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Influence of Acute Epinephrine Infusion on Endotoxin Induced Parameters of Heart Rate Variability: A Randomized Controlled Trial

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Abstract

Objective—To determine whether the acute anti-inflammatory influence of epinephrine (EPI) extends to changes in heart rate variability (HRV) induced by the prototypical inflammatory stimulus, endotoxin (LPS).

Summary Background Data—HRV reflects fluctuating cardiac autonomic inputs and is acutely reduced during the systemic inflammation induced by LPS as well as during severe critical illnesses such as sepsis and traumatic injury. While EPI may diminish proinflammatory cytokine release it is unknown whether this net anti-inflammatory activity extends to HRV.

Methods—Healthy volunteers (n=17) were randomized to either saline+LPS (2ng/kg) or LPS + antecedent EPI infusion (30ng/kg/min) from −3 to 6 hours relative to LPS. HRV and blood samples were obtained prior to EPI and LPS as well as hourly afterwards. Plasma cytokines were measured by ELISA. Statistical analysis was by repeated measures ANOVA. This study was registered at Clinicaltrials.gov and is listed under the following ID number: NCT00753402

Results—LPS acutely influenced all measured parameters of HRV including SDANN, pNN50 and RMSSD, HF, LF, LF/HF and VLF (all $p<0.01$). EPI infusion reduced the inflammatory cytokine response to LPS as measured by decreased TNFα, IL-6 and IL-8 ($p<0.01$). Relative to the saline+LPS group, antecedent EPI infusion was associated with further reductions in parameters of HRV measuring vagal/parasympathetic activity including, pNN50, RMSSD and HF ($p<0.05$).

Conclusion—Prior EPI exposure exerts anti-inflammatory influences but also may reduce vagus nerve activity. Hence, acute EPI administration may be protective against early inflammatory challenges but diminish vagal nerve responsiveness to subsequent stimuli.

Introduction

An acute stress response is observed following traumatic injury [1] and surgical intervention.[2] Stressful events lead to pro-inflammatory mediator release (e.g., TNFα, IL-6)[1, 3] from both

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immune cells and parenchymal tissues that result in a systemic inflammatory phenotype. Localized inflammatory signals may further enhance immune activation that serve to control infection or injury.[4] Unremitting systemic inflammation, as observed during sepsis, is associated with immune impairment [5] and adverse outcomes.[6] Attempts have been made to modulate several of the purported pro-inflammatory mediators of excessive systemic inflammation or the inducible systemic and cellular reactions to severe infection. [7-9] However, there has been only limited success toward improving outcome[10] with such anti-inflammatory strategies based perhaps on variations in the extent or magnitude of existing non-infectious stress influences. Clinical assessments of inflammatory risk, such as those encompassed in vital signs and biochemical analysis, presently lack sufficient sensitivity and specificity to direct therapeutic intervention and might be further improved by dynamic quantification of host and organ systems functional capacity.

Measures of heart rate variability (HRV) are non-invasive assessments that may reflect real-time alterations of physiologic status. [11-13] Under such normal circumstances, variability parameters reflect homeostatic feedback between organ systems such as the central nervous system and heart whereas decreased variability implies physiologic decomplexification[14] manifested by diminished organ responsiveness to autonomic signaling.[15] Decreases in variability of some components of heart rate have been suggested to have prognostic value for poor outcomes in patients with heart failure [16], myocardial infarction,[17] diabetes,[18] sepsis[19] and traumatic injury.[13]

Catecholamines have both anti-inflammatory as well as vasoactive properties.[20, 21] In vitro, both norepinephrine [22, 23] and epinephrine (EPI) have been shown to inhibit immune cell secretion of inflammatory cytokines such as TNFα[24], IL-1β[20], IL-8[25] and shown to potentiate the secretion of IL-10,[26] primarily through the action of β-2 receptors.[27] We have previously confirmed the in vivo activity of epinephrine by infusion of this catecholamine prior to intravenous administration of LPS in healthy subjects and observed reduced levels of several pro-inflammatory cytokines and blunted manifestations of the systemic inflammatory response.[20, 28]

The acute systemic inflammatory condition mediated by endotoxin (LPS) administration in healthy volunteers is also associated with a transient decrease in some HRV parameters. [29 30, 31] Given the acute sympathetic activation resulting from experimental endotoxemia and the propensity for endotoxin induced inflammation to occur against a clinical background of catecholamine inducing stressors, such as surgery or trauma[28], we sought to assess whether antecedent epinephrine would modulate the known alteration in HRV and autonomic balance resulting from LPS.

Methods

Subjects

Healthy adult male and female subjects were recruited by public advertisement and screened for normal health status under approved guidelines of the Institutional Review Board of the Robert Wood Johnson Medical School. Inclusion criteria for the study were: good general health as demonstrated by medical history and physical examination, complete blood count and basic metabolic panel screening within normal lab limits. Exclusion criteria included a history of any acute or chronic disease, arrhythmia, recent history of alcohol, drug or medication ingestion, pregnancy or prior exposure to LPS in the experimental setting. Once informed, written consent was obtained; all subjects received an initial recording of heart rate and electrocardiogram (EKG) to screen for any arrhythmic patterns or irregular heartbeats. Only subjects with a normal standard EKG were considered for admission to the protocol.

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Study design and procedures

Upon accrual to the study, seventeen healthy male (n=12) and female (n=5) subjects between 18 and 37 years of age were admitted to the Clinical Research Center (CRC) at UMDNJ-Robert Wood Johnson Medical School the afternoon prior to the acute endotoxin study. Upon admission, a repeat history and physical examination confirmed that no change in health status had occurred since enrollment. Female subjects underwent a urine pregnancy test upon admission.

Following admission, subjects were randomized to one of two study groups, those who would receive a placebo infusion of physiologic saline prior to LPS administration (saline + LPS n=10; males=7, females=3) and those who would receive an infusion of EPI, beginning 3 hours prior to endotoxin administration (EPI + LPS n=7; males=5, females=2). Patients were fasted from midnight of the admission day and received an overnight intravenous fluid infusion (5% dextrose and 0.45% sodium chloride-1.5ml/kg/hr) via a peripheral venous catheter. Both investigators and subjects were blinded as to whether EPI or saline was being administered prior to LPS. The unblinded research pharmacist who was not part of the research team was responsible for preparing and labeling the study drug/placebo intravenous bags. The treatment group received EPI (30ng/kg/min)[24], administered intravenously by continuous infusion for 9 hours beginning at 06:00 on day 1 of the study. As previously described[32], a radial arterial catheter was placed at 07:00 the morning of study day. The arterial catheter was utilized to monitor heart rate and blood pressure as well as for periodic blood sampling at defined time points before and after endotoxin administration. A rectal thermometer was placed for continuous monitoring of core body temperature. As previously described, a onetime dose of endotoxin (2ng/kg, CC-RE, Lot #2)[31] was administered over a one minute-period through a separate peripheral intravenous catheter at approximately 09:00 (time point 0) on study day 1.

Clinical monitoring

Vital signs, including heart rate and mean arterial blood pressure (MAP), were recorded every 30 minutes from the arterial monitoring system for the first 6 hours post-LPS (09:00-15:00 hours) and then periodically taken manually for up to 24 hours after LPS administration. Core temperatures were recorded every 30 minutes for 6 hours post-LPS (09:00-15:00 hours) via rectal thermometer, then orally for up to 24 hours after LPS administration.

At 6 hours after LPS bolus, the arterial catheter and rectal thermometer were discontinued. The peripheral intravenous catheter infusing saline solution (5% dextrose and 0.45% sodium chloride-1.5ml/kg/hr) was removed once each subject tolerated a regular diet. Subjects remained in the CRC overnight and were discharged to home the following morning after blood samples and HRV measurements were obtained at 24 hours post LPS administration.

Assessment of heart rate variability parameters

Base-line determinations of parameters of HRV were obtained at the time of admission as well as hourly from 0 to +6 hours following LPS challenge and at +9 and +24 hours after LPS challenge. Each recording interval consisted of two consecutive 5-minute epochs. During such determinations, heart rate and respiration were monitored using a continuous electrocardiography (EKG) technique with three standard limb leads, a respiratory belt, and CardioPro® 2.0 software with one Infiniti and one Procomp Plus encoder (Thought Technology, Ltd., Montreal, P.Q., Canada). HRV parameters and inter-beat intervals were collected using EKG data at a rate of 256 samples/second. The respiration channel collected respiratory data at a rate of 32 samples/second. However, because movement artifact occasionally influenced computer-detected respiration rates, this measure was also scored by

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hand. Minor fluctuations in the tracing, due either to movement or to changes in inhalation patterns during individual breaths were not counted in tallying respiration rate.

Parameters of HRV were analyzed for both time domain and frequency domain measures. Time domain measures included, 1) the standard deviation of the average beat to beat intervals over a 5 minute period (SDANN), a measure of total heart rate variability and overall system adaptability, 2) the square root of the mean squared differences of successive interbeat intervals (RMSSD), that is considered to be influenced predominantly by the vagus nerve system, and the percentage of interval differences of successive interbeat intervals greater than 50 ms (pNN50), that is generally associated with respiratory sinus arrhythmia and therefore, vagus nerve activity. Frequency domain measures included, 1) high frequency variability (HF)[0.15-0.4Hz] that correlates with parasympathetic and vagal tone, 2) low frequency variability (LF)[0.05-0.15Hz], a measure associated with both parasympathetic and sympathetic activation, 3) very low frequency variability (VLF) [0.005-0.05 Hz] that is associated with thermoregulatory function and sympathetic contribution to vascular regulation, and 4) the LF/HF ratio, that is hypothesized to be associated with sympathetic:parasympathetic balance.[14, 33, 34]

In a continuous EKG record, each QRS complex was detected and the “normal-to-normal” (NN) intervals (all intervals between adjacent QRS complexes resulting from sinus node depolarization) were tabulated, thus providing a record of instantaneous heart rate.[33] For each epoch, noise artifact and irregular heartbeats were manually edited by visual inspection and interpolation prior to calculation of interbeat intervals using CardioPro software. We analyzed each epoch as previously described [31] and excluded complete measurement epochs where events such as extra systolic heartbeats, skipped beats, and other arrhythmias comprised greater than 10% of the total epoch. The power spectral density then was calculated using a Fast Fourier transformation algorithm.[33, 35] All signals were exported in standard ASCII format to Excel and EAS 9.0 for analysis and graphics.

Analysis of Blood Samples

Blood samples were collected at time points −24, 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hours in relation to endotoxin administration. Blood-derived plasma was then analyzed by ELISA for measurement of soluble inflammatory markers (TNFα, IL-6 and IL-8).[36]

Statistical Analysis

Analysis of vital signs and metabolites—We computed descriptive statistics and analyzed vital signs and soluble inflammatory mediator measures by two-way analysis of variance with repeated measures on time using Statistica version 6.1 (StatSoft, Inc., Tulsa, OK).[37] P-values less than 0.05 were considered to be statistically significant. The group (EPI+LPS vs. saline+LPS) versus time interactions are reported below for assessment of an effect of EPI on the response to LPS.

Analysis of HRV parameters—For respiration and HRV measures, we used mixed model analyses with repeated measures using Proc Mixed program from the SAS system (v. 9.1) as previously described. [31] The variance-covariance structure for repeated measures, determined by the Akaike’s Information Criterion (AIC) [38] was modeled with the auto-regressive model (order of one) that assumes stronger correlations for measurements closer in time. Analysis involved data measured upon admission, the evening prior to EPI and/or LPS exposure, as well as 0, 1, 2, 3, 4, 6, 9 and 24 hours relative to LPS exposure. A log transformation was applied to provide more normally/symmetrically distributed data. A linear contrast was constructed to examine the differences from time-point 0 to 3 hours for

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the placebo (saline+LPS). A separate model including 0 and 24 hour was used to assess the difference for each variable. Significance was assessed at 0.05.

Because respiratory sinus arrhythmia (RSA) may be affected by respiration rate independently of vagus nerve traffic [39] we used a recommended[40] statistical control for respiration rate in all HRV analyses. Notable, however was that none of the subjects exceeded 24 breaths per minute during the study period, so that HF could be analyzed as reflecting respiratory sinus arrhythmia, as is usually the case.

Results

Vital Signs

Antecedent EPI infusion resulted in a differential temperature response to LPS over time ($p<0.001$), however the clinical relevance is unclear given a similar trend towards baseline at 9hrs. EPI infusion (+LPS) also was associated with a significantly higher heart rate over time vs. saline + LPS ($p<0.05$) (Figure 1).

Inflammatory Markers

There was a significant EPI related diminution of the proinflammatory cytokine response to LPS. TNFα was significantly higher in the saline+LPS group (peak concentration of 427 ± 336 pg/ml) versus the EPI+LPS group (peak concentration of 98 ± 66 pg/ml) ($p<0.001$, group × time effect). IL-8 levels were also significantly higher in the saline+LPS group (peak concentration of 294 ± 148 pg/ml) versus the EPI+LPS group (peak concentration of 64 ± 75 pg/ml) ($p<0.001$). IL-6 exhibited a more rapid rise to peak concentration at 1.5 hours in the EPI+LPS group and return to baseline levels at 4 hours post LPS vs. the saline +LPS group, which reached peak concentration at 2 hours and returned to baseline at 6 hours post LPS ($p<0.001$, group × time effect). All of the above inflammatory mediators were undetectable by 24 hours after LPS challenge (Figure 2).

HRV

None of the HRV parameters varied between groups at baseline determinations on the evening prior to acute EPI and/or LPS exposure. In both groups the greatest changes in HRV occurred between time-points zero and +3hr. Studying the immediate effects of LPS alone on HRV in the placebo group, using a post hoc analysis from time-point zero to time-point +3hr, we observed that LPS acutely influenced all time domain parameters of HRV measured in this study including SDANN, pNN50 and RMSSD ($p<0.01$) as well as frequency domain parameters of HRV including LF, HF, LF/HF and VLF ($p<0.01$) (Figure 3) (Figure 4).

In measuring HRV from baseline to +24 hours relative to the saline+LPS group, antecedent EPI infusion significantly reduced the time domain HRV parameters of vagal/ parasympathetic activity, RMSSD ($p< 0.005$) and pNN50 ($p<0.01$), as well as the frequency domain parameter HF HRV ($p<0.05$). There were no significant differences between groups for the other HRV parameters SDANN, LF/HF, VLF and LF when measured over the entire study period (Figure 3) (Figure 4).

Discussion

Injury and critical illness propagate both pro- and anti-inflammatory mediator cascades[41], alter adrenergic responses to systemic inflammation [42] as well as induce acute changes in some parameters of HRV.[11] It is currently unknown to what extent a sterile injury background might influence subsequent responses to infectious ligands. In order to further

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our understanding of these complex fluctuations, we examined the relationship between the known anti-inflammatory effects of the sympathomimetic agent EPI [24] and parameters of HRV manifested in response to endotoxin-induced systemic inflammation[29, 31] in healthy, subjects. As determined for example by reduced SDANN after LPS, we confirmed previous reports that endotoxin challenge mediates an acute reduction of time domain measures of HRV.[29, 31] In addition, we observed an endotoxin mediated diminution of measured parameters of HRV reflecting parasympathetic/vagal activity, including pNN50, RMSSD and HF. Importantly, a brief period of antecedent EPI excess also appears to exert a vagolytic effect that may limit vagally mediated anti-inflammatory pathways.[43]

The present study models an acute sterile stress condition that may modulate innate immune system activation [28] and result in altered inflammatory mediator release.[44] These pro-inflammatory mediators are counter-regulated by several endogenous mechanisms including, anti-inflammatory molecules such as soluble cytokine receptors [45], as well as components of the autonomic nervous system and the pituitary-adrenal axis.[46, 47] Following sterile tissue injury in animals, there is an increased susceptibility to infectious challenge that is mediated, in part by decreased release of inflammatory mediators (TNFα, IL-6) and reduced neutrophil chemotaxis.[48]In a murine model of initial sterile injury, there is substantial gene expression homology in mice subjected to surgical instrumentation only and between both those subjected to instrumentation and later hemorrhagic shock. [49] During persistent systemic inflammation, subsequent insults have been associated with exaggerated inflammatory profiles as have been observed in traumatically injured patients who then require surgical intervention.[3] This enhanced response was observable despite the relatively short time course between injury and surgery. In addition, pneumonia following surgical intervention has been associated with increased mortality[50] further demonstrating the influence of additional inflammatory stimuli. Thus, sentinel sterile stressors, including surgery and traumatic injury likely lead to activation of pathways that share similar inflammatory profiles as well as influence outcomes from later infectious challenge.[51]

Recent data supports the role of efferent, vagus nerve activity as a modulator of systemic responses to inflammation and infection.[52] As an effector arm of the parasympathetic nervous system, vagal activity may regulate inflammation through several mechanisms. The vagus nerve has sensory components and afferent impulses may develop in response to peripheral stimuli including, inflammatory ligands and cytokines.[52] Vagal sensory afferent activity mediates HPA axis activation, and the increased secretion of soluble mediators, such as cortisol. [53] Vagus nerve efferent signals have been shown to reduce production of the pro inflammatory cytokine TNFα via the interaction of acetylcholine (Ach) with nicotinic receptors containing α-7 subunits on mononuclear phagocytes of the reticulo-endothelial system [43, 52, 54]. Vagotomy has been demonstrated to enhance inflammatory mediator release, including TNFα, IL-1β and IL-6, in a murine model of intraperitoneal sepsis.[55] Pharmacologic activation of nicotinic receptors has also been shown to reduce TNFα release from alveolar macrophages exposed to LPS.[56] We have recently confirmed that antecedent transcutaneous administration of the known α-7 agonist, nicotine, reduced systemic phenotypic and pro-inflammatory mediator responses to endotoxin in humans.[32]

In healthy individuals, HRV is predominantly modulated by parasympathetic/vagus nerve activity[12] and recent data has demonstrated an inverse relationship between HRV determined parasympathetic activity and ex-vivo LPS stimulated TNFα and IL-6 production by circulating immune cells.[57] In animal models, the predominant source of TNFα production appears to be the spleen where resident immune cells are modulated by vagal nerve activity.[58] Hence the apparent modulation of circulating immune cell mediator responses by parasympathetic/vagal activity in humans suggests that centrally mediated neural inflammatory control may extend beyond tissue-fixed immune cells.

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Parameters of HRV may also serve as a surrogate for the interactions between reflex mechanisms accompanying autonomic and respiratory changes and also reflect cardiac responsiveness to underlying parasympathetic and sympathetic activity.[14, 33] HRV has been adapted as a bedside assessment in both pediatric [59] and adult patients.[60] In response to initial traumatic injury, Norris and colleagues have observed an increased mortality in trauma patients with decreased time domain measures of HRV that may be evident within hours of admission.[11, 61] Similarly, Proctor and colleagues have observed an association between confirmed traumatic brain injury and reduction in HRV parameters including SDNN and RMSSD.[60] In addition, decreased sympathetic outflow as measured by altered HRV was associated with head injury severity in pediatric trauma patients. [59] Among patients with infection-induced inflammation, altered parameters of HRV have also been observed in both pediatric and adult patients following the onset of severe sepsis. [19, 62] Interestingly, in critically ill trauma patients with presumptive adrenal insufficiency, restoration of HRV measures was associated with increased survival after exogenous glucocorticoid administration.[63]

In comparison to saline+LPS control subjects, we observed a tendency towards decreased vagal parasympathetic signaling (pNN50, HF and RMSSD) resulting from a brief, three-hour period of antecedent EPI infusion prior to LPS exposure. A relative reduction of vagal tone would be expected given the sympathomimetic properties of EPI.[64] The dose of EPI (30ng/kg/min) administered in this trial was based on earlier studies wherein this EPI infusion dose resulted in significant systemic anti-inflammatory and anticoagulant effects.[20, 21, 24] Despite diminished pro-inflammatory mediator levels and decreased systemic manifestations of endotoxin induced inflammation, overall adaptability as assessed by SDANN was not significantly altered by EPI infusion prior to and following LPS exposure. It was also notable, that imputed parameters of sympathetic activity reflected by VLF, LF and the LF/HF ratio were not significantly different between groups. This is consistent with our recent observations with steroid administration prior to LPS.[31]

In addition to HRV, other measures have been suggested to quantify the physiologic complexity between organ systems including multiscale entropy (MSE)[65]. Entropy measures the disorderliness within datasets and thus increases with greater variability between values and decreases with decreased variability or increased regularity between values. HRV and MSE both quantify the complexity of interactions between organ systems and not surprisingly generate equivalent results when evaluating similar populations. For example, HRV decreases with age in healthy human subjects[66] and similarly, decreased MSE is also associated increased age in healthy humans[65, 67]. The concordance between HRV and MSE extends beyond healthy subjects as both measures predict mortality in ICU[11, 68] and trauma patients [61, 69]. Specifically, MSE has been shown to correlate with SDANN in predicting mortality [68]. In this study we did not calculate MSE since we were interested in studying not only organ system uncoupling but also the influence of EPI on vagus nerve activity in the setting of acute systemic inflammation. Given that SDANN was not significantly different between groups it is unlikely that MSE analysis would be further revealing. In future studies however, we plan to integrate MSE analysis to better understand the influence of interventions on physiologic complexity.

We have previously observed that the anti-inflammatory influence of EPI is of limited duration[24] and may represent a dynamic cellular adaptation to catecholamine stress. In the initial hours, during EPI infusion, an anti-inflammatory EPI effect mediated by β-2 receptors[27] appears to dominate. Over a longer time period[24], the vagolytic influence of EPI may dampen the peripheral capability of cholinergic anti-inflammatory pathways. A persistent EPI induced vagolytic effect would potentially heighten pro-inflammatory activity as β-2 receptor activity decreases[70] and may permit an exaggerated immune response to

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additional inflammatory stimuli. A longer EPI infusion may be revealing since it is yet unknown whether the vagolytic effect of EPI persists or attenuates over time. These results also underscore the complexity of interpreting the role of vagal nerve signaling during conditions of ongoing stress.

The limitations of this study include the infusion of a single hormonal modulator of the LPS response. While it is recognized that the infusion of an adrenergic agent, such as epinephrine, does not replicate the full spectrum of stress induced neuro-endocrine activators, the present model does provide opportunity to dissect individual hormone influences.[28] The relatively brief infusion period of EPI may also limit the scope of our results as our previous reports have demonstrated that the anti-inflammatory effect of EPI diminishes over a 24 hour infusion period prior to LPS administration.[24] Despite the relatively young age of our study group, there may also be age related inflammatory responses[71] that might strictly limit our observations to a younger population. Although age does influence parameters of HRV[72] and endocrine responsiveness,[73] older subjects appear to maintain innate immune activity.[71] Hence we propose that the observations of acute catecholamine influences reported herein may also apply to older populations.

Conclusion

Increased catecholamine secretion accompanies even modest injury and infection. EPI has been shown to have anti-inflammatory properties as evidenced by reduced proinflammatory mediator production during systemic inflammatory responses inducible by LPS. [24, 26] It was previously unknown what influence EPI infusion might have on the LPS induced decrease in HRV. EPI exerts anti-inflammatory effects as well as potentially pro-inflammatory effects by reducing vagus nerve activity. Hence, acute EPI administration can be protective against inflammatory stimuli whereas prolonged exposure may lead to exaggerated immune reactions to subsequent inflammatory stimuli possibly by diminishing vagal anti-inflammatory responses.

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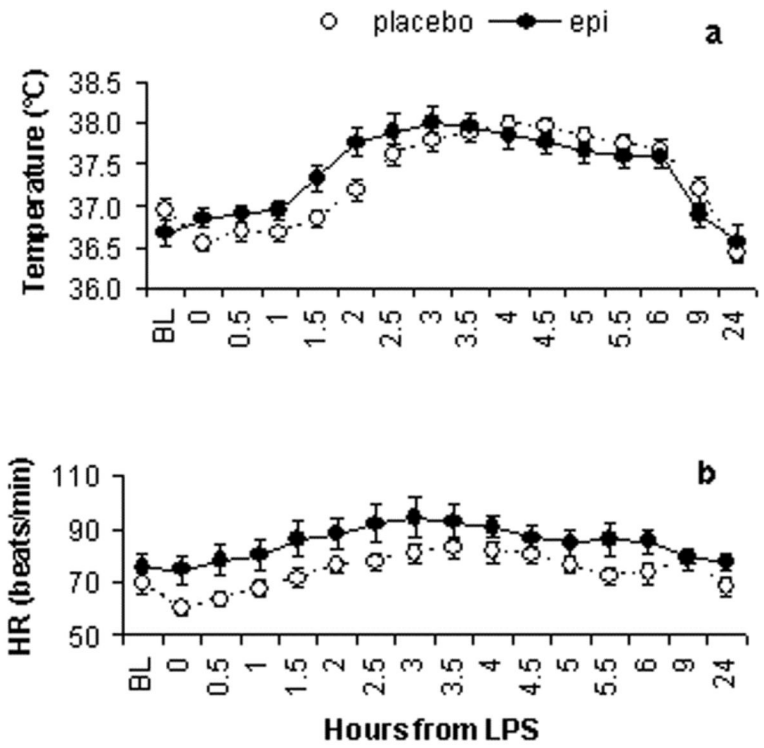

Figure 1 (a,b).

Legend: Temperature (a) and heart rate (b) as a function of time after intravenous LPS administration, given at time-point zero, in human subjects that received either a placebo infusion of physiologic saline (n=10) or those that received an infusion of epinephrine (n=7) (30 ng/kg per min) for 3 hours prior to LPS administration and was continued until +6hr. Results are expressed as mean ± SE. In the EPI+LPS group; temperature (a) more rapidly peaked and returned to baseline after LPS ($p<0.001$) and heart rate (b) was significantly greater ($p<0.05$) compared to the saline+LPS group over time. BL-baseline

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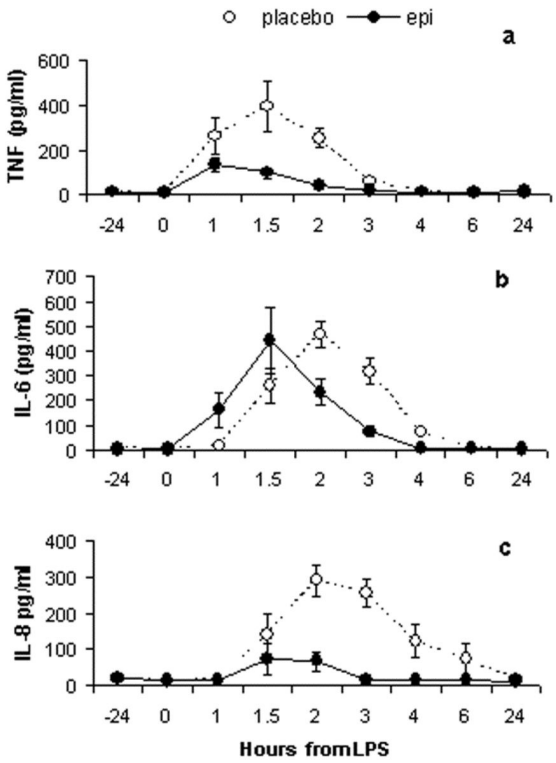

Figure 2 (a,b,c).

Legend: TNFα (a), IL-6 (b) and IL-8 (c) as a function of time after intravenous LPS administration, given at time-point zero, in human subjects that received either a placebo infusion of physiologic saline (n=10) or those that received an infusion of epinephrine (n=7) (30 ng/kg per min) for 3 hours prior to LPS administration and was continued until +6hr. Results are expressed as mean ± SE. LPS induced inflammatory mediator release as observed by elevated TNF (a), IL-6 (b) and IL-8 (c) in the placebo group. EPI infusion attenuated the release of both TNFα ($p<0.001$) (a) and IL-8 ($p<0.001$) (c) and mediated a more rapid peak and return to baseline of IL-6 ($p<0.001$) (b). All of the above inflammatory mediators were undetectable by +24 hours. BL-baseline

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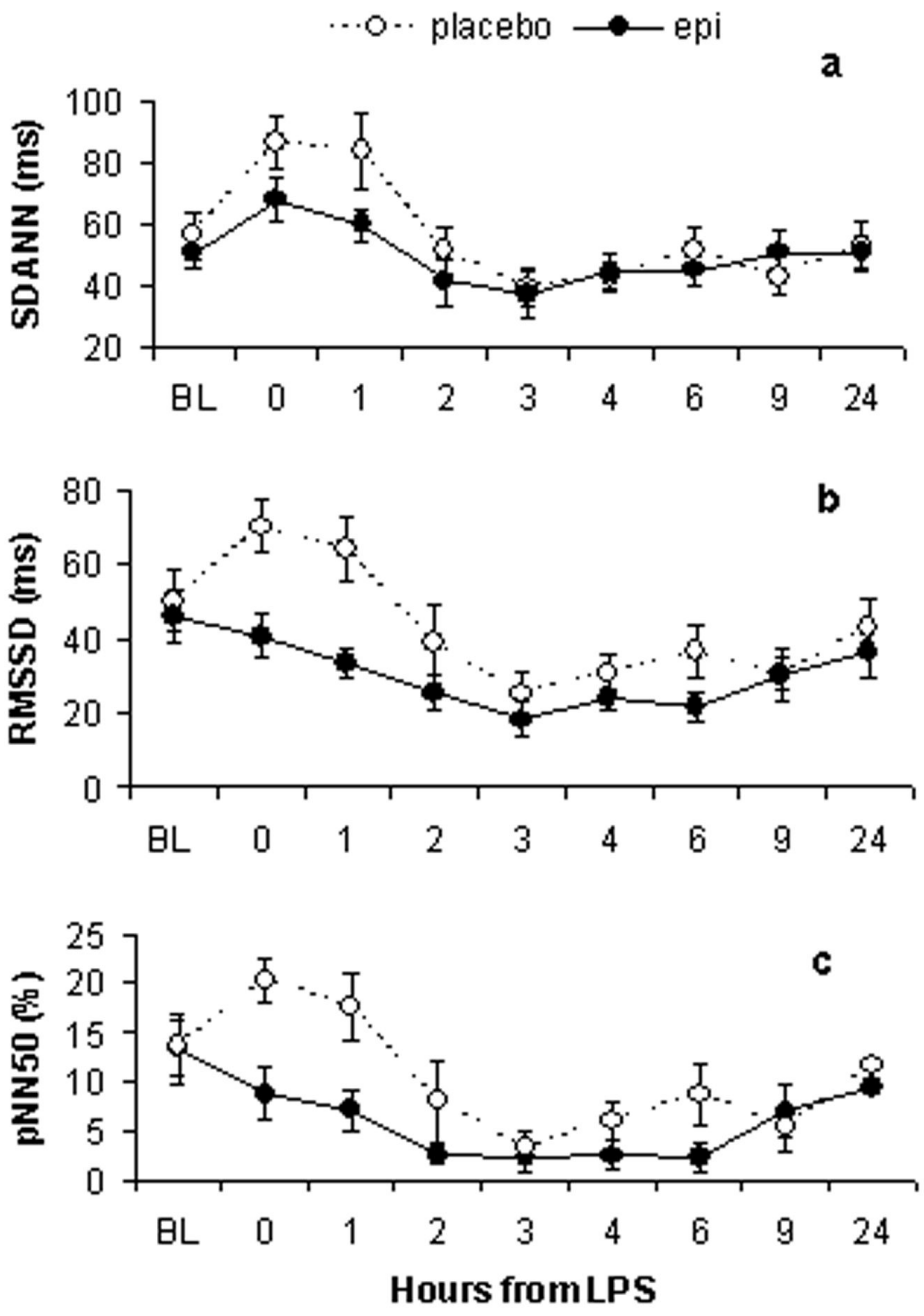

Figure 3 (a,b,c).

Legend: Time domain measures of HRV, SDANN (a), RMSSD (b), pNN50 (c) as a function of time after intravenous LPS administration, given at time-point zero, in human subjects that received either a placebo infusion of physiologic saline (n=10) or those that received an infusion of epinephrine (n=7) (30 ng/kg per min) for 3 hours prior to LPS administration and was continued until +6hr. Results are expressed as mean ± SE. In the saline+LPS group from time-point 0hr to +3hr, LPS mediated a decrease in SDANN ($p<0.01$) (a), RMSSD ($p<0.01$) (b), and pNN50 ($p<0.01$) (c). From BL to +24hr, compared to saline+LPS, EPI mediated a decrease in RMSSD ($p< 0.005$) (b) and pNN50 (c) ($p<0.01$), while SDANN (a) was not significantly different between groups. BL-baseline

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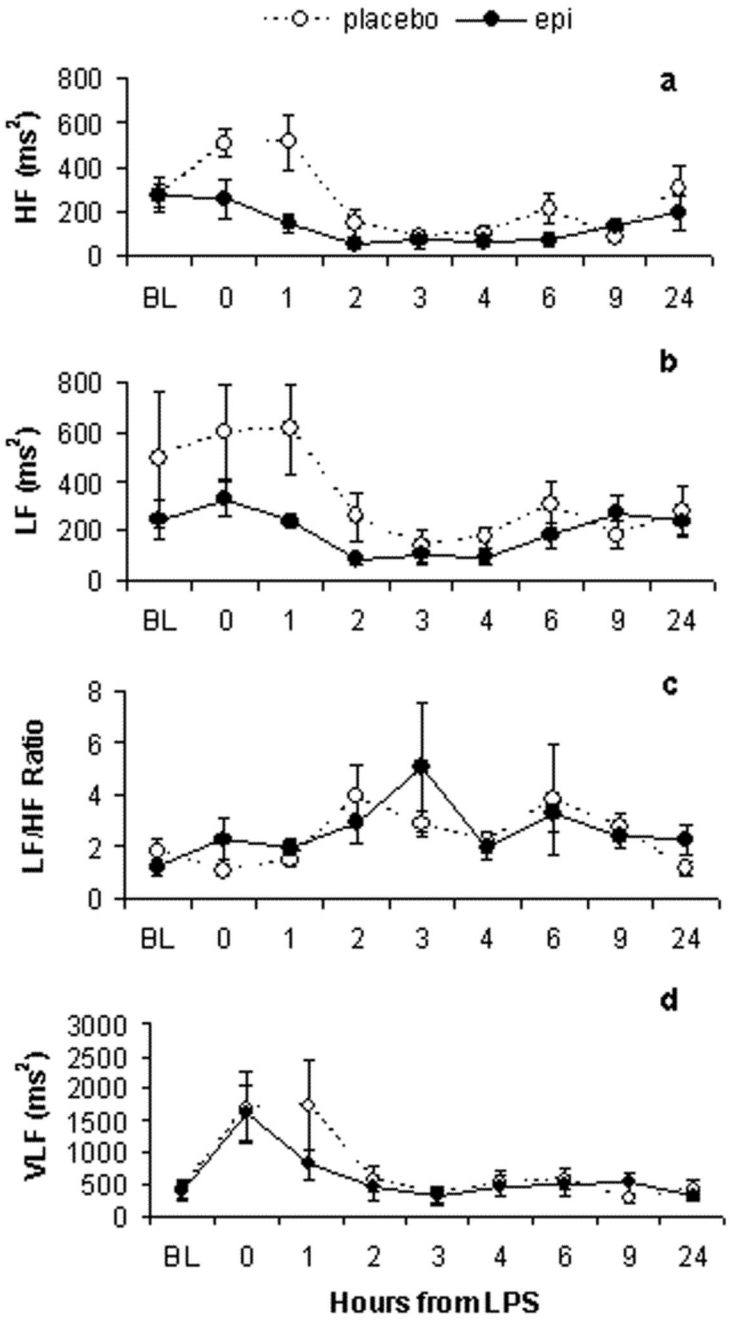

Figure 4 (a,b,c,d).

Legend: Frequency domain measures of HRV, HF (a), LF (b), LF/HF (c) and VLF (d) as a function of time after intravenous LPS administration, given at time-point zero, in human subjects that received either a placebo infusion of physiologic saline (n=10) or those that received an infusion of epinephrine (n=7) (30 ng/kg per min) for 3 hours prior to LPS administration and was continued until +6hr. Results are expressed as mean ± SE. In the saline+LPS group from time-point 0hr to +3hr, LPS mediated an increase in LF/HF ($p<0.01$) (c) and also mediated a decrease in HF ($p<0.01$) (a), LF ($p<0.01$) (b) and VLF ($p<0.01$) (d). From BL to +24hr, compared to saline+LPS, EPI mediated a decrease in HF ($p<0.05$) (a)

while LF (b), LF/HF (c) and VLF (d) were not significantly different between groups. BL-baseline

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