# A multiscale modeling approach to inflammation: A case study in human endotoxemia

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# 1 Abstract

Inflammation is a critical component in the body's response to injury. A dysregulated inflammatory response, in which either the injury is not repaired or the inflammatory response does not appropriately self-regulate and end, is associated with a wide range of inflammatory diseases such as sepsis. Clinical management of sepsis is a significant problem, but progress in this area has been slow. This may be due to the inherent nonlinearities and complexities in the interacting multiscale pathways that are activated in response to systemic inflammation, motivating the application of systems biology techniques to better understand the inflammatory response. Here, we review our past work on a multiscale modeling approach applied to human endotoxemia, a model of systemic inflammation, consisting of a system of compartmentalized differential equations operating at different time scales and through a discrete model linking inflammatory mediators with changing patterns in the beating of the heart, which has been correlated with outcome and severity of inflammatory disease despite unclear mechanistic underpinnings. Working towards unraveling the relationship between inflammation and heart rate variability (HRV) may enable greater understanding of clinical observations as well as novel therapeutic targets.

**Keywords:** Systems biology, heart rate variability, circadian, microarray, autonomic dysfunction

# 2 Introduction

Inflammation is the complex, multiscale physiological response of an organism to biological stressors that is required for immune surveillance and regeneration after injury. Under normal circumstances, the endpoint of inflammation is a favorable outcome as homeostasis is

restored. However, when anti-inflammatory processes fail to sufficiently counteract pro-inflammatory signals, inflammation becomes prolonged and can lead to uncontrolled systemic inflammation which, in turn, can eventuate in various disease conditions or aggravate an already existing disease process. Clinically, this presents a huge challenge in inflammatory diseases such as sepsis [1], as therapies for the management and control of inflammation in septic patients are limited.

It has long been well established that the control of inflammation plays a key role in a variety of inflammation-related disorders. Novel therapies aimed at treating many inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, with anti-cytokine therapies have made great strides in recent years, but similar strategies have not produced positive results in sepsis [2, 3]. One of the primary challenges hampering the discovery of new therapies is the redundant, interacting pathways involved in the inflammatory response which give rise to complex, unintuitive dynamics which resist straightforward reductionist study [4]. Thus, it is becoming increasingly evident that further progress requires a systems-level understanding of inflammation [5-8]. This has motivated the investigation of computational models of inflammation [9]. Inherent in these models are quantitative, explicit representations of hypotheses, at a wide range of scales. Simple, reduced models of inflammation allow for investigation of broad patterns and detailed mathematical analysis of system dynamics [10-12]. More complex models, often incorporating features like spatial heterogeneity and stochasticity [13], allow for the exploration of more nuanced components on a more detailed system [14]. This type of work has significant translational potential in areas such as rationalizing drug development and clinical trials to optimizing patient care [15, 16], which are particularly critical tasks in inflammatory diseases such as sepsis, in which current treatment options are limited and

mortality rates are high [17]. Critical in translational applications of models of inflammation are experimental and computational work studying these responses in humans.

A great deal about the initial human response to infection has been learned from the elective administration of endotoxin (lipopolysaccharide, LPS) [18-20], a major component of the outer membrane of Gram-negative bacteria that activates the innate immune system, leading to inflammation. Although acute, systemic inflammation is but one component of sepsis syndrome, a variety of useful surrogate experimental paradigms have been established that avoid the complex pathophysiology and co-morbidities of human sepsis [21]. Human endotoxemia precipitates signs and symptoms characteristic of clinical sepsis [21, 22], acute respiratory distress syndrome (ARDS) [23], and trauma [24]. The administration of a low dose of endotoxin to human subjects elicits significant dynamic transcriptional changes as well as hemodynamic and neuroendocrine responses that mimic acute injury and early sepsis [25]. At the cellular scale, innate immune cell activation leads to the production and release of both pro-inflammatory and anti-inflammatory cytokines, which are proximal mediators of the systemic inflammatory response and of the compensatory anti-inflammatory response syndrome, respectively. At the higher level, the central nervous system (CNS) regulates the immune response through activation of the sympathetic and parasympathetic branches of the autonomic nervous system as well as the hypothalamic-pituitary-adrenal (HPA) axis [26]. Further, heart rate variability (HRV), a system-level physiologic signal, is also diminished by low-dose endotoxin to human subjects.

In recent years, analysis of HRV has become attractive as a readily-available physiologic metric that may give insight into the progression and recovery from diseases involving systemic infection and inflammation. This phenomenon raises intriguing possibilities for understanding the loss of inter-organ communication and coupling observed in critical illness [27, 28].

Decreases in HRV have also been studied and characterized as generalized responses to human endotoxemia [29], raising intriguing possibilities for the disruption of signal output variability of many organ systems as but one manifestation of systemic loss of complexity, as observed in critical illness [25, 30]. In addition to this, autonomic imbalance, manifested as diminished cardiac vagal function and prevalence of sympathetic control of heart rate, is also elicited during the acute inflammatory condition mediated by endotoxin administration in healthy volunteers [25]. It has been hypothesized that a reduction in HRV and cardiac vagal tone reflect increased isolation of the heart from other organs. The hypothesis, originally introduced by Godin and Buchman [31], suggests that healthy organs behave like biological oscillators coupled to one another. Thus, reduced HRV reflects systemic-level loss of high level signal variability which is associated with a less "healthy" state in hospitalized critically ill patients. This reduction in complexity may have diagnostic value. Technology based on analysis of HRV signals is on the verge of moving from the investigatory stages into the realm of clinical practice, as recently evidenced by a successful clinical trial that reduced mortality from neonatal sepsis through increased HRV monitoring [32]. Recent studies have also shown clinical applications of HRV analysis as a predictive tool in trauma patients [33-36] as well as sepsis in adults [37] through an increasingly-expanding library of HRV metrics [38].

Despite successes in correlating HRV with disease state, the underlying physiological processes linking the recognition of danger signals by immune cells with systemic changes are not well defined. The application of mathematical models to physiological dynamics is one promising modality through which these goals could be accomplished [39-41]. In an effort to establish quantifiable relationships among the components of the inflammatory response, we proposed a multiscale model linking human endotoxemia and HRV, as a prototype model of

acute inflammation and autonomic dysfunction in humans. In this paper, we review this computational model and highlight its potential utility as derived from incorporating multiple temporal and spatial scales, culminating in the overall network structure depicted in Figure 1. First, transcriptional responses to human endotoxemia were identified from DNA microarray experiments [18]. These responses were linked together through physicochemical modeling, producing a quantitative model of the progression and resolution of systemic inflammation [42]. To account for endogenous and exogenous hormonal regulation of inflammation, pharmacokinetic/pharmacodynamic models of epinephrine and cortisol were incorporated to account for their immunomodulatory effects [43]. Then, dynamics of these molecular and cellular patterns are linked to HRV through a continuous-discrete model. The output of the combined model is a series of discrete heart beats, which are post-processed to determine HR and HRV.

# 3 Data-driven physicochemical modeling of inflammation

Deciphering the connectivity and dynamics of emerging network architectures is a critical task in the analysis of biological systems. The advent of methods that facilitate this task is largely based on the rapid advances in monitoring changes at the cellular and molecular scales, and especially by developments in measuring gene expression at the genome-wide scale [18] as well as multiplexing techniques for analogous measurements of multiple proteins simultaneously. Characterizing the behavior of a dynamic system requires defining the system's state space as it evolves over time. At the genome scale, orchestrated patterns in the expression of genes define the transcriptional state of the system. Further, advances in "bedside" technologies monitor the stage of a disease by measuring vital signs that define an individual patient's clinical outcome. As this technology has matured, what started as an attempt to classify

temporal patterns has evolved into sophisticated analyses capable of providing semi-mechanistic disease progression models [44]. In this section, the process of linking the transcriptional dynamics identified in high-throughput analysis techniques with biological function is discussed within the context of data-drive physicochemical models of disease progression.

## *3.1 Identifying critical transcriptional responses to human endotoxemia*

As described above, there has recently been a growing interest in modeling the inflammatory response as a set of key components that are considered to play a critical biological role in the dynamics of the host response when exposed to various stressors such as infection, trauma, hemorrhage shock, or other inflammatory stimuli [45, 46]. Thus, there is emphasis on reducing the complexity of the computational models of inflammation by identifying a limited number of time-dependent interactions amongst key elements that are highly sensitive to specific modes of initiation and modulation of the response. This type of approach has been applied in modeling system-level disease processes, like sepsis [47]. A number of prior studies [10-12, 45, 48] have placed significant emphasis on simulating inflammation based on the kinetics of well-accepted constituents of the acute inflammatory response at the final effector level (typically functional proteins or free radicals and their reaction products). One of the key features of these models is the *a priori* postulation of certain components that are consistent with biological knowledge to play a major role in triggering the inflammatory response; thus, the computational integration of these well-vetted components can provide us with significant insight of how such components behave over time, empowering their translational application as predictive controls in clinical settings.

The analysis discussed here follows a different but complementary approach, using gene expression data from human endotoxemia experiments as originally reported by Calvano *et al.* [18]. Blood samples were drawn from healthy human volunteers before as well as 2, 4, 6, 9, and 24 hours after LPS administration. From these samples, leukocytes were recovered through centrifugation and subsequently total leukocyte RNA was isolated. Gene expression was assessed through hybridization onto Affymetrix Hu133A and Hu133B oligonucleotide arrays, yielding a total of 44,924 measured probe sets for each individual at each time point.

Such high-throughput experimental data allow for a more unbiased approach to identifying key components of the inflammatory response. However, a major challenge is the systematic identification of such representative biological features, based on experimental data that can adequately represent the complex dynamics of a host undergoing an inflammatory response. This process requires the decomposition of the non-linear dynamics of the response into an elementary set that can serve as a surrogate for predicting the collective behavior of the system. One approach used in the systems biology field has been to define principal drivers of a response using techniques such as principal component analysis [49]. These methods, based on a starting population of high-dimensional data at the protein level, are being employed to define principal drivers in the response to trauma/hemorrhage [50], endotoxemia [14], traumatic brain injury [51], and related inflammatory challenges in both animals and humans. Principal components are orthonormal linear combinations of the data vector, with the property that they carry the largest variances in several orthogonal directions. This is a method that reduces the dimensionality of a problem by concentrating on just a few (usually up to five or six) statistically most significant orthonormal linear combinations. Next, the "principal drivers" of inflammation are identified by summing the weights of each analyte present in each of the principal

components. These principal drivers can then be interlinked via mechanistic computational models [7, 8, 50, 52].

An alternative answer to this problem can be identified through the analysis of gene expression data aimed at monitoring the dynamics of the host response to an inflammatory agent, exploring the idea that cellular responses correspond to dynamically converging high-dimensional transcriptional trajectories. Decomposing the intrinsic dynamics of the entire system into a reduced, modular set of responses enables us to both project and understand the complex dynamics of the system by studying the properties of its essential dynamic parts. A central tenet of Translational Systems Biology is that computational models should be calibrated against the type of data that are available in the clinical setting [15, 53, 54]. Bedside sampling and transcriptional profiling analysis of human blood leukocytes has become methodologically viable [18], motivating the hypothesis that the genes that are most responsive to endotoxin are governed by defined mechanisms and have concerted changes in their expression profile.

A systematic computational framework was recently proposed that decomposes high-dimensional microarray data into an elementary set of temporal responses [55]. The underlying hypothesis of this work is that there is a defined network structure underlying the emerging dynamic inflammatory response. A corollary to this hypothesis is that these *core inflammatory responses* might serve as surrogates for the dynamic evolution of the host response due to endotoxin stimulation. In order to hone in on this core response, a micro-clustering approach is first applied. The method is based on a symbolic transformation of time series data, which assigns a unique integer identifier (*hash value)* to each expression motif. The symbolic transformation of the expression motifs and the subsequent assignment of hash values to each expression profile produces a distribution of motif values for all the available probes.

Having assigned the temporal expression profiles to distinct motifs, the next task is to select expression motifs that appear to be highly non-random, thus generating a sub-set of transcriptional motifs which are considered to be the most characteristic of the target response (*e.g.* exposure of the host to endotoxin). Based on these significantly enriched expression motifs from the initial large set of micro-clusters, one next needs to identify a discriminating set of critical temporal shapes that best characterize the intrinsic dynamic response of the system. Due to the global nature of the transcriptional measurements and the fact that one does not select a limited set of responsive genes *a priori*, the entirety of the transcriptional response is expected to exhibit a Gaussian-type of response with no clear defining responses. The transcriptional state (TS) of the system is defined as the overall distribution of expression values at a specific time point, and the deviation of the system is then quantified at each time point versus a baseline distribution (control time point) by applying the Kolmogorov-Smirnov test to compare a subset of genes with the entire population of genes. Given the aforementioned metric, we are interested in identifying the minimum number of expression motifs that characterize the maximum deviation of the system. This selection problem is a combinatorial optimization problem for which a stochastic simulated annealing optimization algorithm is applied.

When this analysis was applied to data on human volunteers subjected to endotoxemia, three critical expression motifs were identified as enriched in critical and relevant biological pathways: (i) Early up-regulation response (Pro-inflammatory component), such as genes in the Toll-like receptor signaling pathway and members of the NF-κB/RelA family; (ii) Late up-regulation response (Anti-inflammatory component), including genes in the JAK-STAT cascade as well as IL10RB, which is assumed to be indicative of the IL-10 signaling cascade; and (iii)

Down-regulation response (Energetic component), including genes that are mainly involved in cellular bio-energetic processes.

This approach of high-throughput transcriptional screening of human blood leukocytes in endotoxemia to gain insight into systemic inflammation is valuable in that it provides an *in vivo* look into leukocyte-level responses in humans, rather than in an *in vitro* or animal model [18]. An animal model, for instance, would allow for the evaluation of systemic inflammation in a variety of tissues, which is an important consideration given that blood leukocytes reflect only one level of the inflammatory response. The *in vitro* nature of these experiments means that the gene expression data reflects, in part, the complex regulatory structure in the human inflammatory response which cannot be recapitulated through analysis of human cell lines.

An important caveat in evaluating gene expression data is potential differences the dynamics of gene transcripts and proteins. While a more complete understanding would be obtained through additional proteomic analysis, lacking such available experimental data, the validity of analyzing gene expression data in human endotoxemia is supported by experimental evidence showing correlations between mRNA and protein expression for key inflammatory mediators in human endotoxemia [56] as well as the overrepresentation of inflammation-linked genes and pathways identified in our analysis. Furthermore, because the data was modeled at the level of clusters rather than accounting for the specific expression values of individual genes, these mathematical models are valid with regard to specific predictions and insights. Therefore, the analysis of gene expression data represents an effective technique to assess the dynamics of the human endotoxemia response.

## 3.2 Physicochemical modeling of transcriptional processes

Physicochemical modeling seeks to describe essential biological processes in terms of equations that can be physiologically interpretable. Such models provide means to merge prior knowledge with experimental data and underlying principles about processes whose components (i.e. "pathway signals") and connectivity are relatively well established [57]. Considering the leukocytes as a well defined system, the purpose of traditional experiments is to qualitatively characterize the cellular dynamics. The purpose of a systems biology approach, on the other hand, is to reverse engineer quantifiable representations of the intracellular dynamics by identifying (i) appropriate constitutive elements; (ii) the topology of the interactions among these elements; and (iii) the quantitative relations among these elements. In the previous section, we addressed the first issue, whereas now we will discuss how to construct the topology of the underlying network that describes the dynamics at the leukocyte level.

The state of the art in mechanistic simulations of inflammation was recently discussed by Vodovotz and coworkers [9]. Although black-box modeling has found widespread applications in systems biology, the transcriptional analysis described above allows for the development of a more mechanism-based model As such, a cellular physicochemical host response model is developed to serve as transmittable repositories of knowledge, linking extracellular signals with intricate signaling cascades essential for the onset and propagation of the host response. One of the key assumptions underpinning this modeling effort is that intracellular signaling cascades activate inflammation-specific transcriptional responses [58], which in turn lead to the expression and modification of proteins that carry out the biological functions of inflammation.

In the endotoxin injury model, the inflammatory response is activated when endotoxin is recognized by pathogen recognition receptors, initiating a complex signaling cascade that

ultimately triggers essential signaling modules for the activation of pro–inflammatory transcription factors. Although a large number of transcription factors are known to be involved in inflammation, the NF-κB module is central, and has been the focus of much computational modeling activity. An inadequate control of the transcriptional activity of NF-κB is associated with the culmination of a hyper-inflammatory response, making this transcription factor a desired therapeutic target in sepsis [59].

Each essential transcriptional motif is considered to be the manifestation of a process involving synthesis and degradation terms. Specifically, the upstream activated signaling complex (i.e. NF-κB module) serves as the "active signal" that indirectly gives rise to the "first-line" transcriptional response represented by pro-inflammation. Such stimulation is particularly expressed as a linear function with respect to NF-κB activity ("pathway signal") that affects the production rate of the pro-inflammatory response triggered upon the recognition of LPS by its receptor, which in turn is mathematically approximated as a standard ligand-receptor interaction [57]. Further, the manifestation of the other emergent transcriptional events (i.e. anti-inflammation, energetic response) is hypothesized to be the downstream effect with respect to the initiation of the early pro-inflammatory response. For instance, pro-inflammatory influences stimulate the energetic response whilst a dysregulation in the cellular bio-energetic processes serves as a positive feedback danger signal to the pro-inflammatory response. The anti-inflammatory response, on the other hand, serves as the essential immunomodulatory signal that contributes to resolution of inflammation by inhibiting the production rate of the relevant components that involve the pro-inflammation and energetic responses. The prototypical responses to inflammatory stimuli are resolution, in which host dynamics favor a return to the baseline state, or sustained (and perhaps irreversible) responses characterized by an ongoing,

self-maintaining presence of inflammation driven by endogenous damage-associated molecular pattern molecules.

In total, this physicochemical model of human endotoxemia captures experimental results on the progression of the inflammatory response at the transcriptional scale. Thus, computational models permit relevant steady states to exist, which in the case of inflammation can be equated with “recovery/self-limited” or “uncontrolled/sustained inflammation” responses that might reflect the clinical phenotype of critically ill patients. However, keeping in mind the ultimate goal of translational applications, both the effects of immunomodulatory treatments and the assessment of more clinically-accessible markers are critically important.

# 4 Modeling human endotoxemia

The model described in the previous section is comprised of molecular-level physicochemical equations fit to gene expression data from microarray experiments. To further account for the phenomena observed in human endotoxemia, this single scale model must be extended to represent multiscale data and processes.

## *4.1 PK/PD models of immunomodulatory hormones*

Anti-inflammatory drugs such as corticosteroids play a critical role in modulating the progression of inflammation and significant prior research efforts have attempted to elucidate the mechanisms driving corticosteroid activity. Such studies simulate the pharmacogenomic effect of glucocorticoids at the transcriptional scale taking their mechanistic (signaling) action into account by modeling (i) the binding of the corticosteroid to its cytosolic receptor; (ii) the subsequent formation of the corticosteroid-receptor complex; (iii) the translocation of the cytosolic complex to the nucleus that alters the transcriptional machinery, activating or repressing numerous genes; and finally (iv) the auto-regulation of the gene transcript of the

glucocorticoid receptor. As such, the development of a physicochemical model of human inflammation is developed that couples pro-inflammatory pathways with a pharmacokinetic model of corticosteroids, to be used as a template for assessing anti-inflammatory intervention strategies [43]. Typical responses involve a self-limited inflammatory response that resolves within 24 hours post-endotoxin administration; the progression of an unconstrained inflammatory response due to an increase in host's susceptibility to endotoxin stimulus, as well as due to a dysregulation in NF-κB signaling dynamics; and finally, the possibility of acute hypercortisolemia "reprogramming" the dynamics of the system in favor of a balanced immune response [60]. However, current challenges in developing such models include limitations in prior knowledge and the specification of model structure. Oftentimes, prior knowledge is sparse and the manifestation of a perturbation is difficult to describe explicitly using elementary kinetic reactions. In the following section we will discuss possible mathematical representations that can address this type of challenge.

Indirect response (IDR) models have been widely used in pharmacokinetic/pharmacodynamic models simulating the physiological response of a system exposed to an external signal or perturbation [61-63]. Our inability to explicitly model complex signaling mechanisms using physicochemical principles makes indirect response modeling appealing. The underlying assumption of IDR models is that external signals indirectly affect the synthesis and/or degradation terms of the response. As a result, the existence of such signals can either stimulate or inhibit the production and degradation rates of the response of interest. In the absence of any external signal, the system lies at homeostasis and the baseline of the probed response is defined by the balance of these two parameters. However, the presence of external signals that perturb the dynamics of the system away from its equilibrium can be quantitatively

represented by appropriate functions (stimulatory or inhibitory) that affect the production and/or degradation terms of the manifested response.

Along these lines, we have also explored the relationship between circadian rhythmicity and human endotoxemia [64]. Circadian rhythms are biochemical, behavioral, or physiological processes that are entrained to a 24-hour periodic cycle, as have widely been observed in humans and other animals from the scale of biochemical reactions, such as hormone production, to behavioral patterns, such as regular sleeping and feeding times. Understanding the impact of these rhythms is an important clinical challenge, given that it has been observed that sepsis patients have a heightened risk of mortality between 2 a.m. and 6 a.m. [65].

Circadian rhythms may be important in human endotoxemia because several key components of the inflammatory response have significant circadian patterns [66]. Cytokines undergo circadian variations in plasma concentrations, typically peaking in the night [67-71]. Plasma cortisol concentration also exhibits a circadian pattern, peaking in the early morning. Due to the immunomodulatory effects of glucocorticoids and the strong circadian pattern of plasma cortisol concentration, cortisol has been implicated in the circadian entrainment of cytokine production by leukocytes [69]. However, cortisol stimulates the production of anti-inflammatory cytokines while inhibiting the production of pro-inflammatory cytokines [72, 73]. So, as most cytokines peak near the same time in the night, a simple relationship with cortisol cannot explain the circadian rhythms in cytokine production [69].

Another potential circadian regulator, melatonin, has also been implicated in the mediation of crosstalk between the immune system and the suprachiasmatic nucleus (SCN) [66]. Plasma melatonin concentration peaks transiently in the night while resting at very low values the rest of the day, and melatonin has been shown to stimulate the production of cytokines [74-

76]. Based on this, we developed circadian models of melatonin and cortisol, based on a "two rates" pharmacodynamic model [77]. Then, these circadian hormones drive the circadian variation in all of the other model variables. This produces circadian rhythms throughout the model, leading to a predicted time-of-day-dependent responses to inflammatory stimuli as shown in Figure 2.

To this point, we have largely described modeling at the molecular scale, such as cytokine and hormone responses. While changes in these inflammatory mediators play critical roles in the progression of the inflammatory response, they do not reveal the full inflammatory state of the host and they are difficult to assess clinically. For these reasons, we have explored modeling approaches aimed at linking these molecular processes with changes in HRV, a clinically accessible variable which has been shown to correlate with disease state in inflammatory disorders [78].

## *4.2 Heart rate and heart rate variability*

From a phenomenological perspective, it has been long appreciated that stresses such as sepsis lead to reduced physiological complexity manifesting in part as reduced HRV. However, a comprehensive conceptual framework linking the inflammatory processes described above with changes in HRV is lacking. One reason for the lack of a mechanistic underpinning that connects inflammation and physiological complexity is the existence of multiple scales and hierarchies of biological organization [13, 53, 79]. The mirroring of this complexity impedes the successful transfer of information from the pre-clinical to the clinical stage, as seen primarily in attempts to develop effective therapies for diseases resulting from disorders of internal regulatory processes [13]. Progress in treating these processes requires effective translational methodologies that concatenate mechanisms across multiple scales of biological hierarchy. Focusing on the

study of inflammation, the acute systemic inflammatory condition mediated by endotoxin administration in healthy volunteers elicits a complex network of multiscale interactions between the immune system and the central nervous system (CNS) that can result in reduced HRV [29]. Dissecting the relevance of neuroimmunomodulation in controlling inflammatory processes requires an understanding of the interplay between CNS and the immune response.

Previously, we approached these problems through ordinary differential equation models linking endotoxemia with changes in both HR [80] and HRV [81]. These models began to explore physiological changes occurring in inflammation which are transduced through the autonomic nervous system to the heart, producing changes in beating rate and pattern. However, this modeling approach treats HR and HRV as continuous processes that evolve through distinct models, when in reality they are both statistical quantities derived from a discrete time-varying signal, a series of heart beats. Thus, a major challenge in more mechanistic modeling of HRV changes in endotoxemia is reconciling relatively smooth, continuous quantities (hormone and cytokine concentrations) with a discrete, noisy process (the beating of the heart). We approached this problem in two phases [82]: First, by developing a continuous model of autonomic influence on the heart; and second, combining this with a discrete model to generate output in the form of a series of heart beats. This output can then be assessed to calculate HR and HRV.

### 4.2.1 Autonomic modulation of the sinoatrial node

The sinoatrial (SA) node of the heart is known as the heart's pacemaker because its action potentials initiate the periodic contraction of cardiac tissue giving rise to heart beats. The SA node is innervated by the sympathetic and parasympathetic branches of the autonomic nervous system, thus allowing fluctuating amounts of autonomic neurotransmitters to modulate the firing pattern of SA node cells away from their free running rhythm. Variability in the

activity of the SA node, and thus variability in HR, is largely governed by changes in autonomic output.

Keeping in mind our ultimate goal of linking systemic-level changes in HRV with molecular and cellular processes involved in inflammation, a first step towards studying this is to model autonomic regulation at the SA node. The rhythmic signals transducer by the autonomic nervous system to the SA node reflect underlying biological control systems, and the characteristics of these rhythms as manifested in HRV give some insight into the physiological state of the host. Three periodic signals are most apparent in HRV:

**HF (high frequency) rhythms** occur in the frequency range of 0.15-0.4 Hz [83] and has a relatively clear physiological underpinning, driven mainly by breathing pattern and communicated to the heart via the vagus nerve [84]. This is further supported by experiments showing that atropine, an inhibitor of vagal signaling, almost entirely eliminates HF oscillations [85]. **LF (low frequency) rhythms** ranging from 0.04-0.15 Hz [83]. LF oscillations, historically used as an indicator of sympathetic activity, is now generally viewed to reflect fluctuations in both branches of the autonomic nervous system [84]. **Circadian rhythms**, with a period of 24 hours matching the light-dark cycle and thus at a much longer timescale than LF and HF oscillations, impose a distinct pattern in HRV [86] due to circadian changes in both sympathetic and parasympathetic activity.

By accounting for these oscillatory factors, as well as their disruption in human endotoxemia, we developed a model of autonomic modulation of the SA node in the form of a continuous algebraic equation [82]. Yet to truly assess the variability in this signal, similar to how variability in HR is assessed from discrete RR interval series experimental data, it must be

processed to produce an output similar to how the heart responds to autonomic modulation by discrete yet rhythmic beating patterns.

### 4.2.2 Discrete-continuous modeling

SA node neurons function by sensing local neurotransmitter output from the autonomic nervous system and firing when a threshold is crossed, thus initiating electrical impulses that propagate through the cardiac tissue and provoke contraction. As the model described in the previous section gives an approximation of the postsynaptic neurotransmitter, an integrate-and-fire neuron model can translate the continuous, oscillatory model to a discrete series of events representing heart beats. The input signal is repeatedly integrated until a threshold is reached, and each time the threshold is hit, an event (heart beat) occurs. This type of procedure has been used in the context of integral pulse frequency modulation (IPFM) models [87], which have previously been used to investigate the effect of autonomic modulation of the SA node [88, 89]. This translation from a continuous oscillatory system to a variable discrete output is critical in modeling HRV. Clinically, HRV can be measured by a variety of time domain, frequency domain, and nonlinear metrics, all aimed at gaining some biological or clinical insight from the pattern of heart beats. Through our discrete modeling, we can similarly apply post-processing techniques to calculate various HRV metrics. This is important as, both in the modeled results and in clinical data, different HRV metrics change in different ways in different scenarios. Furthermore, through our integrated model of human endotoxemia, this allows us to assess the effects of systemic inflammatory mediators on the dynamics of heart beat patterns by applying HRV metrics to the heart beat time series. An example of this is shown in Figure 3, in which the discrete nature of heart beats allows for the analysis of a series of heart beat intervals through

Poincaré plots. Such plots can give insight into both short-term and long-term changes in HRV dynamics [90].

# 5 Conclusions

The modeling work described herein summarizes our efforts towards reconciling known physiological processes occurring in inflammation with a systemic-level clinical measurable, HRV. The clinical relevance of our model is increased due to the fact that it is built on several different modalities of data from human endotoxemia experiments, ranging from gene expression of peripheral blood leukocytes to systemic hormone concentrations to EKG-derived data. This diversity of data is matched by a variety of data analysis and modeling techniques to identify key patterns in the data, link them through physicochemical modeling, account for pharmacodynamic hormonal effects, and ultimately produce a discrete, noisy output of heart beats.

Multiscale mechanistic models which link cellular and molecular processes to changes in HRV are particularly intriguing, as HRV has been shown to correlate with disease state in a variety of situations yet the precise physiological underpinnings of modulated HRV are not clear. Fundamentally, rhythmic variations in HR are driven by negative feedback control systems, and changes in the character of physiological oscillators reflect the state of these regulatory systems. Thus, if we are able to successfully model changes in HRV through this approach, the result will be increased mechanistic insight on how changes in homeostatic regulatory function are related to the progression of inflammation. Effectively, this work is part of a transition from correlating a signal with an outcome to asking why, from a physiological perspective, a signal is changing [91]. Because of this, the model described here has great potential in translational applications [15, 16], from informing clinical trials to rationalizing drug discovery to optimizing patient care.

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# 8 Figures

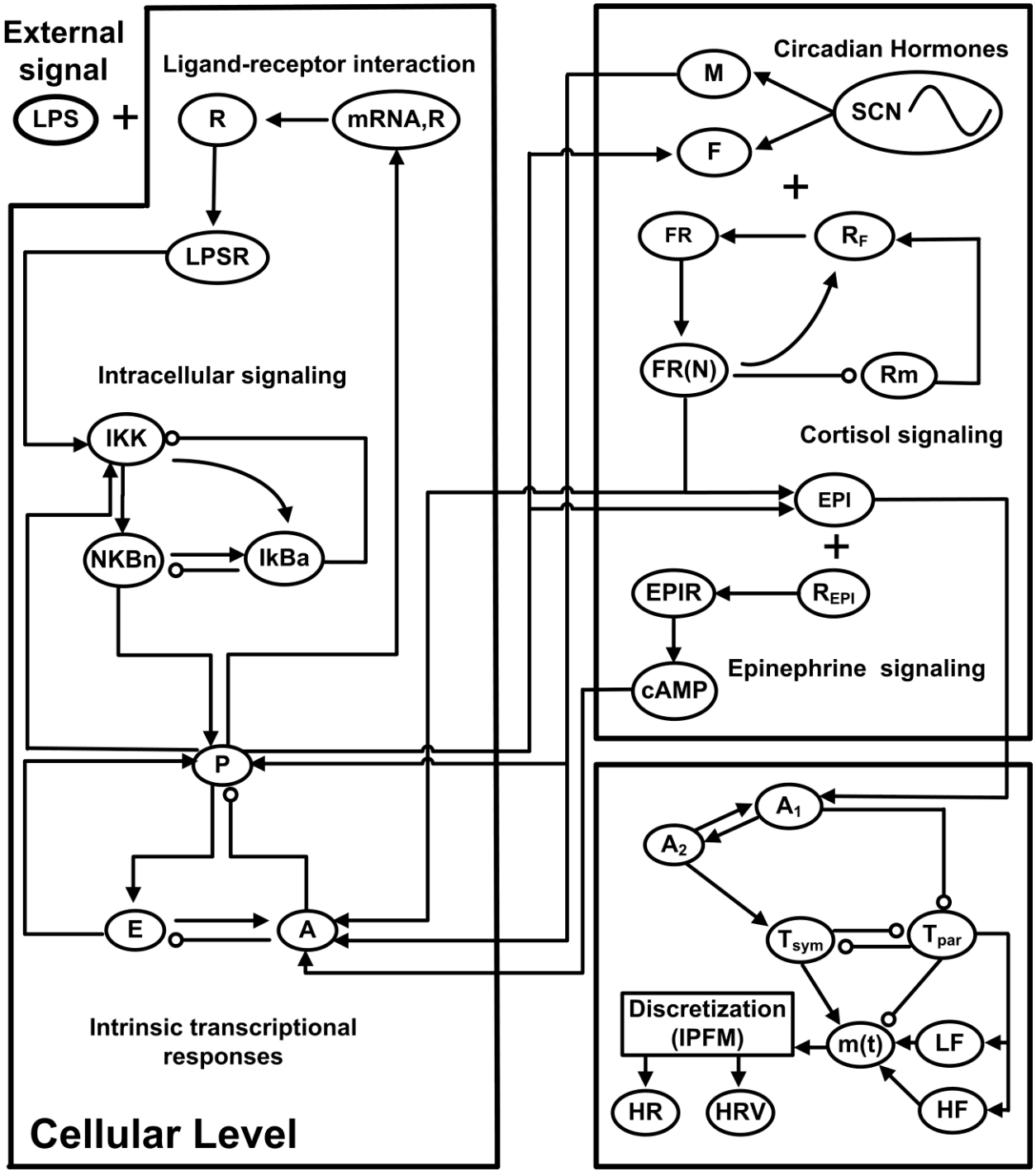


Figure 1: **Network structure of a model of human endotoxemia.** At the cellular scale, LPS binds to TLR4 (R), activating the NF-κB signaling cascade that activates the transcriptional

response to endotoxemia that includes pro-inflammatory signaling (P), anti-inflammatory signaling (A), and a decrease in cellular bioenergetic processes (E). Neuroendocrine-immune crosstalk results in the production of immunomodulatory hormones cortisol (F) and epinephrine (EPI), which aid in the restoration of homeostasis. Circadian rhythms permeate the model based on signaling originating from the circadian hormones cortisol (F) and melatonin (M). Finally, these signals propagate to the heart, where both HR and HRV are governed by circadian rhythms and exhibit acute responses to endotoxemia.

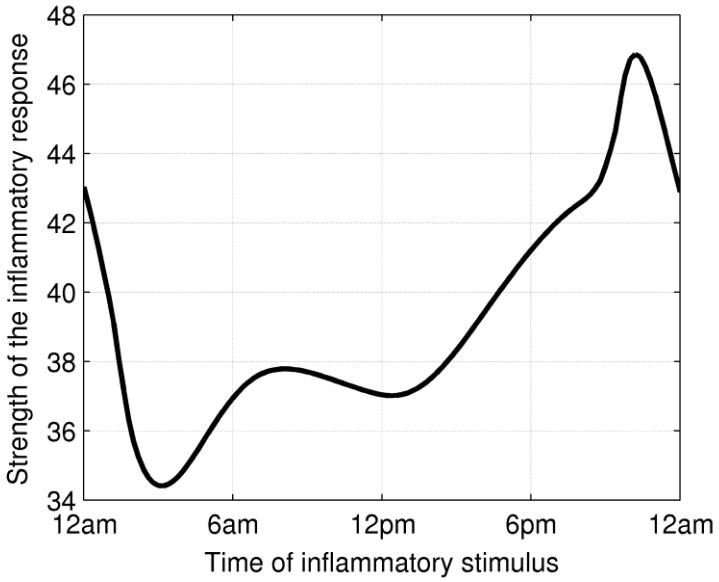


Figure 2: **Circadian changes in the strength of the inflammatory response.** The strength of the inflammatory response to identical levels of LPS varies throughout the day, as illustrated here by the maximal response of pro-inflammatory signaling, which has its largest response in the night when homeostatic pro-inflammatory signaling is highest [64].

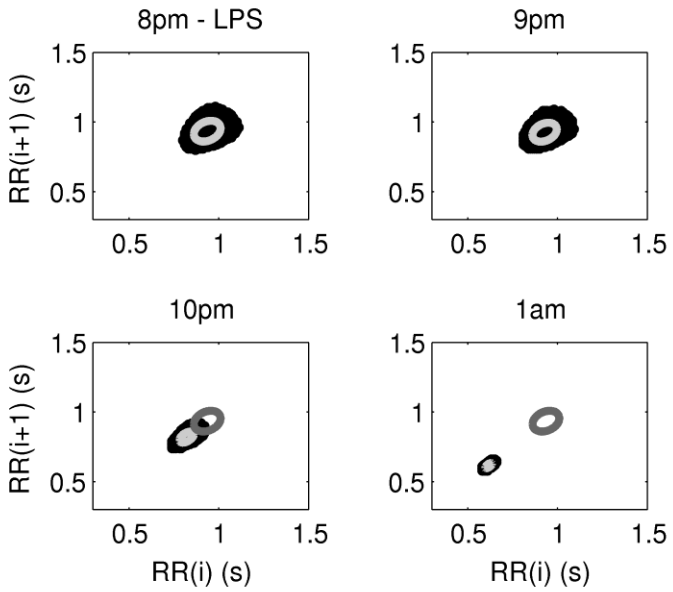


Figure 3: **The effect of LPS on the beating of the heart.** These four panels contain Poincaré plots showing the RR intervals of heart beats in response to a dose of LPS given at 10pm [82]. After injection, the points on the plot shift down and to the left and become more tightly distributed, reflecting shorter RR intervals and thus decreased HR as well as the loss of HRV. The ellipses have axes are equal to the standard deviation of points on each axis so that the sizes of the ellipses are related to the variability in each panel. The pre-LPS fitted ellipse from the first pre-LPS panel is repeated in later panels to illustrate the difference in both the mean and the distribution of points during the acute endotoxemia response.