which occurred in all 10 mice, peaked 2–4 h after LPS administration and lasted 12–15 h. The mean of the median HRs (±SD) from 2–4 h after high-dose LPS was 694 ± 31 compared with 547 ± 41 in the 2 h prior to LPS administration (P < 0.01).

Four out of ten mice demonstrated an early response to LPS characterized by a decrease in temperature to <35°C and concurrent decrease in HR peaking 30–60 min after administration (Fig. 2).
Time- and frequency-domain analyses of high-dose LPS effect on HRV. We compared four time-domain and two frequency-domain measures of HRV in mice treated with high-dose LPS (10 mg/kg, \( n = 10 \)) (Fig. 3). For each mouse, median values for every 2-min interval from 24 h before to 24 h after LPS administration were calculated, and the mean of the median values for 10 mice is shown. LPS induces a low HRV state in which both high- and low-frequency variations are reduced equally, and long pauses (indicated by pNN6) are eliminated. This demonstrates that the observed reduced HRV is independent of the algorithm used to quantify HRV.

Dose dependence of LPS effect on HRV. The effect of LPS on HRV was dose dependent, with significant depression of HRV occurring at 0.01 mg/kg and higher (Fig. 4). The duration of HRV depression was also dose dependent, with the threshold dose of 0.01 mg/kg LPS inducing a response lasting 3–6 h and the highest dose of 10 mg/kg depressing HRV for 12–15 h.

LPS effect on blood pressure and HRV. To determine the contribution of blood pressure changes to HRV changes following LPS, we implanted a catheter in the carotid artery to continuously monitor blood pressure and heart rate via radiotelemetry. Five mice were given 10 mg/kg LPS ip. Two of the five mice had a significant drop in blood pressure within 30 min of LPS administration with return to baseline blood pressure by 1 h (Fig. 5A). Two other mice had low blood pressure from 2–6 h after LPS, and one mouse had no significant change in blood pressure. Importantly, HRV (SDNN) was only weakly correlated with mean blood pressure (Spearman’s rank correlation coefficient: \( r = 0.22 \)) (Fig. 5B).

Temporal correlation of cytokine elevation and HRV response to LPS. As expected, administration of 10 mg/kg LPS to mice caused a significant rise in plasma levels of multiple cytokines. The dynamics of the cytokine response were temporally correlated with the time course of altered HRV. In preliminary experiments, we analyzed 23 cytokines at 1, 3, 6, and 9 h post-LPS, and using hierarchical clustering analysis (Fig. 7A), we selected 10 cytokines most responsive to LPS. TNFα and IL-10 showed peak expression 1 h after LPS (Fig. 7B, top), coinciding temporally with the drop in heart rate and temperature 1 h after LPS administration, which occurred in 4 of 10 mice (Fig. 2). Peak expression of IL-1β, IL-6, IL-12p40, macrophage inflammatory protein-1β (MIP-1β), monocyte chemoattractant protein (MCP-1), and KC occurred 3 h after LPS administration (Fig. 7B, middle), coinciding with the peak in HR (Fig. 1D) and depression in HRV (Figs. 3 and 4) that occurred in all mice. Granulocyte colony-stimulating factor (G-CSF) and regulated upon activation, normal T cell expressed and presumably secreted (RANTES) continued to show high-level expression 6–9 h after LPS administration (Fig. 7B, bottom), when HRV was still depressed with LPS administration alone but was normalizing in mice treated with dexamethasone prior to LPS (Fig. 6A).

Dexamethasone effects on HRV and cytokines. To elucidate the role of cytokines in LPS-induced changes in HR and HRV, we pretreated mice with dexamethasone (DEX). As expected, DEX pretreatment significantly attenuated LPS-induced production of TNFα, IL-6, IL-12p40, IL-1β, MCP-1, KC, and MIP-1β (Fig. 7B). There was no significant change in levels of IL-10, RANTES, or G-CSF with dexamethasone.

![Fig. 3. Time- and frequency-domain analyses show comparable endotoxin-induced depression of heart rate variability (HRV). ECG data were collected 24 h pre- and post-LPS in 10 mice administered 10 mg/kg LPS and analyzed using time- and frequency-domain methods. Median values for every 30-min interval are shown. Units are as follows: R-R interval (ms); SDNN, Standard deviation of normal R-R intervals (ms); coefficient of variance (SDNN/mean R-Ri); pNN6: percentage of normal R-R intervals differing from the previous one by >6 ms; low-frequency power (0.4–1.5 Hz) (AU); and high-frequency power (1.5–4 Hz) (AU).](http://ajpregu.physiology.org/content/297/10/2821/F3.large.jpg)
methasone pretreatment. DEX pretreatment had no effect on the magnitude of the initial response of depressed HRV following high-dose LPS administration (Fig. 6A). However, the duration of reduced HRV was shortened by about half. Interestingly, dexamethasone alone significantly increased HRV. This was seen in both low- and high-frequency power spectra (Fig. 6B).

TNFα administration mimics LPS effects on HR, HRV, and cytokines. Because peak plasma TNFα expression at 1 h correlated temporally with LPS-induced decrease in temperature and heart rate seen in 4 of 10 mice (Fig. 2), we administered recombinant TNFα to mice and analyzed changes in temperature, HR, and HRV. As with LPS, there was an early response to TNFα administration in half of the mice (3/6), characterized by a decrease in temperature and heart rate 30–60 min after TNFα administration (Fig. 8). HR data were available for 6 h post-TNFα administration in all six mice and for 24 h post-TNFα in three mice. From 6 to 14 h following TNFα administration, we found a noticeable decrease in the normalized SDNN that was significant in the time period 8–10 h following administration (P<0.05, paired t-test) with a trend toward significance (P < 0.1) at other times. TNFα has a short half-life in vivo, but it induces production of cytokines including itself. Six hours after recombinant TNFα administration, at the time of maximal depression of HRV, we found a significant increase in 8 of 10 cytokines studied (Fig. 9B).

DISCUSSION

Systemic administration of endotoxin to mice or humans causes a well-characterized sepsis-like physiological response, including hypotension, tachycardia, and temperature instability. Effects of endotoxin on heart rate variability are less well characterized but are important to study because of the possibility of continuous monitoring strategies that may allow early diagnosis of severe infection. For example, we have found reduced HRV and brief HR decelerations preceding clinical signs of illness in neonatal sepsis and developed a HRC index for continuous monitoring (4, 11, 13, 22). We hypothesized that circulating cytokines alter HRV and that studies linking abnormal HRV with increases in inflammatory markers during sepsis could lead to better diagnostic strategies for early detection of this life-threatening condition. In our mouse model, we show that high-dose LPS induced a biphasic response with an initial decrease in temperature and HR in some mice, followed by a prolonged period of increased HR with reduced HRV correlating with peak expression of multiple cytokines. Administration of...
recombinant murine TNFα was sufficient to induce a similar response, whereas blunting the cytokine response to LPS with dexamethasone shortened the duration of reduced HRV and substantially increased HRV when given alone.

**LPS and TNFα effects on heart rate and heart rate variability.** Reduced HRV from about 2–15 h after LPS or TNFα administration was consistent in all mice. On the other hand, the early response of decreased temperature and heart rate 30–60 min after administration occurred in 4/10 LPS-treated mice and 3/6 TNFα-treated mice. The variability of this early response was not obviously due to any technical issues. In fact, we have found that the cytokine response to LPS is itself quite variable, even among inbred, genetically highly similar mice (Fig. 7B, note 1–3 h SE bars). This may account, at least in part, for the inconsistency of the very early physiological responses to LPS with regard to temperature and blood pressure.

Decreased HRV during experimental endotoxia has been previously reported in both animal models (8, 21) and humans (2, 7), consistent with findings in neonates and adults with sepsis (6, 10, 13, 22, 25). The mechanism of this effect is not well understood but could be through direct or indirect effects of LPS on pacemaker cell electrophysiology or on autonomic nervous system activity. Although we provide evidence of a causal link between cytokines and abnormal HRV as discussed below, other mechanisms could be at play as well, given the complex nature of both HRV regulation and the systemic inflammatory response. In vitro administration of LPS has been shown to decrease pacemaker currents in human right atrial appendage myocytes (33) and decrease beating rate variability in neonatal rat cardiomyocytes (29), suggesting an effect independent of autonomic modulation. On the other hand, an in vivo study showed that administration of LPS to wild-type and nitric oxide synthase knockout mice decreased HRV in intact animals but not in isolated beating atria from mice previously treated with LPS (21), suggesting an intact autonomic nervous system is required for LPS to depress HRV.

It is unclear to what extent spectral analysis of HRV, at best, a very indirect measure of autonomic nervous system function, reflects sympathovagal balance in endotoxemic mice. We, nonetheless, performed these analyses and showed that LPS depresses both low- and high-frequency power in roughly equal proportion. This is consistent with results of Chang et al. (5) in studies of human neonatal sepsis. We cannot, however, rely on frequency domain analysis to completely rule out selective modulation of sympathetic or parasympathetic tone during endotoxemia or sepsis.

In addition to effects on SA node cells or autonomic tone, LPS may also alter HR and HRV indirectly through its pathophysiological effects on temperature or blood pressure (31). Our studies show that LPS causes an early (30–60 min) drop in temperature, HR, and blood pressure in some but not all mice. The depression in HRV 2–15 h following LPS administration occurred in all mice, was not correlated with hypothermia and only weakly correlated with hypotension, and persisted after normalization of HR. Thus, we show that simple physiological coupling is not responsible for all of the LPS effects on heart rate control.

**LPS and steroid effects on cytokines and HRV.** We provide both indirect and direct evidence supporting a role of cyto-

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Fig. 6. Dexamethasone shortens the duration of LPS-induced HRV depression and increases HRV. Mice were given dexamethasone alone 0.7 mg/kg (n = 4), dexamethasone followed 90 min later by LPS (n = 5), LPS 10 mg/kg alone (n = 10), or vehicle (n = 4). A: median SDNN for every 2-min interval 9 h pre- to 24 h post-LPS, normalized to pre-LPS baseline (horizontal line at “1”) B: median low- (0.4–1.5 Hz) and high (1.5–4 Hz) -frequency power spectra from 24 h predexamethasone to 24 h postdexamethasone alone (n = 4). Arbitrary units are shown.
kines in abnormal HRV in our model, including a temporal correlation between cytokine elevations and HRV depression post-LPS and direct evidence linking TNFα to abnormal HRV. The kinetics of LPS-induced cytokine elevation and changes in HRV are closely linked in our model, with the majority of cytokines peaking at 3–6 h after LPS administration during the peak depression of HRV. Clinical studies have linked elevations in IL-6 with decreased HRV in adults with heart disease (15), diabetes (9), renal failure (26), and sepsis (32), and we found very high levels of IL-6 post-LPS.

Fig. 7. Profiling the cytokine response to LPS ± dexamethasone. Cytokines were analyzed in mouse plasma from 0 to 9 h following LPS 10 mg/kg alone (LPS, n = 3–6) or 0.7 mg/kg dexamethasone followed 90 min later by LPS 10 mg/kg (DEX, n = 3). A: hierarchical clustering analysis of 23 cytokines was used to generate a dendrogram depicting the similarity of individual cytokine profiles and identify the cytokines most responsive to LPS. B: time course of expression (pg/ml) of select cytokines to LPS (solid lines) or DEX followed by LPS (dotted lines). Six cytokines peaked at 3 h post-LPS, whereas two peaked at 1 h and two peaked at 6–9 h. *P < 0.05 LPS vs. time 0, **P < 0.05 LPS vs. time 0 and Dex+LPS vs. LPS alone.
temporally associated with the nadir of HRV 3 h after LPS administration. We show that administration of TNF alone was sufficient to depress HRV, though TNF also potently induced multiple other cytokines, which likely contributed to the effect.

Dexamethasone blunted but did not block LPS-stimulated cytokine production and, similarly, shortened but did not eliminate LPS-induced HRV depression. A study of healthy human volunteers also showed that administration of hydrocortisone before endotoxin reduced cytokine levels but did not affect HRV (2). This may indicate that even low levels of cytokines are sufficient to alter HRV. In our studies, dexamethasone reduced most cytokines to baseline levels and concurrently restored normal HRV 6 h after LPS administration, showing at least a temporal if not a causal link between the two.

Interestingly, we found that giving dexamethasone alone resulted in a dramatic increase in HRV lasting more than 12 h. The dexamethasone effect was opposite the LPS effect and involved increases in both low- and high-frequency spectra. Although acute glucocorticoid administration is known to increase blood pressure, effects on HRV have not been well defined. Fetal HRV has been found to increase acutely following maternal administration of glucocorticoids for induction of lung maturity, but maternal HRV was not measured in these studies (1, 23, 30). There is also evidence that endogenous glucocorticoids play a role in regulation of HRV. In a study of critically ill intensive care unit patients, adrenal insufficiency was linked to depression of HRV, and glucocorticoid replacement normalized HRV (1, 23). Despite this in vivo evidence linking steroids and HRV in both healthy and ill states, the mechanism of this effect is not known. Possibilities include effects on blood pressure or depression of baseline cytokine expression.

We developed this mouse model as a means to further understand the changes in heart rate characteristics that occur in patients with sepsis. Although a single dose of

![Fig. 8. TNFα causes early (1 h) decrease in heart rate and temperature in 3 of six mice. Continuous ECG and temperature were recorded in six mice administered recombinant murine TNFα 0.2 mg/kg ip at time 0. A: heart rate (bpm) and temperature 2 h pre- to 2 h post-TNFα in a representative mouse, which showed decrease in HR and temperature 30–60 min after TNFα. B: median heart rate (bpm) for each 2-min interval 2 h pre- to 2 h post-TNFα in individual mice (gray lines) and average of all mice (black line) C: temperature curve for the same mice shown in B (gray lines), and average of all mice (black line). Three of six mice had decreased temperature <35°C and concurrent decrease in HR in the early period following TNFα administration.](http://ajpregu.physiology.org/)

![Fig. 9. TNFα causes decreased HRV and increased cytokines similar to LPS. A: recombinant murine TNFα 0.2 mg/kg ip was administered to mice undergoing continuous radiotelemetry (n = 6). Median heart rate variability for all mice from 9 h pre- to 24 h post-TNFα. Data points represent median SDNN normalized to pre-TNFα baseline (“1” indicated by horizontal line). B: recombinant murine TNFα 0.2 mg/kg ip was administered to a separate group of mice not undergoing telemetry (n = 6), and blood was drawn 6 h after TNFα administration for quantitation of 10 cytokines. Fold increase at 6 h post-TNFα compared with baseline is shown (means ± SE) *P < 0.05 at 6 h vs. baseline.](http://ajpregu.physiology.org/)
endotoxin mimics some aspects of sepsis, further research is required to determine how these findings translate to the more complex condition of sepsis with replicating pathogens. Our group has shown that continuous monitoring of heart rate variability and decelerations can identify neonatal intensive care unit patients in the early stages of sepsis, often prior to onset of symptoms (10, 12, 14). Although this physiomarker is useful for identifying patients who warrant additional clinical scrutiny, its positive predictive accuracy for sepsis is less than 50% (10). On the basis of our findings in the mouse that abnormal heart rate characteristics coincide with rises in blood cytokines, we speculate that addition of a cytokine profile at the time of abnormal heart rate characteristics could further identify infants in early stages of sepsis, potentially leading to earlier treatment and improved clinical outcomes.

GRANTS
This work was supported by the following funding sources: National Institute of Child Health and Human Development Grant 5K08HD051609-02 (to K. D. Fairchild), National Institute of General Medical Sciences Grant 64640 (JRM), The Wallace H. Coulter Foundation Translational Research Award, and the University of Virginia Children’s Hospital.

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