Modeling the influence of circadian rhythms on the acute inflammatory response

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Abstract: A wide variety of modeling techniques have been applied towards understanding inflammation. These models have broad potential applications, from optimizing clinical trials to improving clinical care. Models have been developed to study specific systems and diseases, but the effect of circadian rhythms on the inflammatory response has not been modeled. Circadian rhythms are normal biological variations obeying the 24-hour light/dark cycle and have been shown to play a critical role in the treatment and progression of many diseases. Several of the key components of the inflammatory response, including cytokines and hormones, have been observed to undergo significant diurnal variations in plasma concentration. It is hypothesized that these diurnal rhythms are entrained by the cyclic production of the hormones cortisol and melatonin, as stimulated by the central clock in the suprachiasmatic nucleus. Based on this hypothesis, a mathematical model of the interplay between inflammation and circadian rhythms is developed. The model is validated by its ability to reproduce diverse sets of experimental data and clinical observations concerning the temporal sensitivity of the inflammatory response.

Keywords: Systems biology, inflammation, PK/PD modeling, signaling and regulation, circadian rhythms

Introduction

The acute inflammatory response is a critical component of the body's defense against a variety of harmful stimuli, such as an invading pathogen or trauma. Inflammation consists of a complex, coordinated set of interactions between the immune system and the neuroendocrine system to initiate the restoration of homeostasis, either through the removal of the pathogen or the repair of damaged tissue. Typically, inflammation is tightly regulated, activating when necessary and abating after healing has been initiated. However, inflammation does not always resolve appropriately; in some cases, a heightened level of inflammation persists, which can damage healthy tissue. Prolonged systemic inflammation comes with severe consequences, often leading to organ failure and death. This type of overwhelming inflammatory when accompanied by an infection is called sepsis. There are approximately 750,000 cases of severe sepsis every year in the United States alone, leading to over 200,000 deaths annually (Angus *et al.*, 2001). Thus, the management of inflammation is a major challenge in the treatment of critically ill patients.

Despite our understanding of the importance of this problem and extensive research towards the development of effective therapies, current treatment options (Annane *et al.*, 2002; Bernard *et al.*, 2001) remain limited and other novel therapies remain elusive (Freeman and Natanson, 2000). This is likely due to the inherent challenges in applying reductionist techniques to nonlinear systems (Seely and Christou, 2000). In fact, it may be impossible to predict the outcome of perturbing a pathway involved in inflammation given only a knowledge of its isolated behavior (Vodovotz *et al.*, 2004). For this reason, there is interest in applying techniques from systems biology towards the development of models of inflammation, with the goal of attaining a systems-level understanding of the key interactions in the inflammatory response.

In recent years, a number of models have been developed by applying different modeling techniques (agent based modeling or equation based modeling), at different scales (molecular, cellular, systemic, or a combination), and focusing on different specific problems (acute inflammation, trauma, or the response to a specific disease) (An, 2008; Foteinou *et al.*, 2009c; Jit *et al.*, 2005; Kumar *et al.*, 2008; Li *et al.*, 2008; Lipniacki *et al.*, 2006; Mi *et al.*, 2007; Prince *et al.*, 2006; Zuev *et al.*, 2006). These models have been developed with the practical goals of impacting healthcare through translational systems biology (Foteinou *et al.*, 2009d; Vodovotz *et al.*, 2008) and rationalizing the design of experiments and clinical trials (Clermont *et al.*, 2004). Because of the large number of components involved in inflammation, existing models make assumptions about which interactions are most important, either by simplifying or neglecting certain elements. One aspect that has not previously been studied from the perspective of systems biology is the interplay between circadian rhythms and inflammation.

Circadian rhythms are periodic processes that are synchronized to the 24 hour light/dark cycle. This rhythmicity is widely observed in humans from the scale of biochemical reactions, such as hormone production, to behavioral patterns, such as regular sleeping and feeding times. In the

context of healthcare, mouse and rat models have shown that the same dose of a drug can be lethal at certain times and ineffective at others (Levi and Schibler, 2007). Thus, it is not surprising that there is also a circadian component to inflammation; in fact, many of the elements typically included in models of inflammation (leukocytes, cytokines, and hormones) are known to have strong diurnal patterns (Coogan and Wyse, 2008). The importance of these variations is apparent by observing that sepsis patients have a heightened risk of mortality between 2am and 6am (Hrushesky *et al.*, 1994).

This paper presents a mathematical model of the interplay between circadian rhythms in inflammation that synthesizes disparate biological knowledge about these systems. Circadian variability is introduced into our previous multiscale model of inflammation (Foteinou *et al.*, 2010) under the hypothesis that the observed circadian variations in the inflammatory response are governed by the hormones cortisol and melatonin and their interactions with immune cells. The model is validated by its ability to reproduce experimental results from a variety of sources and its qualitatively accurate predictions of diurnal variability in the strength of the inflammatory response.

Model

Modeling inflammation

In vivo human endotoxin challenge is a commonly-used model for studying acute inflammation because it evokes signs and symptoms of systemic inflammation along with significant transcriptional and neuroendocrine responses (Lowry, 2005). Lipopolysaccharides (LPS, endotoxin), found in the outer membrane of gram-negative bacteria are pathogen-associated molecular patterns (PAMPs) that are recognized by innate immune system pattern recognition receptors (PRRs), most notably Toll-like receptor 4 (TLR4), thus eliciting an inflammatory response. Based on data generated from the human endotoxemia model, we have previously developed a semi-mechanistic mathematical model of human endotoxemia (Foteinou *et al.*, 2009a; Foteinou *et al.*, 2009b; Foteinou *et al.*, 2009c; Foteinou *et al.*, 2010). These previous efforts are based on three critical concepts: (1) essential transcriptional dynamics are computationally discovered through the analysis of gene expression data (Yang *et al.*, 2009); (2) physicochemical modeling (Aldridge *et al.*, 2006) is used to model the signaling cascades that lead to the transcriptional responses; and (3) indirect response (IDR) (Jusko and Ko, 1994) modeling is used to represent the implicit relationships between model components.

The binding of LPS to its receptor TLR4 (*R*) (Eq. 1a-1d) leads to the activation of the NF- κB, which initiates the transcriptional response to inflammation. NF-κB is normally sequestered in the cytoplasm in an inactive form when it is bound to its inhibitor IκBα. LPS stimulates the activation of IKK, which initiates the degradation of IκBα. Then, NF-κB can move into the nucleus where it regulates the transcription of a number of genes, including its inhibitor IκBα, creating a negative

feedback loop. The NF-κB module is based on a reduced model of NF-κB dynamics that includes IKK (Eq. 1e), nuclear (activated) NF-κB (Eq. 1f), and IκBα (Eq. 1g, 1h) (Ihekwaba *et al.*, 2004). The fundamental transcriptional processes found in the gene expression data are the pro-inflammatory response (Eq. 1i), the anti-inflammatory response (Eq. 1j), and the energetic response (Eq 1k).

$$\frac{dLPS}{dt} = k_{lps,1} \cdot LPS \cdot (1 - LPS) - k_{lps,2} \cdot LPS \quad (1a)$$

$$\frac{dR}{dt} = k_{syn} \cdot mRNA, R + k_2 \cdot (LPSR) - k_1 \cdot LPS \cdot R - k_{syn} \cdot R \quad (1b)$$

$$\frac{d(LPSR)}{dt} = k_1 \cdot LPS \cdot R - k_3 \cdot (LPSR) - k_2 \cdot (LPSR) \quad (1c)$$

$$\frac{d(mRNA, R)}{dt} = k_{in,mRNA,R} \cdot (1 + H_{mRNA,R,P}) - k_{out,mRNA,R} \cdot mRNA, R \quad (1d)$$

$$\frac{dIKK}{dt} = k_3 \cdot (LPSR) / (1 + IkBa) - k_4 \cdot IKK + P \cdot \left(\frac{IKK^2}{1 + IKK^2} \right) \quad (1e)$$

$$\frac{dNFkBn}{dt} = \frac{k_{NFkB,1} \cdot IKK \cdot (1 - NFkBn)}{(1 + IkBa)} - k_{NFkB,2} \cdot NFkBn \cdot IkBa \quad (1f)$$

$$\frac{dmRNA_{IkBa}}{dt} = k_{in,IkBa} \cdot (1 + k_{IkBa,1} \cdot NFkBn) - k_{out,IkBa} \cdot mRNA_{IkBa} \quad (1g)$$

$$\frac{dIkBa}{dt} = k_{I,1} \cdot mRNA_{IkBa} - k_{I,2} \cdot (1 + IKK) \cdot (1 - NFkBn) \cdot IkBa - k_{I,1} \quad (1h)$$

$$\frac{dP}{dt} = k_{in,P} \cdot (1 + H_{P,NFkBn}) \cdot (1 + H_{P,E}) / A - k_{out,P} \cdot P \quad (1i)$$

$$\frac{dA}{dt} = k_{in,A} \cdot \left(1 + H_{A,cAMP}\right) \cdot \left(1 + k_{A,FRN} FR(N)\right) \cdot \left(1 + H_{A,E}\right) - k_{out,A} \cdot A \quad (1j)$$

$$\frac{dE}{dt} = k_{in,E} \cdot (1 + H_{E,P}) / A - k_{out,E} \cdot E \quad (1k)$$

$$H_{i,j} = k_{i,j} \cdot J$$

The interplay between the NF-κB pathway and the pro- and anti-inflammatory responses normally leads to a healthy inflammatory response that resolves after LPS has been cleared, but high doses of LPS can lead to a state of persistent inflammation. In addition, NF-κB is regulated by glucocorticoids, both endogenous (cortisol (F)) and exogenous, which allows for the ability to assess potential treatment options. This is modeled by equations governing the inflammation-induced production of cortisol (Eq. 1l) and its receptor (Eq. 1m, 1n) and the intracellular dynamics as the signal is transduced from the cytoplasm (Eq. 1o) to the nucleus (E1. 1p).

$$\frac{dF}{dt} = k_{in,F_{en}} \cdot \left(1 + H_{F_{en},P}\right) - k_{out,F} \cdot F \quad (1l)$$

$$\frac{dR_m}{dt} = k_{syn\_Rm} \cdot \left(1 - \frac{FR(N)}{IC_{50\_Rm} + FR(N)}\right) - k_{\deg} \cdot R_m \quad (1m)$$

$$\frac{dR_F}{dt} = k_{syn\_R} \cdot R_m + r_f \cdot k_{re} \cdot FR(N) - k_{on} \cdot \left(F - 1\right) \cdot R_F - k_{dgr\_R} \cdot R_F \quad (1n)$$

$$\frac{dFR}{dt} = k_{on} \cdot F \cdot R_F - k_T \cdot FR \quad (1o)$$

$$\frac{dFR(N)}{dt} = k_T \cdot FR - k_{re} \cdot FR(N) \quad (1p)$$

This work has recently been extended to study the effects of endotoxemia on autonomic dysfunction (Foteinou *et al.*, 2010). The hormone epinephrine has been shown to modulate immune function (Padgett and Glaser, 2003). Epinephrine is secreted by the sympathetic nervous system (SNS), which is stimulated by the pro-inflammatory response (Elenkov *et al.*, 2000) and ultimately leads to an increase in anti-inflammatory signaling, mediated by cAMP (van der Poll, 2001), as shown in Eq. 1q-1t. Heart rate variability (HRV) is an important clinical marker for autonomic dysfunction. A decrease in HRV is one aspect of the diminished physiological variability caused by endotoxemia (Godin *et al.*, 1996). HRV is incorporated into the model by a non-linear potentiation by pro-inflammatory activity in Eq. 1u-1x.

$$\frac{dEPI}{dt} = k_{in,EPI} \cdot \left(1 + H_{EPI,P}\right) - k_{out,EPI} \cdot EPI \quad (1q)$$

$$\frac{dR_{EPI}}{dt} = k^0_{R_{EPI}} - \left[k_{1,R_{EPI}} \cdot \left(1 + H_{R_{EPI},EPI}\right) + k_{2,R_{EPI}}\right] \cdot R_{EPI} \quad (1r)$$

$$\frac{dEPIR}{dt} = k_{1,R_{EPI}} \cdot \left(1 + H_{R_{EPI},EPI}\right) \cdot R_{EPI} - k_{3,EPIR} \cdot \left(EPIR + 1\right) \quad (1s)$$

$$\frac{dcAMP}{dt} = \frac{1}{\tau} \cdot \left(EPIR^n - cAMP\right) \quad (1t)$$

$$\frac{df_P}{dt} = \left(1 + \tanh\left(P - w\right) - f_P\right) \cdot H_P \quad (1u)$$

$$\frac{dS_f}{dt} = \frac{1}{\tau_S} \cdot \left(H_P \cdot f_P^{n_S} - S_f\right) \quad (1v)$$

$$\frac{dHRV}{dt} = k_{in,HRV} - k_{out,HRV} \cdot \left(1 + k_{HRV,S} \cdot S_f\right) \cdot HRV \quad (1w)$$

$$H_P = \tanh\left(P^{\phi} - 1\right)^{\phi} \quad (1x)$$

Taken together, these elements in Eq. 1 comprise a semi-mechanistic model of human endotoxemia and its relationship to autonomic dysfunction. Further detail is available in previous publications (Foteinou *et al.*, 2009a; Foteinou *et al.*, 2009b; Foteinou *et al.*, 2009c; Foteinou *et al.*, 2010).

Modeling circadian rhythms in inflammation

Many of the components described in the previous section are known to have circadian rhythms. Several studies have shown that numerous pro- and anti-inflammatory cytokines undergo diurnal variations in plasma levels, typically peaking in the night (Hermann *et al.*, 2006; Petrovsky and Harrison, 1997; Petrovsky and Harrison, 1998; Petrovsky *et al.*, 1998; Zabel *et al.*, 1990). Plasma cortisol levels also exhibit a circadian pattern, peaking in the early morning. Cortisol is produced by the actions of the hypothalamic-pituitary-adrenal axis, and the circadian production is due to stimulation from the central circadian clock in the suprachiasmatic nucleus (SCN) (Hermann *et al.*, 2006; Kohsaka and Bass, 2007).

Due to the immunomodulatory effects of glucocorticoids and the strong circadian pattern of plasma cortisol levels, cortisol has been implicated in the circadian entrainment of cytokine production (Petrovsky and Harrison, 1998). However, exogenous glucocorticoid administration is known to have a differential effect on cytokines; it stimulates the production of anti-inflammatory cytokines while inhibiting the production of pro-inflammatory cytokines (Barber *et al.*, 1993; Barnes, 1998). Thus, it seems unlikely that cortisol alone could be responsible for the observed fluctuations in cytokine level, especially in light of the fact that a number of other hormones also vary either in or out of phase with cytokine levels (Petrovsky and Harrison, 1998).

Of particular interest is the hormone melatonin, due to its potential role as a mediator in the crosstalk between the SCN and the immune system (Coogan and Wyse, 2008). Melatonin is tightly regulated to have a peak in production in the night while remaining at very low levels the rest of the day and it has been shown to stimulate the production of cytokines, likely through the melatonin receptors in human leukocytes (Guerrero and Reiter, 2002; Skwarlo-Sonta *et al.*, 2003). This is supported by experimental evidence showing that pinealectomy leads to decreased cytokine production in mice (Delgobbo *et al.*, 1989). Thus, in the model presented herein, melatonin is used as the primary circadian regulator of cytokine production. Melatonin and cortisol drive the circadian variation in all of the model variables.

In (Chakraborty *et al.*, 1999), six different mathematical models are fit to experimental data to reproduce the circadian profile of plasma cortisol levels. They found that several of these models were adequately able to capture the dynamics of the cortisol profiles. To assess which circadian cortisol equation is most effective to incorporate into this multiscale model of inflammation, the different circadian cortisol models were tested and shown to produce qualitatively similar results. Ultimately, this work incorporates the “two rates” model due to its simplicity. In this model, a zero-order production term (*RF*) is set to two different values depending on the time of day and

the circadian pattern is induced by using a high production rate in the morning and a low production rate the rest of the day (Eq. 2a). For comparison, results for the most complex model, consisting of the first three terms of a Fourier series fit to the data (Eq. 2b), are also shown.

$$\frac{dF}{dt} = RF + k_{in,F_{en}} \cdot \left(1 + H_{F_{en},P}\right) - k_{out,F} \cdot F$$
$$RF = \begin{cases} k_{in,RF1}, & t_{F1} < \mathrm{mod}(t,24) < t_{F2} \\ 0, & t_{F2} < \mathrm{mod}(t,24) < t_{F1} \end{cases} \quad (2a)$$

$$RF = a_0 + \sum_{n=1}^{3}\left[a_n \cos(2\pi nt/24) + b_n \sin(2\pi nt/24)\right] \quad (2b)$$

Melatonin is modeled in a similar manner (Eq. 3), using *RM* as a zero-order production term that is large during the night and small during the rest of the day and also including a first-order degradation term. More complex models of melatonin production are not investigated because melatonin levels do not have the type of biphasic pattern that is sometimes apparent for cortisol. However, it is well established that pro-inflammatory cytokines can reduce or even fully suppress the nocturnal peak in melatonin (Couto-Moraes *et al.*, 2009; Fernandes *et al.*, 2006; Jiang-Shieh *et al.*, 2005; Pontes *et al.*, 2006; Pontes *et al.*, 2007; Skwarlo-Sonta *et al.*, 2003) and corticosteroids can antagonize this effect by stimulating melatonin production (Fernandes *et al.*, 2009; Fernandes *et al.*, 2006; Ferreira *et al.*, 2005). The indirect effect of these two substances on melatonin production is modeled by including an indirect stimulus term for cortisol and an indirect inhibition term for pro-inflammatory cytokines on the production rate of melatonin.

These models for cortisol and melatonin (Eq. 2, 3) are fit to experimental data (Grivas and Savvidou, 2007; Hermann *et al.*, 2006) to ensure that the peak levels of hormones in the model occur at the correct times.

$$\frac{dM}{dt} = RM \cdot \left(1 + \frac{F}{1+F}\right) \cdot \left(1 - \frac{P}{1+P}\right) - k_{out,RM} \cdot M$$
$$RM = \begin{cases} k_{in,RM1}, & t_{M1} < \mathrm{mod}(t,24) < t_{M2} \\ k_{in,RM2}, & t_{M2} < \mathrm{mod}(t,24) < t_{M1} \end{cases} \quad (3)$$

Melatonin has been shown to stimulate the production of both pro- and anti-inflammatory cytokines (Petrovsky and Harrison, 1997; Raghavendra *et al.*, 2001). This is modeled by adding a stimulating term to the production rates of P and A (Eq. 4). The strength of these interactions is calibrated based on experimental data for IL-1α (P) (Petrovsky *et al.*, 1998) and IL-10 (A) (Petrovsky and Harrison, 1997).

$$\frac{dP}{dt} = k_{in,P} \cdot (1 + H_{P,NFkBn}) \cdot (1 + H_{P,E}) \cdot (1 + H_{P,M}) / A - k_{out,P} \cdot P \quad (4a)$$

$$\frac{dA}{dt} = k_{in,A} \cdot \left(1 + H_{A,cAMP}\right) \cdot \left(1 + H_{A,E}\right) \cdot \left(1 + H_{A,FRN}\right) \cdot (1 + H_{\mathrm{A,M}}) - k_{out,A} \cdot A \quad (4b)$$

Cortisol produced in the adrenal cortex directly interacts with the adrenal medulla, stimulating epinephrine production (Wurtman *et al.*, 1972). This matches up well with available experimental data which shows that plasma epinephrine levels lag cortisol levels (Dimitrov *et al.*, 2009; Kronfol *et al.*, 1997). This is modeled by letting cortisol stimulate the production rate of epinephrine (Eq. 5a). The normal circadian pattern of HRV is roughly sinusoidal with a peak in the night (Massin *et al.*, 2000); this behavior is likely driven by sleep patterns and a decrease in sympathetic activity at night (Ewing *et al.*, 1991). In this model, epinephrine is used as a surrogate for sympathetic activity, which inhibits the production rate of HRV. Experimental data are used to validate the responses of epinephrine (Kronfol *et al.*, 1997) and HRV (Massin *et al.*, 2000).

$$\frac{dEPI}{dt} = k_{in,EPI} \cdot \left(1 + H_{EPI,P}\right) \cdot \left(1 + H_{EPI,FRN}\right) - k_{out,EPI} \cdot EPI \quad (5a)$$

$$\frac{dHRV}{dt} = k_{in,HRV} / EPI - k_{out,HRV} \cdot HRV \quad (5b)$$

It is difficult to draw precise, quantitative conclusions about specific levels of the variables in this model because often, experimental data is not sufficient to calibrate the model. For instance, the measurements of cytokines that are used are indirect measurements that only give relative levels of cytokines (Petrovsky and Harrison, 1997; Petrovsky *et al.*, 1998). Thus, when plotted, all variables are scaled to be between 0 and 1 in the baseline case when there is no inflammatory stimulus (Fig. 2) by subtracting the minimum and dividing by the difference between the maximum and minimum. These scalings are then consistently used throughout the other figures.

All of the parameters used in the following simulations are shown in Table 1. After fitting the model to the data, sensitivity analysis is performed to gain insight into the model's dependence on the newly-introduced parameters. As in(Ihekwaba *et al.*, 2004; Yue *et al.*, 2006), for each parameter, the sensitivity coefficient is calculated as

$$S_p^m = \frac{\delta m / m}{\delta p / p} \quad (6)$$

where *p* represents the parameter that is varied, *δp* is an incremental perturbation in the parameter, *m* is the response of the original system, and *δm* is the incremental change in *m* due to the perturbation *δp*. Then, *m* is defined as the minimum value of *HRV*, i.e. maximum *HRV*

depression, throughout the entire time course in response to a low dose of LPS that results in a self-limited inflammatory response. Effectively, this sensitivity analysis measures how the perturbations in the parameter values affect the overall systemic response to the stimulus. Because this model responds differently depending on the time of dosing, due to the circadian nature of the baseline, the sensitivity analysis is run 24 different times to capture the response to LPS at the 24 different hours of the day.

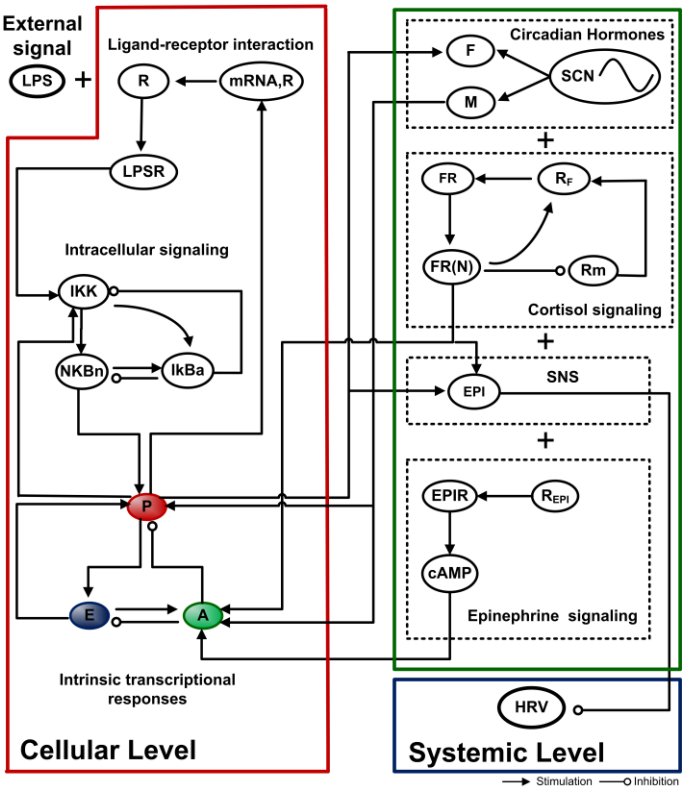

Fig. 1: Network diagram of the components of the model.

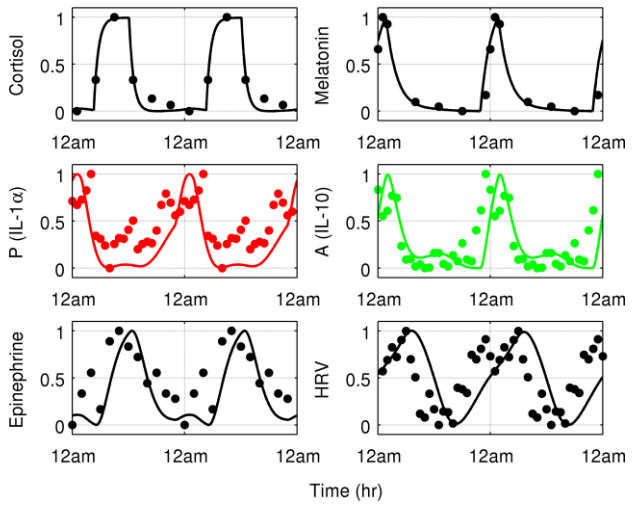

Fig. 2: Simulation of the model when there is no inflammatory stimulus.

Results

Eq. 2, 3, 4, and 5, combined with the remaining unmodified equations from Eq. 1, comprise a model of human endotoxemia that takes into account circadian variations in most of its variables. A network diagram of these interactions is shown in Fig. 1. The model consists of several interacting modules representing various different scales; at the cellular level, the three essential transcriptional responses (pro-inflammatory P, anti-inflammatory A, and energetic E) are regulated by NFkB signaling and the recognition of LPS, as shown in the cellular level in Fig. 1. The circadian hormone section of Fig. 1 shows how the diurnal components of the system are driven by SCN-regulated circadian rhythms in cortisol and melatonin production. Interactions between peripheral inflammation and the neuroendocrine axis are accounted for by incorporating

the inflammatory effects of the hormones cortisol, epinephrine, and melatonin along with the systemic level influences on heart rate variability.

The model is designed to reproduce experimental data from a variety of sources (Grivas and Savvidou, 2007; Hermann *et al.*, 2006; Kronfol *et al.*, 1997; Massin *et al.*, 2000; Petrovsky and Harrison, 1997; Petrovsky *et al.*, 1998), as shown in Fig. 2. In this figure, a simulation is run with no inflammatory stimulus, giving the normal baseline condition for the model variables. While there is a link between cortisol and the anti-inflammatory response, variations seen in both the pro- and anti-inflammatory responses are primarily driven by melatonin levels. Cortisol is responsible for modulating the production of epinephrine, resulting in epinephrine levels peaking during the day slightly after cortisol does. Then, HRV is inhibited by epinephrine levels.

In addition to the simple "two rates" model (Eq. 2a) of cortisol used to generate Fig. 2, a more complex model based on the Fourier series (Eq. 2b) was also tested as shown in Fig. 3. This model accounts for some of the small deviations from the simpler model, such as the small secondary peak after the diurnal decrease in cortisol levels is already

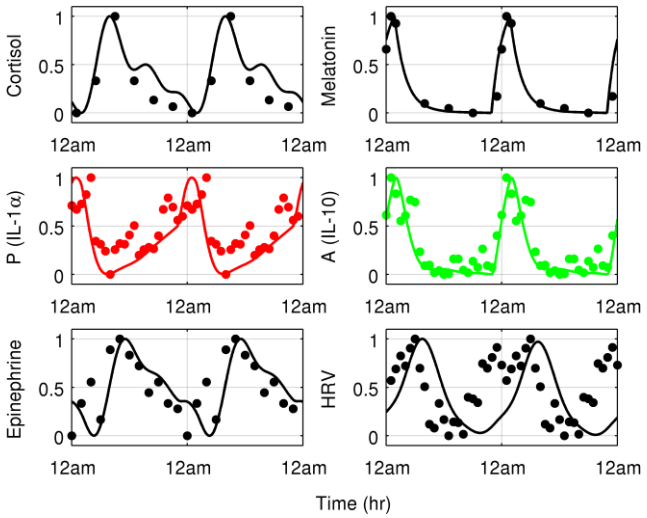

Fig. 3: Simulation of the model, using an alternative equation for cortisol, when there is no inflammatory stimulus.

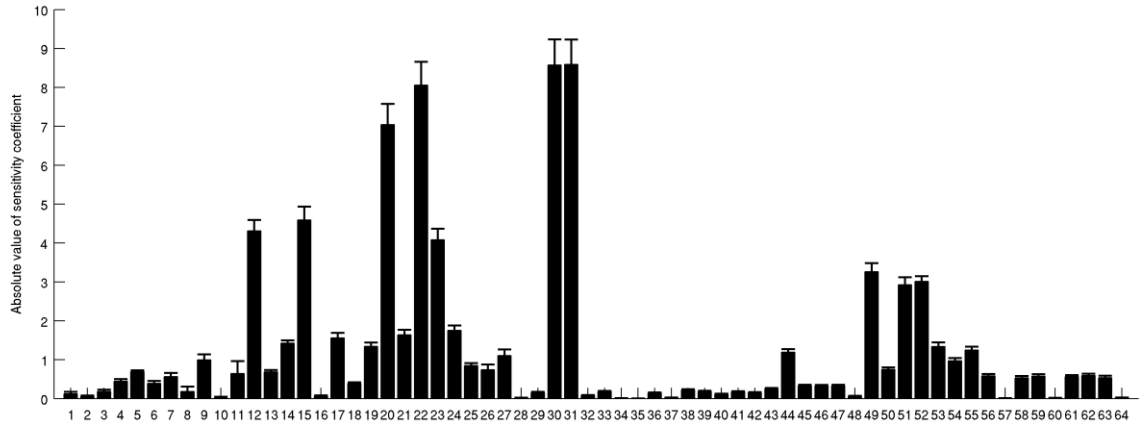

Fig. 4: Sensitivity analysis on the model parameters. Sensitivity coefficients are calculated by using Eq. 6 with δp=0.01. Error bars represent the standard deviation of the sensitivity coefficients for stimuli given at different times during the day. The numbered labels on the x-axis correspond to the parameters in Table 1.

underway. This allows for a better fit for the epinephrine data, which shows that the epinephrine levels increase faster than they decline. However, it also leads to a worse fit for HRV. Overall, the predictions do not qualitatively improve when using the more complex model in Fig. 3; thus, further results presented use the "two rates" model as in Fig. 2.

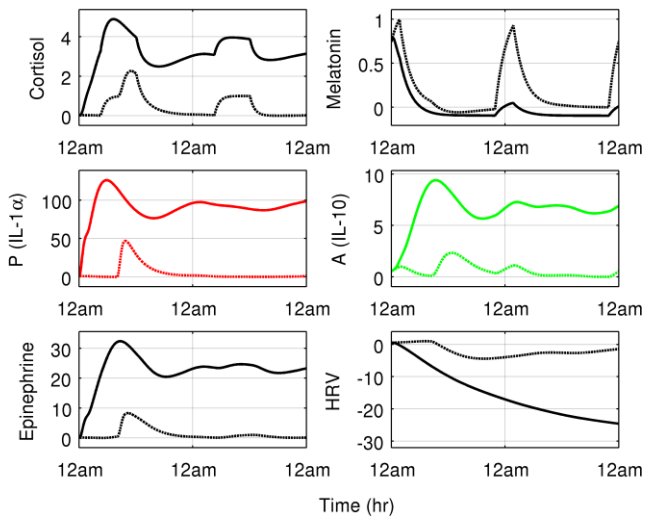

Fig. 5: Simulation of the model for stimuli at two different times. An inflammatory stimulus is given at 8am (dashed lines) or 12am (solid lines). At 8am, the system is able to recover from the inflammatory stimulus, but at 12am, the same exact stimulus sends the system into an unresolved inflammatory state.

To determine the sensitivity of the system with respect to the parameters, sensitivity analysis is performed by calculating the sensitivity coefficient (Eq. 6) for each parameter. The simulations are run for the case when the inflammatory stimulus is LPS_0=1, which leads to a self-limited inflammatory response, and the response is tested for dosing times at each of the 24 hours of the day. Fig. 4 shows the results, with the large bars equal tothe mean sensitivity coefficients and the small error bars equal to the standard deviation. .

Fig. 5 shows simulations of the application of an identical large inflammatory stimulus (LPS_0=10) at two different times. First, at 8am (dashed lines), cortisol levels are high while cytokine levels are low. Thus, the cytokines have less ability to initiate an inflammatory response, and they are countered by the anti-inflammatory influence of cortisol. When the inflammatory stimulus is given at 8am, it provokes an acute response that resolves normally; within several hours, all of the variables have returned to their baseline values. But at midnight (solid lines), cortisol levels are very low and cytokine levels are high; thus, in this scenario, the system is more susceptible to inflammation. This is illustrated by the unresolved inflammatory response that is provoked by the inflammatory stimulus. Interestingly, even in the unresolved inflammatory state, the circadian oscillations persist in cortisol, epinephrine, and the pro- and anti-inflammatory responses. These oscillations are in phase with the normal oscillations in Fig. 2.

Melatonin levels also respond differently in the two cases in Fig. 5. In the case when inflammation resolves (dashed lines), there is almost no change in melatonin relative to the normal conditions in Fig. 2. This is because the transient peaks in *P* and *A* occur during the day when melatonin levels are already low, so the cytokines cannot further suppress melatonin production. But in the case when inflammation does not resolve (solid lines), melatonin levels remain suppressed. However, a

transient inflammatory response can still lead to a decrease in melatonin production, as shown in Fig. 6 when the inflammatory stimulus is given towards the beginning of the period when melatonin production is high.

The temporal variation in the inflammatory response to LPS is illustrated in Fig. 7. In this plot, the model is run as the time of the inflammatory stimulus (LPS_0=1) is varied. Then, the peak of the pro-inflammatory signal (P_{max}) is recorded as a representation of the overall strength of the inflammatory response. There is a significant diurnal variation in this signal, which peaks at night and is low during the daytime.

Discussion

Circadian rhythms are of critical importance in inflammation because so many of the biological components that regulate the outcome of inflammation are themselves under circadian regulation. This work presents the first model that incorporates the effect of circadian variability on the inflammatory response. Proper treatment of inflammatory diseases requires an appreciation of circadian effects (Hrushesky and Wood, 1997), so a quantitative understanding of diurnal variations on inflammation is important in efforts to translate computational systems biology approaches in inflammation to clinical relevance (Foteinou *et al.*, 2009d; Vodovotz *et al.*, 2008).

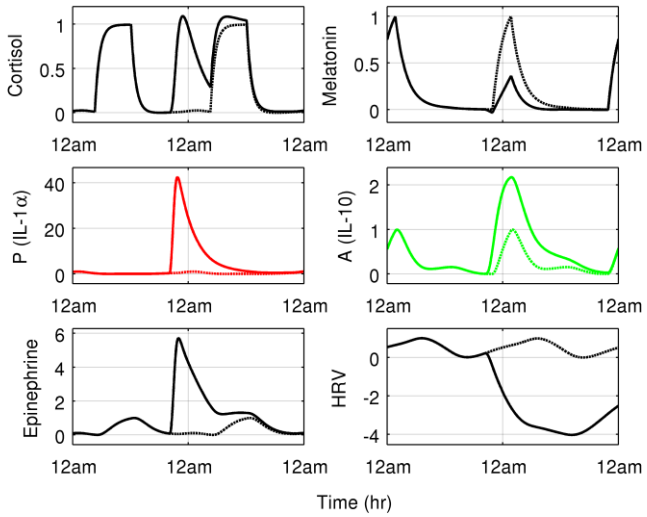

Fig. 6: The inflammatory response can suppress melatonin levels. The solid lines show an inflammatory response (LPS_0=1) initiated at 8pm so that the inflammation is heightened when melatonin production is beginning to increase. The dashed lines show the baseline conditions (as in Fig. 2) for comparison. Pro-inflammatory cytokines suppress the production of melatonin, leading to suppressed nocturnal melatonin levels. However, normal melatonin production returns the following night when the pro-inflammatory signal has resolved.

The sensitivity analysis shown in Fig. 4 illustrates the relative influence of the values of all model parameters on the outcome of the model. The outcome is defined as the minimum value of *HRV* after an inflammatory stimulus because heart rate variability is known to have prognostic value in critically ill patients. Because the sensitivity is measured with respect to changes in *HRV*, it is not surprising that some of the most sensitive parameters are in the equations for *EPI* ($k_{in,EPI}$ (20), $k_{out,EPI}$ (22) and k^0_{REPI} (23)), which is closely linked to *HRV* in the model, and *HRV* itself ($k_{in,HRV}$ (30) and $k_{out,HRV}$

(31)). Parameters governing the behavior of both pro-inflammatory cytokines ($k_{in,P}$ (49), $k_{P,E}$ (51), and $k_{out,P}$ (52)) and anti-inflammatory cytokines ($k_{in,A}$ (12) and $k_{out,A}$ (15)) also have high sensitivities. Of the ten most sensitive parameters, eight represent the production and degradation terms for the four variables mentioned (*HRV*, *EPI*, *P*, and A). The other two are k^0_{REPI} (23), the production rate of epinephrine's receptor, and $k_{P,E}$ (51), which links cellular energetic activity to changes in the pro-inflammatory response. Functionally, many of the most sensitive parameters relate to the communication between the different modules of the system. The acute inflammatory response relies on this signaling to activate other components of the neuroimmune system and provoke a systemic response to inflammation, and this is reflected by high sensitivities in parameters governing cytokine and hormonal signals.

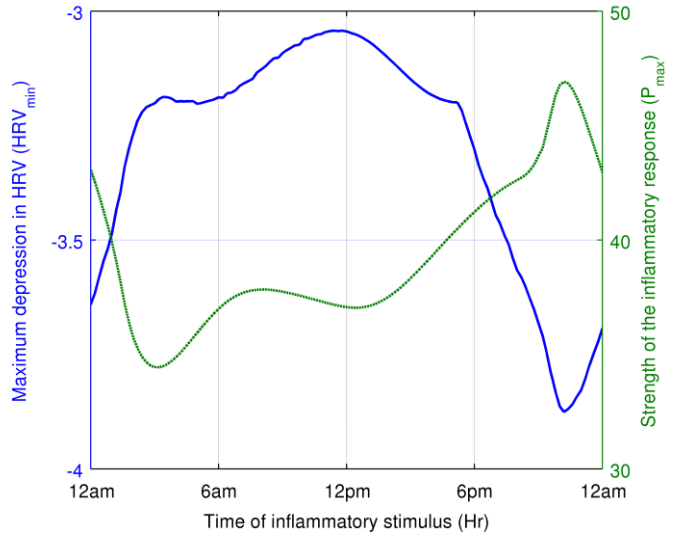

Fig. 7: Diurnal changes in the strength of the inflammatory response. The strength of the inflammatory response varies, as illustrated by monitoring either the maximal response of pro-inflammatory cytokines (P_{max}) or the maximum depression in HRV. Both of these variables have their maximum response in the night when normal levels of pro-inflammatory cytokines are elevated, and the minimum responses occur during the morning when cortisol levels are peaking.

The new parameters added to the model to account for circadian rhythms, labeled 1-11 in Fig. 4, have relatively low sensitivity coefficients compared to the most sensitive parameters from the original model that does not incorporate circadian effects, indicating that the model retains its diurnal response even when the new parameters are not precisely set. Yet although the sensitivities for the circadian parameters are less than the sensitivities of some of the other parameters mentioned earlier, this should not be taken to mean that the circadian components added to the model are unimportant in determining the outcome of the system. This is illustrated by the time-dependent responses found for identical inflammatory stimuli, as shown in Fig. 5-7.

The persistent inflammatory state shown in Fig. 5 (solid lines) is interesting because this type of persistent inflammation, either along with a persistent infection or after the pathogen is successfully cleared, has been observed clinically (Alberti *et al.*, 2002; Bone, 1996). The suppression of the circadian release of melatonin, shown in the simulation in Fig. 6, illustrates the ability of the model to capture critical aspects of the neuroimmune feedback on the production of circadian hormones. A similar diminished nocturnal melatonin release in response to inflammation

has been observed experimentally (Fernandes *et al.*, 2006). Furthermore, the observed temporal dependence of the inflammatory response, as shown in Fig. 5 and Fig. 7, has important implications in translational medicine, where the goal is to translate current scientific discoveries into tools that can be applied to clinical problems. Specifically, modeling circadian variations in inflammation could lead to optimized clinical treatment times. Models could potentially be used to optimize the treatment of individual patients in an effort towards fulfilling the promise of personalized medicine. In inflammation, this is particularly important because it has been repeatedly observed that patients with sepsis have a significantly increased risk of mortality at night, but if they survive until the morning rise in cortisol levels is underway, they are likely to survive at least until the next night (Hrushesky and Wood, 1997). This qualitatively matches the results shown in Fig. 7, where the potential for an inflammatory response is greatest at night and is significantly lower during the daytime; furthermore, P_{max} reaches its minimum early in the morning when the risk of death from sepsis is decreased. The observed differences in P_{max} mainly arise due to the variations in cortisol and in both pro- and anti-inflammatory cytokines. When cortisol levels are high, the system is protected from a heightened inflammatory response. But when cortisol levels are low, natural variations in cytokine levels result in periods of time when the system is primed for an inflammatory response.

One key aspect of the interplay between circadian rhythms and inflammation that is not adequately considered in this work is the feedback from inflammation to circadian rhythms. There is some evidence suggesting that immune mediators can directly influence the circadian clock by modulating the strength of expression of clock-related genes and by shifting the phase of circadian rhythms (Coogan and Wyse, 2008). Melatonin has been implicated mediating these processes; additionally, inflammatory cytokines are known to influence the production of melatonin (Fernandes *et al.*, 2006; Mundigler *et al.*, 2002), likely facilitating bidirectional information transfer between the neuroendocrine and immune systems.

The relationship between circadian rhythms and inflammation may be of particular importance in understanding the effects of chronic stress. In response to chronic stress from a variety of stimuli, such as depression (Yehuda *et al.*, 1996), obesity (Rosmond *et al.*, 1998), psychological stress (Polk *et al.*, 2005), and various types of cancer (Mormont and Levi, 1997), diurnal variations in plasma cortisol concentration are diminished while overall cortisol levels remain high. The loss of the circadian nature of autonomic and neuroendocrine signaling in chronically stressed patients may be linked to a patient's overall potential to mount a healthy response to an inflammatory stressor (Lowry, 2009). Furthermore, an extended period of stress hormone exposure results in diminished anti-inflammatory capacity as manifested by dynamic alterations in circulating levels of the anti-inflammatory cytokine IL-10, similar to subjects exposed only to LPS (van der Poll *et al.*, 1996a; van der Poll *et al.*, 1996b). The clinical relevance of the circadian component of inflammation, particularly as it relates to chronic stress, is illustrated by the fact that diminished diurnal variability in cortisol is associated with increased mortality in patients with breast cancer (Sephton

et al., 2000). The model presented here provides a solid foundation towards future work exploring the intricacies of these interactions.

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#	Parameter	Value	Units	Description
1	$k_{in,RM1}$	0.406	pg/mL/hr	Production rate of M during the night
2	$k_{in,RM2}$	0.0318	pg/mL/hr	Production rate of M during the day
3	$k_{out,RM}$	0.421	1/hr	Clearance rate of M
4	$k_{in,F1}$	0.992	ng/mL/hr	Circadian production rate of F
5	$k_{EPI,FRN}$	0.0901	mg L/nmole	Strength of indirect stimulus on EPI by FR(N)
6	$k_{P,M}$	0.973	mL/pg	Strength of indirect stimulus on P by M
7	$k_{A,M}$	1.00	mL/pg	Strength of indirect stimulus on A by M
8	T_{F1}	4.62	hr	Start time for when cortisol production is heightened
9	T_{F2}	12.1	hr	End time for when cortisol production is heightened
10	T_{M1}	21.9	hr	Start time for when melatonin production is heightened
11	T_{M2}	1.73	hr	End time for when melatonin production is heightened
12	$k_{in,A}$	0.461	1/hr	Base production rate of A
13	$k_{A,cAMP}$	0.145	1	Strength of indirect stimulus on A by cAMP
14	$k_{A,E}$	0.534	1	Strength of indirect stimulus on A by E
15	$k_{out,A}$	0.810	1/hr	Clearance rate of A
16	$k_{A,FRN}$	0.401	mg L/nmole	Strength of indirect stimulus on A by FR(N)
17	$k_{in,Fen}$	0.843	ng/mL/hr	Base production rate of F
18	$k_{Fen,P}$	0.256	1	Strength of indirect stimulus on F by P
19	$k_{out,F}$	1.06	1/hr	Clearance rate of F
20	$k_{in,EPI}$	5.92	pg/mL/hr	Base production rate of EPI
21	$k_{EPI,P}$	0.231	1	Strength of indirect stimulus on EPI by P
22	$k_{out,EPI}$	7.29	1/hr	Clearance rate of EPI
23	k^0_{REPI}	11.0	1/hr	Production rate of REPI
24	$k_{1,REPI}$	3.01	1/hr	Base binding rate between EPI and REPI
25	$k_{REPI,EPI}$	0.845	1	Stimulus on binding rate between EPI and REPI by REPI
26	$k_{2,REPI}$	5.47	1/hr	Clearance rate of REPI
27	$k_{3,EPIR}$	5.55	1/hr	Dissociation rate between EPI and REPI
28	τ	0.0525	hr	cAMP mean transit time
29	n	5.51	1	cAMP shaping factor
30	$k_{in,HRV}$	1.19	1	“Production rate” of HRV
31	$k_{out,HRV}$	1.05	1/hr	“Clearance rate” of HRV
32	$k_{lps,1}$	4.50	1/hr	Growth rate of LPS
33	$k_{lps,2}$	6.79	1/hr	Clearance rate of LPS
34	k_{syn}	0.0200	1/hr	Translation rate of R
35	k_2	0.0400	1/hr	Dissociation rate between LPS and R
36	k_1	3.00	1/hr	Binding rate between LPS and R
37	k_3	5.00	1/hr	Decay rate of LPSR
38	k_4	2.24	1/hr	Decay rate of IKK

39	$k_{in,mRNA,R}$	0.0914	1	Base transcription rate of mRNA,R
40	$k_{mRNA,R,P}$	1.74	1	Strength of indirect stimulus on mRNA,R by P
41	$k_{out,mRNA,R}$	0.251	1/hr	Decay rate of mRNA,R
42	$k_{NFkB,1}$	16.3	1/hr	Base transport rate for NFkB into the nucleus
43	$k_{NFkB,2}$	1.19	1/hr	Base transport rate for NFkB out of the nucleus
44	$k_{in,IkBa}$	0.463	1/hr	Base transcription rate of $mRNA_{IkBa}$
45	$k_{IkBa,1}$	13.3	1	Strength of indirect stimulus on $mRNA_{IkBa}$ by NFkBn
46	$k_{out,IkBa}$	0.463	1/hr	Decay rate of $mRNA_{IkBa}$
47	$k_{I,1}$	1.40	1/hr	Translation rate of IkBa
48	$k_{I,2}$	0.870	1/hr	Strength of indirect effects of IKK and NFkBn on IkBa
49	$k_{in,P}$	0.0331	1/hr	Base production rate of P
50	$k_{P,NFkBn}$	29.7	1	Strength of indirect stimulus on P by NFkBn
51	$k_{P,E}$	9.05	1	Strength of indirect stimulus on P by E
52	$k_{out,P}$	0.333	1/hr	Decay rate of P
53	$k_{in,E}$	0.0800	1/hr	Base production rate of E
54	$k_{E,P}$	2.210	1	Strength of indirect stimulus on E by P
55	$k_{out,E}$	0.257	1/hr	Decay rate of E
56	k_{syn_Rm}	2.900	fmole/g/hr	Base transcription rate of R_m
57	IC_{50_Rm}	26.2	nmole/L/mg	Concentration of FR(N) producing half the maximum effect
58	k_{deg}	0.112	1/hr	Decay rate of R_m
59	k_{syn_R}	1.12	1	Translation rate of R_F
60	r_f	0.490	1	Strength of stimulus on R_F by FR(N)
61	k_{re}	0.570	1/hr	Transport rate of FR into the nucleus
62	k_{on}	0.00329	L/nmole/hr	Binding rate between F and RF
63	k_{dgr_R}	0.0572	1/hr	Decay rate of R_m
64	k_T	0.630	1/hr	Transport rate of FR(N) out of the nucleus

Table 1: List of parameters used in the simulation of the model. Parameters 1-11 are the new parameters that were added to the previous model to incorporate the circadian effects. Parameters 12-64 are identical to those used in previous modeling efforts that did not account for diurnal variability. Many of the variables are dimensionless, so many of the parameters have units of either 1 or 1/hr.