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Research Article

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Posted Date: October 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11536584/v1

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Additional Declarations: The authors declare no competing interests.

Computational Modelling of Glucocorticoid Chronotherapy for Diseases with Cortisol Circadian Dysregulation

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Abstract:

Unnatural exposure to nighttime light, shift work, and chronic disturbance in sleep/ wake and feeding cycles are known to cause circadian rhythm (CR) disruption of the hypothalamic-pituitary axis. Dysregulated cortisol CR is associated with metabolic dysfunction, sleep disorders, mood, and cognitive dysfunction. Addressing cortisol circadian dysregulation to restore normal peripheral physiology could be a potential option. We used an ODE-based computational model to study the complexity of the HPA-Inflammation system and the time-dependent effects of glucocorticoids on systems CR. Further, the model was validated with the reported human circadian physiology of system state variables. A treatment response analysis (TRA) based on phase and amplitude responses of the state variable was used to identify chronotherapeutic dosing of dexamethasone (Dex) to correct cortisol CR. The TRA suggest optimal time ranges for Dex dosing: 5-6 AM for Hypocortisolemia and 8-9 AM for lower-grade inflammation. Several cortisol rhythm defects are simulated to generate training data for a neural network (NN) to predict the optimal time for Dex dosing in cases of cortisol CR dysregulation. The NN protocol forecasted chronotherapeutic dosing for psychiatric, neuroendocrine, and inflammatory diseases involving cortisol CR dysfunction, such as hyper- or hypocortisolemia and CR phase shifts. We demonstrate a hybrid method in which mechanistic models generate detailed synthetic clinical data to train NN models to predict glucocorticoid chronotherapy, a task that would otherwise be difficult to perform clinically.

Keywords: Circadian rhythm; Cortisol dysregulation; Phase Response Curve; Deep learning model; Chronotherapy

Introduction

Pernicious stimuli like overtime work (work-stress), psychological stress, psychosocial stress, disrupted sleep-wake cycles, etc., have been known to disrupt the circadian rhythm (CR) of various physiological processes. Continuous irregularities in these stimuli onset disturbances in the light/dark phase and feeding habits, which in turn dysregulate the cortisol response curve (mainly expression amplitude and phase) that may contribute to the pathophysiology of systemic disease in an individual (Bass & Takahashi, 2010; Bass & Lazar, 2016). A significant portion of the world population is getting exposed to pernicious stimuli, such as nighttime light pollution (~80%), which increases their risk of cortisol circadian dysregulation and its associated comorbidities (Walker et al., 2020; Tomatsu, Abbott & Attarian, 2025). Studies on circadian misalignment-related comorbidities, such as inflammation, hyperglycemia, insulin resistance, obesity, post-traumatic stress disorder, seasonal affective disorder, sleep disorders, and mood or cognitive dysfunction, have reported a significant association between long-term stress and circadian dysregulation of cortisol signaling (Nicolaides et al., 2017; Iob & Steptoe, 2019). Most peripheral biological processes are regulated by the natural light-dark (LD) cycle (~24hr) after receiving an entrainment signal from the master clock (i.e., the suprachiasmatic nucleus; SCN).

The SCN governs circadian periodicity by relaying peripheral rhythms via the Hypothalamus-Pituitary-Adrenal (HPA) axis through cortisol signaling. Corticosteroids regulate transcriptional processes and govern homeostasis in the peripheral system (Devi & Shankar, 2008; Zamanian, Dehghani & Hashemi, 2013). The glucocorticoid receptors (GRs) play a pivotal role in relaying SCN LD entrainment to peripheral core clocks via plasma cortisol levels, making the GR-cortisol complex an important controller of multiple feedback responses in the peripheral system. Cortisol signaling influences major peripheral processes, including immune function, blood glucose regulation, protein balance, respiratory health, and blood pressure control (Nicolaides et al., 2017; Waddington Lamont et al., 2007; Bunney et al., 2015). SCN adjusts the molecular levels of cortisol and melatonin in accordance with the normal LD cycle, thereby controlling the (~24 hr) rhythm of sleep and wakefulness. The normal cycle of sleep/wake helps synchronize different processes, such as healing, metabolism, neuronal remodeling, the motor system, and energy storage/usage. Frequent changes in work shift conditions disrupt lifestyle (e.g., feeding, sleep, social behavior, and work hours), triggering a cortisol response to acclimate

peripheral circadian processes to external changes. But in the long run, circadian disturbances cause a fault in the cortisol response (due to perturbed GR regulation), which negatively affects many peripheral processes related to inflammation, the liver core clock, cellular signaling, and metabolism (Wulff et al., 2010; Geuze et al., 2012; Gotovac et al., 2003). Also, the irregularities in light exposure disturb the sleep/wake cycle, leading to fatigue, confusion, mood disorders, and cognitive impairment (Wulff et al., 2010; Wright et al., 2015). The sleep therapies failed to cure these dysregulations, which resulted from the loss of synchrony among the various entraining cues of the homeostatic system, necessitating glucocorticoid/circadian therapies (Wright et al., 2015; Wulff et al., 2010).

The immune system-governing cells express pro- and anti-inflammatory cytokines in accordance with the circadian pattern of the LD cycle, as read by the SCN (Scheiermann, Kunisaki & Frenette, 2013). This expression dynamics changes in response to external stimuli sensed by signaling pathways, such as the HPA axis. Wherein, the cortisol, along with GR, keeps in check the response of pro-inflammatory cytokines (like IL-6, TNF, IL-1) by regulating the levels of anti-inflammatory cytokines (like IL-1B, TGF, IL-10), during stress conditions and pathology (Wright et al., 2015; Somvanshi et al., 2020). The perturbed circadian rhythm (phase) of cortisol expression (amplitude) under prolonged stress conditions leads to elevated pro-inflammatory responses, as reported in previous studies (Bauer et al., 1994; Cutolo, 2019; Lehrke et al., 2008; Bethin, Vogt & Muglia, 2000). Dexamethasone (Dex) is a synthetic corticosteroid used as an immunosuppressant to control overactive inflammatory responses, the autoimmune system, or adrenal suppression. Dex mimics cortisol's function within a biological system and activates anti-inflammatory signaling as negative feedback to counteract an altered proinflammatory response during prolonged stress conditions. However, Dex effectiveness is time- and dose-dependent, which needs to be managed (time of administration and drug dose) according to circadian physiology to reduce side effects (such as adrenal suppression, muscle weakness, and immune suppression) on peripheral systems (Pincus, Humeston & Martin, 1997; Bijlsma & Jacobs, 2008; Scherholz, Schlesinger & Androulakis, 2019). In the long run, suboptimal timing of Dex concentration will exacerbate circadian physiology in GR and reduce regulation of the proinflammatory system, potentially worsening disease physiology.

Hence, a therapeutic regimen to correct cortisol dysregulation, tailored to individual circadian physiology, is needed to alleviate peripheral circadian dysregulation while minimizing the risk of side effects. To study the complex interconnections among these biological processes, cortisol, and the time-

dependent effects of glucocorticoids on the circadian rhythm, we have used a mathematical framework to model crosstalk and circadian expression patterns of the HPA axis, GR signaling, and the inflammatory system. Further, we have perturbed model parameters to simulate hypocortisolemia and a low-grade inflammatory state to study the effects on the circadian rhythm of model variables and studied the time-dependent effect of Dex on the disease state of the HPA-Inflammation model to screen the optimal time for Dex dosing. Previous studies on model predictive control of pharmaceuticals have discussed the potential of phase response curve (PRC) analysis to study the phase-dependent effects of Dex administration (Abel et al., 2019; Murdoch, Aiello & Doyle, 2023; Abel, Chakrabarty & Doyle III, 2017). Similarly, we used PRC and amplitude changes to assess the changes in time-dependent effects of Dex on circadian physiology during the disease state relative to the normal state (**Figure 1A**). The method provides an effective way to monitor and regulate Dex administration over time, informed by human physiology, and helps identify the optimal dosing regimen.

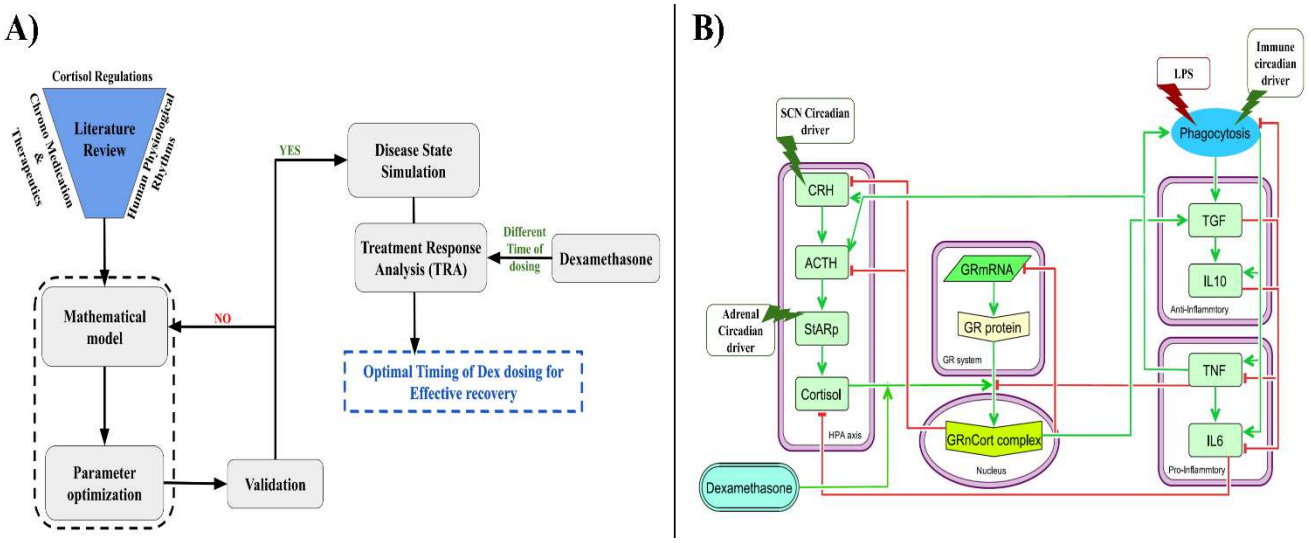


Figure 1. Flowchart and Model Crosstalk Summarization: (A)The flowchart summarizes the methodology for validating the mathematical model and its implementation in simulating the disease states. The disease state models were further used in treatment response analysis (TRA) to study the effect of Dex on the system and to identify the optimal time for Dex dosing. (B) Schematic flowchart of the HPA-Inflammation model, depicting crosstalk between the HPA axis and the Inflammatory modules. The model considers three circadian drivers: SCN-generated circadian signalling, the adrenal circadian drive of cortisol, and an immune circadian drive for the inflammatory module, to reproduce respective ~24 hr rhythm into the model variables. The LPS and Dexamethasone represent an injection of an external endotoxin and glucocorticoid into the system. The detailed explanation of the HPA-Inflammation model is provided in the Supplemental S1.

Given limited data on glucocorticoid chronotherapy on human physiology and the complexity of clinical settings, a mathematical model can be used to generate synthetic data on the temporal effects of glucocorticoids. Synthetic data compensates for the lack of real-time data by offering molecular circadian profiles (cortisol, cytokines, metabolites, etc.), reproducing the physiological rhythms, while maintaining the clinical statistics (Pezoulas et al., 2024). Reliable synthetic data generation is achieved primarily through probability, statistical, machine learning/deep learning, and mechanistic mathematical methods (Mwigereri et al., 2025). This data augmentation approach can aid chronotherapeutic research by improving machine learning models that predict the optimal timing of glucocorticoid dosing. These models may help adjust the timing of drug administration based on an individual's physiology, thereby managing steroid-related side effects. To generate a predictive model

for Dex dosing, we employed a mechanistic model and treatment-response analysis to determine the optimal timing of Dex dosing across perturbed (or disease) states of the HPA-Inflammation system. Each perturbed state of the model represents a different clinical scenario of cortisol CR dysfunction. The circadian data (phase and amplitude of cortisol and proinflammatory cytokines) from these simulated clinical scenarios were further used to train a neural network to predict the optimal time for Dex administration.

Methods:

HPA-Inflammation model:

In this study, we used an integrated mathematical model (MM) of the HPA-Inflammation system from prior research, which focuses on the interactions between variations in glucocorticoid receptor (GR) sensitivity and inflammation. (Somvanshi et al., 2020). The model has two modules: first, the HPA axis module (CRH, ACTH, StArP, and Cortisol) along with GR variables (GRmRNA, GRProtein, GR-Cortisol complex, and GRnuclear); second, the Immune system module (LPS, phagocytes, anti-inflammatory cytokines: TGFβ and IL10, Inflammatory cytokines: TNF-α and IL6) (**Figure 1B**). The detailed ODEs and parameters were mentioned separately in the **Supplemental S1**. To define the crosstalk between the modules, various feedback responses were used in the ODE equations, as shown in **Supplemental Table S2**. Each simulation was performed using the Ode15s solver in MATLAB, and the duration of each simulation was 6-week. The numerical errors were controlled by setting the relative and absolute error tolerances at 1e-10. The MATLAB graphical editor was used to plot the 24-hr dynamics of model variables and phase-amplitude plots of the time-dependent effects of Dex.

Parameter Sensitivity Analysis:

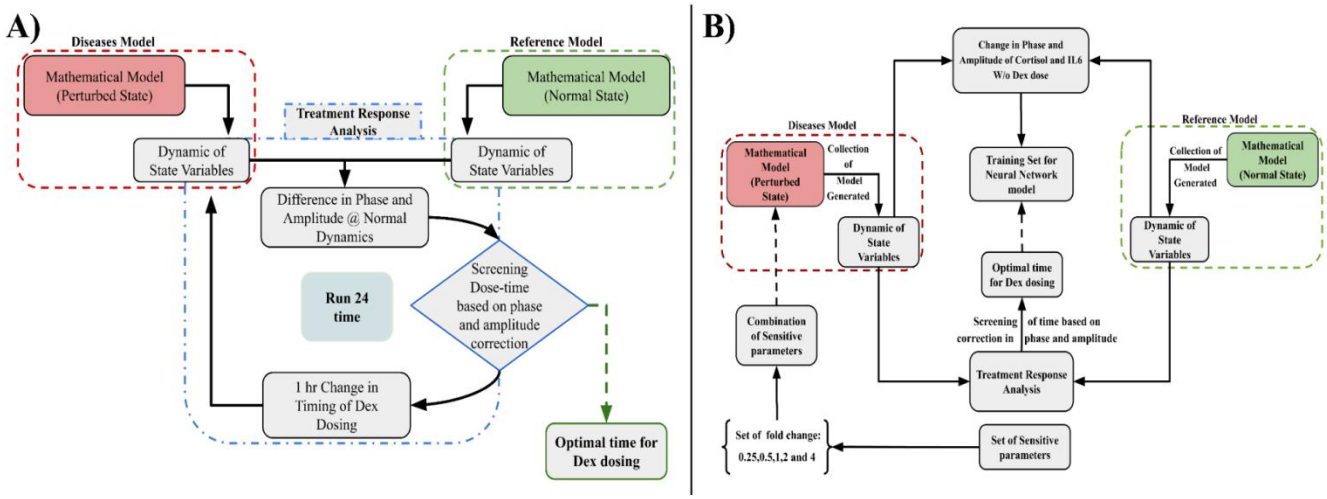
A parameter sensitivity analysis (PSA) was performed to assess the influence of parameter variations on the system response and identify critical parameters for model behavior. The model was iteratively simulated for each altered HPA axis parameter (by ±10%), and the resulting changes in the amplitude and phase of model state variables were recorded. For PSA computation, the recorded changes in cortisol amplitude and phase shift were used in the given formulae,

$$Amplitude\ sensitivity = \frac{\left(Amp_{Cort(+10\%)} - Amp_{Cort(-10\%)} \right)}{Par_{(+10\%)} - Par_{(-10\%)}} * \frac{Par_{basal}}{Amp_{Cort(basal)}}$$

$$Phase\ Difference\ sensitivity = \frac{\left(PhaseDiff_{Cort(+10\%)} - PhaseDiff_{Cort(-10\%)} \right)}{Par_{(+10\%)} - Par_{(-10\%)}} * Par_{basal}$$

Where the Par_{basal}, $Amp_{Cort(basal)}$, Par, and $PhaseDiff_{Cort}$ represent the parameter basal value, cortisol amplitude, parameter value (at ±10% change), and cortisol phase difference, respectively. The Par_{basal}/Amp_{basal} and Par_{basal} were used to normalize the cortisol amplitude and phase-difference sensitivity, respectively. A sensitivity analysis was conducted to pinpoint parameters that significantly

influence cortisol amplitude and phase dynamics, which were subsequently used to model various disease states within the HPA-Inflammation system.



Treatment Response Analysis (TRA):

Figure 2. Treatment Response Analysis and Synthetic data generation: (A) flowchart provides a detailed overview of the TRA for identification of the optimal time of Dex administration for a certain disease state. The flowchart depicts the TRA methodology for evaluating changes in the phase and amplitude of HPA-Inflammation state variables in a disease model in response to Dex dosing, as compared with the normal state (Reference model). This process is repeated for 24 iterations corresponding to each Dex dosing regime as per respective circadian time and the TRA output is used to determine optimal time to administer Dex that can restore normalcy. (B) The flowchart provides a detailed overview of synthetic data generation, using a set of sensitive parameters and the TRA method to screen optimal Dex dosing timings for each disease scenario. Further, the synthetic data was used to train the neural network to learn patterns in the changes of phase and amplitude profiles of the state variables to predict the optimal time for Dex dosing for disease states.

To assess the regulatory effects of Dex on the circadian rhythm of HPA-Inflammation state variables, we performed treatment Response analysis (TRA) by quantifying changes in amplitude and phase of these variables after Dex administration. The normal state (NS) simulation reproduces the reported human circadian physiology of HPA-Inflammation variables, while the simulations with altered parameter

values represent the perturbed (disease) state (DS) of the HPA-Inflammation system. We simulate the effects of Dex dosing on the DS (TDS: Dex treated disease state) by activating the model's dexamethasone dosing variable (Dex: Supplemental S1) at different times of day. The simulation data is further used by TRA to generate response curves for HPA-Inflammation state variables, illustrating the effect of Dex at different times of day. The steps for performing the TRA are shown in Fig. 1B and mentioned below,

1. Amplitude of each state variable in both the normal state (NS) and Dex-treated disease state (TDS) were calculated by: NSAmp or TDSAmp = (maxima - minima)/2 for respective circadian profiles.

2. The timestamp of the maxima from NS and TDS of the system was used to record the peak time of these state variables: Time of maxima for normal state (NTmax) and for TDS (DTmax)

3. The phase difference is calculated as PD= DTmax - NTmax, reflecting change in phase in response to Dex administration.

4. Repeat steps (1→3) for different timings of Dex administration across the circadian period for DS simulation.

5. PRC generation: the response of Dex treatment was independently recorded for Dex dosing at different times of the day for 24 different dosing scenarios, ranging from time 1st hr as midnight 1:00 AM till 24th hr as 12 AM of the circadian time. The response of each dosing regime was simulated until steady state oscillatory response was achieved followed by the recording of its PD. These PDs were plotted with respect to the circadian time of Dex dosing to generate PRC.

6. Optimal dose time is determined as the dosing for which amplitude difference |NSAmp – TDSAmp | and the phase difference PD get minimized for cortisol, TNF-α and IL6 variables.

These steps were used in various disease simulations to determine the optimal timing of Dex administration to restore homeostasis and reduce peripheral side effects (e.g., in the inflammatory module). Also, reveals the effects of Dex administration at suboptimal timings on the amplitude and phase of each variable.

Disease Simulations

Lower Grade Inflammation

The lower grade inflammation was simulated by perturbing the basal level of LPS (*lps_b* parameter) in the system (from 10^{-7}ng/kg-h to 10^{-6} ng/kg-h), which mimics the condition where the body has persistent low-level inflammation with a marked increase in pro-inflammatory cytokines. Further, the TRA method was applied on this perturbed system to determine the optimal time for Dex dosing (~0.975 mg). The optimal timing indicated by the corrections to the circadian physiology of the proinflammatory module, while preserving the cortisol expression profile.

Hypocortisolemia Simulation

The hypocortisolemia model was simulated by reducing the threshold (*Ki_GR1* from 1.2 to 0.6 nM/ml) for GRn (nuclear Glucocorticoid Receptor)-mediated negative regulation of HPA axis variables, thereby reducing basal cortisol levels and increasing inflammatory cytokine levels. For this perturbed state, the TRA was performed to capture the time-dependent effects of Dex (0.5 mg) on the state variables of the HPA-Inflammation system. Analysis also helps to determine the optimal time for Dex dosing to alleviate the effects of Hypocortisolemia on the peripheral system.

Prediction model for Optimal Time of drug administration:

We developed a Neural Network (NN) model to predict the optimal time window for Dex dosing based on observed clinical pathology associated with disruptions in cortisol dynamics and inflammation. For the robust training and validation of the NN model, synthetic data on circadian rhythm disruption were generated using parameter perturbation. For these perturbed states of the HPA-inflammation system, an optimal Dex dosing time was determined using the TRA framework. This data is additionally included in the synthetic data as classification output, as described in the synthetic data generation section. The Classification Learner app in MATLAB was used to import a three-layer (Hidden layer: first layer size= 60, second layer size= 93, third layer size= 120) fully connected neural network model with Tanh activation, 2000 epochs, and Lambda regularization (1.465E-4) as the NN hyperparameters.

Synthetic Data generation: The sensitive parameters for cortisol response were used in pairs to generate perturbed states (m = 608) with alterations in CR of the HPA-Inflammation variables. These scenarios were generated by varying the model parameters over a 0-4 fold range with fractions of 0.25, 0.5, 1, 2, and 4 times the nominal parameter value. Further, the TRA protocol was applied to identify the optimal timing for 0.5 mg Dex dose. Subsequently, the identified optimal time window for Dex dosing is used as

the classification output in the synthetic data. Whereas, the disease state CR profiles (the changes in amplitude and phase of cortisol and IL6) were used as the learning features in the synthetic data for the NN model. From the synthetic data, 80% (m=486) and 20% (m=122) were used as the training and test sets, respectively. The test set was used to evaluate the trained model's prediction accuracy. The model-predicted optimal chronotherapy was tested through simulations for treating disease scenarios to restore normal dynamics of the cortisol rhythm and inflammatory markers.

Accuracy Evaluation: To assess the prediction accuracy of the trained model, we used the NN model to predict the optimal time for Dex (~0.5mg) administration to correct various perturbed cortisol circadian profiles, as shown in **Table 1**. Each perturbed circadian profile (phase [delay/advance/no-change] and amplitude [increase/decrease/no-change]) of cortisol is generated by changing the parameters (V_acth1, k_starp, n1, k_crh, kre, km_GR1, Ki_GR1, V_starp1, V_cort, k_cort, V_GRprot, Ki_GR, k_GRprot, V_GRmrna, and K_strs) of HPA axis related to the synthesis rate, degradation rate, inhibitory threshold & sensitivity, and stress induced activation of HPA variables **Supplemental Table S3**. A couple of clinical scenarios (Major Depressive Disorder (MDD) and Seasonal Affective Disorder (SAD)) of disrupted cortisol dynamics and inflammatory markers were subjected to the NN model to determine the optimal time and dose of Dex for validating the therapeutic regimen.

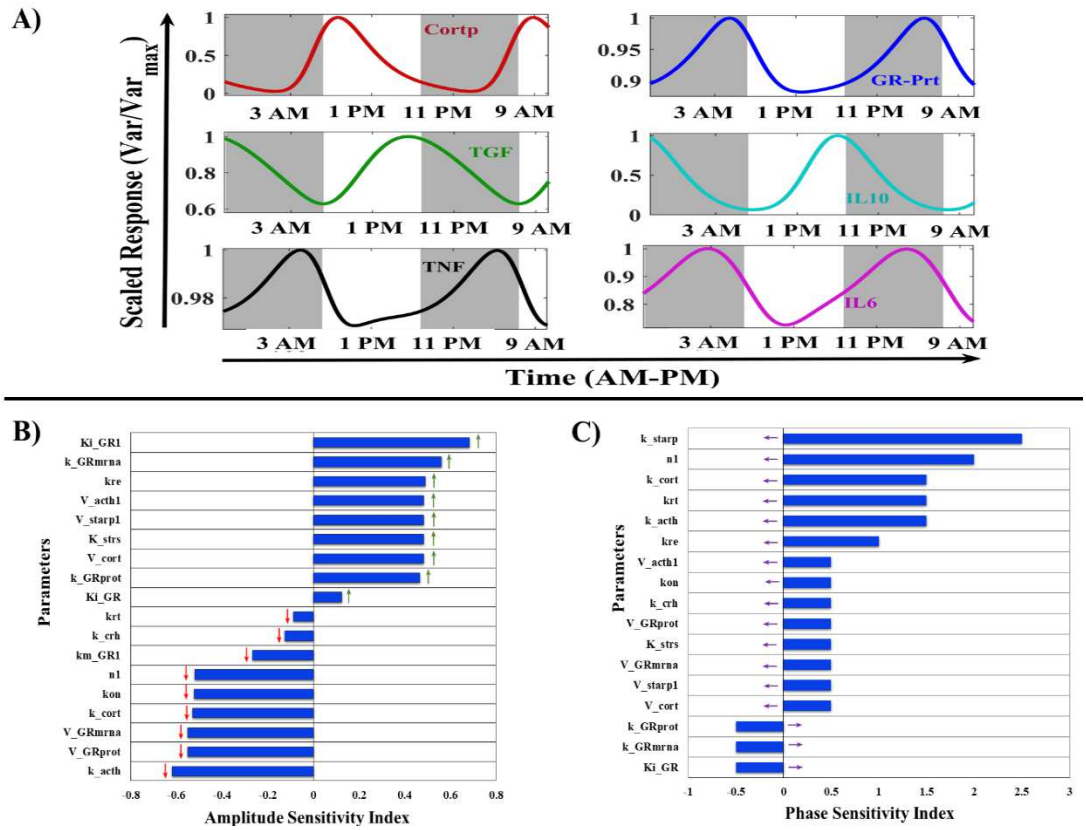
Table 1. The table shows the NN model predicted optimum timing of Dex (~0.5 mg) dose in different dysregulations of circadian profiles (phase [delay/advance/no-change] and amplitude [increase/decrease/no-change]) of cortisol, along with the reported disease etiologies with a similar type of perturbations in cortisol circadian rhythm.

Change in Cortisol Amplitude	Change in Cortisol Phase	PRC recommended time of dosing	Disease Name
Increase	Delay	2AM - 4AM	-
Increase	Advance	8AM - 9AM	MDD, Huntington's disease, acute cortical ischemic stroke (Alesci et al., 2005; Karlović et al., 2012; Adamczak-Ratajczak et al., 2017)
Decreases	Delay	3AM - 6AM	RA, Polymyalgia rheumatica (Kirwan, 2011)
Decreases	Advance	6AM - 8AM	-

Increase	No change	1AM -2AM	Heart failure (Crnko et al., 2023)
Decrease	No change	5AM - 6AM	PTSD, chronic fatigue syndrome (Jerjes et al., 2005; Gotovac et al., 2003)
No change	Delay	2AM - 4AM	Seasonal Affective Disorder (Oren et al., 1996)
No change	Advance	5AM – 7AM	Night shift work (Archer et al., 2022; Jensen et al., 2016)

Result

Previous studies found a co-occurrence between circadian dysregulation of the HPA axis and metabolic disorders in pathophysiological or systemic conditions such as hyperglycemia, insulin resistance, PTSD, hypocortisolemia, and low-grade inflammation (Wulff et al., 2010; Somvanshi et al., 2020; Bijlsma & Jacobs, 2008). To understand the mechanistic connection between circadian dysregulation and its effects on peripheral circadian rhythms, we have used an ODE-based MM that includes cross-talk between the HPA axis and the peripheral inflammatory system, as reported in Somvanshi et al. 2020 (Somvanshi et al., 2020). The model considers two modules representing the HPA axis and the inflammatory system, which are regulated by the GR signalling (**Figure 1B)**. The ~24 hr rhythm of model state variables is governed by three circadian drivers: the SCN's regulation of hypothalamus, the transmission of circadian signals to adrenal gland, and the endogenous rhythm of the immune system. In the model flowchart, the dexamethasone represents the injection of an external glucocorticoid to study its effect on the circadian physiology of model variables. The endotoxin level in the model is controlled by the LPS injection, which remains near the basal value (10e-7) and can be varied to mimic various inflammatory conditions. The non-linear pattern of GR-mediated regulatory signaling was represented by the feedback equations



based on the Michaelis-Menten and Hill kinetic functions, shown in Supplemental Table S2.

Figure 3. Model circadian dynamics & Parameter sensitivity analysis: The (A) shows model-simulated circadian dynamics of Cortisol (Cort), GR protein (GR_prt), Transforming growth factor (TGF), Tumor necrosis factor (**TNF**), Interleukin 10 (IL10), and 6 (IL6), which matches with the reported circadian human physiology. The Y-axis shows a scaled dynamic response of the state variables, obtained by dividing their profiles by their maximum values. The (B & C) plots depict the cortisol amplitude and phase sensitivity index for the HPA axis and GR module parameters. The blue bar length represents the sensitivity indices of the variable response to a perturbation in the parameter, whereas the bar direction indicates the magnitude of sensitivity to increasing or decreasing the amplitude/phase of cortisol. The increase and decrease in cortisol amplitude are represented by the ↑ and ↓ arrows, respectively. Whereas, the delay and advance in the cortisol phase are shown by the → and ← arrows, respectively.

Further, the ~24 hr rhythmic response of the model variables (elements of the HPA axis and the inflammation module) was validated and adjusted to align with reported human circadian physiology. The validation study showed that the HPA variables exhibited circadian expression patterns similar to those reported in previous studies. As shown in (**Figure 3A)**, the model generated circadian dynamics of cortisol peaks (~ 20.04µg/dL) and hits trough (~ 0.526µg/dL) during the morning (~8:30 AM) and midnight (~1:00 AM) time {amplitude: 9.755µg/dL}, which matches with the reported circadian physiology of cortisol (WEITZMAN et al., 1971). Similarly, the HPA axis and GR variables, viz. CRH, ACTH, and GR protein also showed concordance with the reported human physiological expression levels. The circadian profile of inflammatory variables did not reproduce the physiologically reported rhythmic expression (Bauer et al., 1994; Wright et al., 2015). The clinical data show a maximum expression of IL6 around ~6 hr (peak) before the cortisol expression peaks (around ~ 8:30 AM). Based on the reported data, we corrected the phase of inflammatory cytokine expression by adding a circadian driver (cir3(t)) to phagocytosis equations [**Supplemental S1]**. This drive aligns their 24-hour peak-and-trough cycles with the physiological reported rhythm (**Figure 3A)**. After applying circadian drive on the inflammatory module, the peak/trough for IL6 (amplitude: 0.182pg/mL) and TNF (amplitude: 0.0163pg/mL) is set at 2:30 AM(1.322pg/mL)/11:00 AM(0.96pg/mL) and 4:15 AM(1.034pg/mL)/ 11:00 AM(1.002pg/mL), respectively. Hence, we reset the IL6 peak to approximately 6 hr before the cortisol

peak time (~8:30 AM). The model was reparametrized to cross-validate the clinical data, which showed a similar response pattern in proinflammatory and HPA-axis variables after LPS (~0.4 ng/kg) induction at 2 PM [**Supplemental Figure 1**]. The pattern suggests an elevated response of phagocytosis-induced proinflammatory and anti-inflammatory cytokines, along with increased regulatory responses of the HPA axis after the sudden introduction of LPS. Further, the optimized model was used to simulate cortisol circadian rhythm dysregulation and study its effect on the regulation of inflammatory variables. Also, dexamethasone PK-PD equations were further used to assess the effects of Dex administration on circadian rhythms.

Parameter sensitivity of cortisol amplitude and phase:

The union of parameter subsets from both amplitude and phase sensitivity is mainly composed of HPA axis module parameters, which are *Ki_GR1, k_GRmrna, k_GRprot, V_starp1, V_GRmrna, V_GRprot, kon, kre, km_GR1, Ki_GR, krt, k_starp, n1, V_cort, K_strs, V_acth1, k_acth, k_cort, and k_crh* (**Figure 3B & C)**. Among this subset, the cortisol phase showed higher sensitivity (positively) to perturbations in the *k_acth, krt, k_cort, n1, k_starp*, and *kre* parameters. Cortisol amplitude was found to be highly sensitive, in both positive and negative directions, after changes in the respective sets of parameters: (*k_GRprot, V_cort, K_strs, V_starp1, V_acth1, kre, k_GRmrna, Ki_GR1*) and *(k_acth, V_GRprot, V_GRmrna, K_cort, kon, n1*). The high sensitivity of the cortisol circadian profile to the given set of parameters makes it a critical controller for setting and maintaining the circadian profile and for studying different disease states.

Simulation of Lower-grade inflammation and PRC analysis for Dex dosing time

Lower-grade inflammation is simulated by increasing the basal level of systemic LPS by 10-fold (1e-7 ng/kg-h → 1e-6 ng/kg-h) in the model (as explained in the methodology). That in turn increases the amplitude of IL6 and TNF expression by ~2-fold, without perturbing the cortisol expression profile. In the PRC analysis, we assessed the time window of Dex administration (~0.975mg) during which the proinflammatory circadian profile would be reset to homeostatic levels, driven by increased plasma cortisol. As shown by the PRC analysis, administering Dex at suboptimal times [Other than 8-10 AM] during the LGI condition would lead to dysregulated phase and amplitude of the pro-inflammatory and cortisol responses relative to the required homeostatic range (**Figure 4A)**.

Whereas at an optimal time range [8-10 AM], Dex administration, together with cortisol expression (Cort-Dex), controls the abrupt increase in the amplitude of proinflammatory cytokines without affecting their phase of expression (**Figure 4A)**. Accordingly, administering Dex at 9 AM in the LGI system results in a significant resetting of IL6 and TNF amplitudes (**Figure 4B)**.

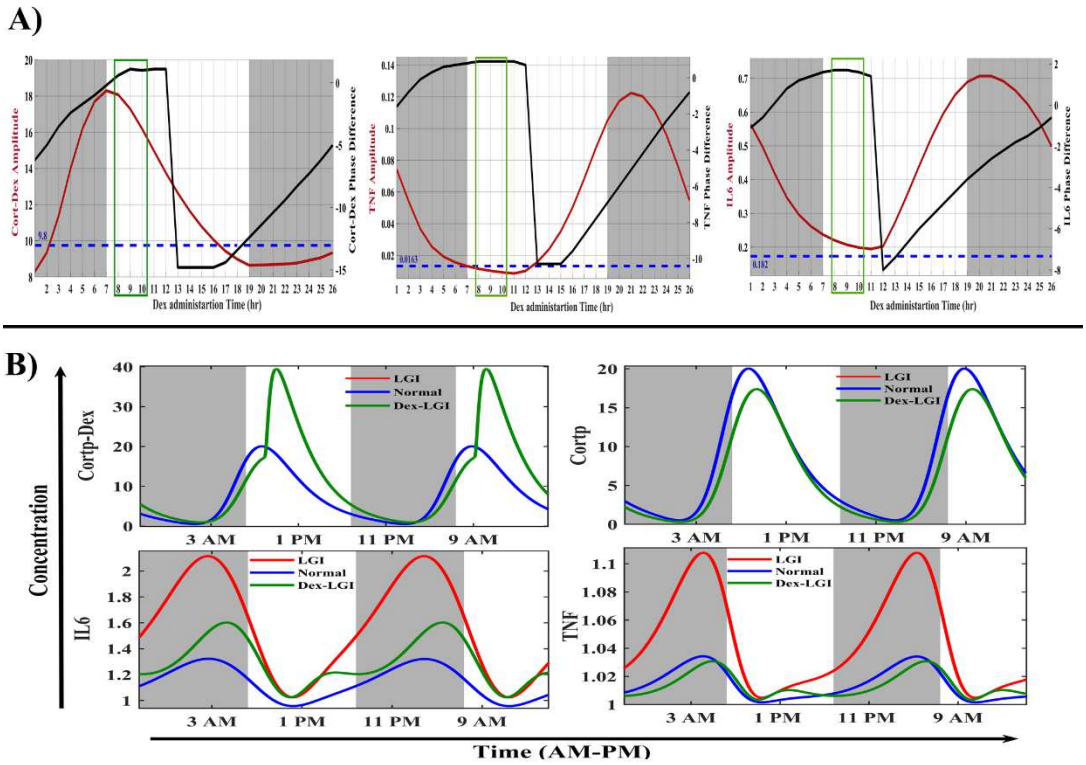
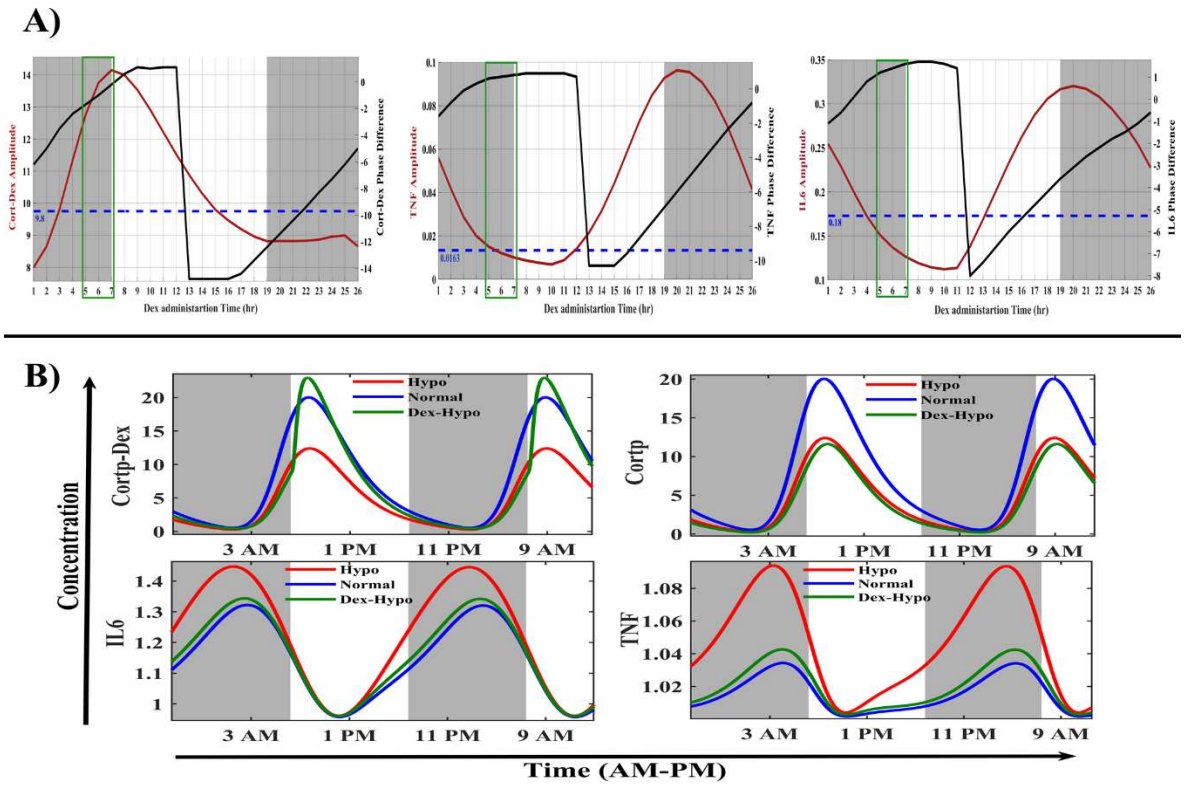


Figure 4. PRC analysis and circadian dynamics of LGI state: (A) PRC depicts the effects of Dex administration at different times of day on the expression profiles of HPA-inflammation variables during the lower Grade Inflammation (LGI) state. The Graph shows changes in phase (Phase Difference) and amplitude of the system variable (LGI state) relative to the system's normal state, where the green box shows the optimal time window for Dex dosing. At this time range [8-10 AM], the phase difference (red) and the amplitude (yellow) of the variables are roughly reset to homeostatic levels (in blue). For the rest of the time, the Dex dosing may lead to adverse physiological states. In the graph, the gray and white areas represent the Dark (night: 19 hr to 6 hr) and Light (day: 7 hr to 18 hr), respectively. (B) represents circadian dynamics of variables in **Normal**, **LGI**, and **Dex** (0.975mg) treated LGI states after administering Dex at 9 AM. Where the Cortisol (Cort) expression, together with Dex (Cort-Dex), alleviates LGI effects on proinflammatory cytokines and rescues normalcy. The fold increase in IL6 and TNF expression is also reduced nearly to their respective homeostatic levels, but still requires a higher Dex dose to fully reset to normal levels. The gray and white areas represent the Dark (night: 7 PM to 6 AM) and Light (day: 7 AM to 6 PM), respectively. The SI units for IL6, TNF, and Cortisol/Cortisol-Dex are pg/mL, pg/mL, µg/dL, and µg/dL, respectively. The blue, red, and green line graphs represent the normal, LGI, and Dex-treated LGI states, respectively. Whereas, the plotted phase difference has A.U.

Simulation of Hypocortisolemia and PRC analysis for optimal Dex dosing time

Similarly, in the case of hypocortisolemia, where the increased negative feedback of GRn (Glucocorticoid within nucleus) on the HPA axis reduces the cortisol expression amplitude by ~0.62 fold, it would parallelly increase the expression amplitude of proinflammatory cytokines by ~2 fold. Timely administration of Dex is needed to compensate for the effect of increased GRn sensitivity, observed between 5-7 AM. The PRC proposes an optimal window of time, 5-7 AM, for Dex administration, which effectively resets the amplitude of each variable (cortisol and proinflammatory cytokines) and shows less effect on their phase of expression (**Figure 5A)**. As shown by the PRC analysis, time ranges other than 5-7 AM for the Dex dosing (~0.5mg) could result in greater dysregulation in the phase and amplitude of the HPA-Inflammation variables, which may lead to the progression of the disease state. Upon administering Dex at 6 AM in the HPA-Inflammation system (in a Hypocortisolemic state), the proinflammatory variables (IL6 and TNF) return to a normal physiological range (**Figure 5B)**. The analysis also shows that Dex, together with cortisol (Cort-Dex), restores normal levels of IL6 and TNF expression. The analysis highlights the importance of understanding systemic crosstalk in determining the optimal dose and timing of corticosteroids to effectively regulate peripheral



circadian dynamics.

Figure 5. PRC analysis and circadian dynamics of the Hypo state: (A) PRC depicts the effects of Dex administration at different times of day on the expression profiles of HPA-inflammation variables during the Hypocortisolemia (Hypo) state. The Graph shows changes in phase (Phase Difference) and the amplitude of the system variable (Hypo state) relative to the system's normal state, with the green box indicating the optimal time window for Dex dosing. At this time range [5-7 AM], the phase difference (red) and the amplitude (yellow) of the variables are roughly reset to homeostatic levels (in blue). For the rest of the time, the Dex dosing may lead to adverse physiological states. In the graph, the gray and white areas represent the Dark (night: 19 hr to 6 hr) and Light (day: 7 hr to 18 hr), respectively. (B) represents circadian dynamics of variables in **Normal**, **LGI**, and **Dex** (0.5mg) treated LGI states after administering Dex at 6 AM. Where the Cortisol (Cort) expression, together with Dex (Cort-Dex), alleviates LGI effects on proinflammatory cytokines and rescues normalcy. The gray and white areas represent the Dark (night: 7 PM to 6 AM) and Light (day: 7 AM to 6 PM), respectively. The SI units for IL6, TNF, and Cortisol/Cortisol-Dex are pg/mL, pg/mL, µg/dL, and µg/dL, respectively. The blue, red, and green line graphs represent the normal, LGI, and Dex-treated LGI states, respectively. Whereas, the plotted phase difference has A.U.

Predictive model for Optimal time Dex dosing:

The trained three-layer neural network achieved a mean validation accuracy of 0.98 (95% CI: 0.97–0.99) using 10-fold cross-validation and a test accuracy of 0.98 (95% CI: 0.97–1.00) across three consecutive runs on model-generated synthetic data (training set, n = 486; test set, n = 122) (**Figure 3C)**. To enable a detailed performance assessment, subsequent analyses were conducted using data from a representative run. During validation, the model demonstrated better classification performance, with an overall sensitivity of 0.88 (95% CI: 0.82–0.93) and specificity of 0.99 (95% CI: 0.98–0.99), indicating robust identification of the optimal time window and discrimination across temporal windows. The receiver operating characteristic (ROC) analysis**, (Figure 6),** also revealed consistently high true positive rates at low false positive rates across all classes, yielding a macro-averaged area under the curve (AUC) of 0.98 (95% CI: 0.96–0.99). Evaluation on the test dataset demonstrated sensitivities of 0.89 (95% CI: 0.77–1.00) and specificities of 0.99 (95% CI: 0.98–0.99), respectively, indicating robust generalization for true-positive detection with a lower chance of misclassification (i.e., negatives as positives). Further, the ROC analysis of the test set yielded an aggregated AUC of 0.98 (95% CI: 0.97–1.00), suggesting that threshold-independent classification performance remained high.

From this data, two perturbed circadian profiles of cortisol were taken to simulate the reported disease states, viz., major depressive disorder (MDD: [change in phase: Advance and amplitude: Increase]) and seasonal affective disorder (SAD: [change in phase: Delay and amplitude: small change]). The MDD state was simulated by increasing the synthesis rate of cortisol (*V_cort*) and sensitivity of GR negative feedback (*n1*) by two times, representing cortisol circadian dysregulation (~1.34 times of normal amplitude and phase advance by ~1.7hr) (**Figure 7A & B)**. The SAD state is simulated by reducing the *kre* (GR nuclear recycling rate) by 2 times and *km_GR1* (GR negative feedback on GR mRNA synthesis) by 4 times, which results in a delay in the cortisol expression phase (by ~1.6hr) without causing significant alterations in amplitude (**Figure 7C & D)**. The NN model suggested morning timings of 8 AM-9 AM and 2 AM-4 AM for administering the Dex dose in MDD and SAD states, respectively. The model-predicted time was used to administer an optimal Dex dose to correct the circadian profile of cortisol and IL6, nearly like normal human circadian physiology. After respective Dex dosing (~0.25 mg in MDD and ~0.43 mg in SAD), the AUC of cortisol expression in MDD and SAD states changes by 0.998 and 1.2 times, respectively, in comparison to the normal AUC. The administered timing of the Dex dose corrects the perturbed phase of cortisol expression (in MDD and SAD states), as shown by the polar plot. This represents the potential use of the model to predict the optimal time for glucocorticoids and further optimize the dose concentration accordingly.

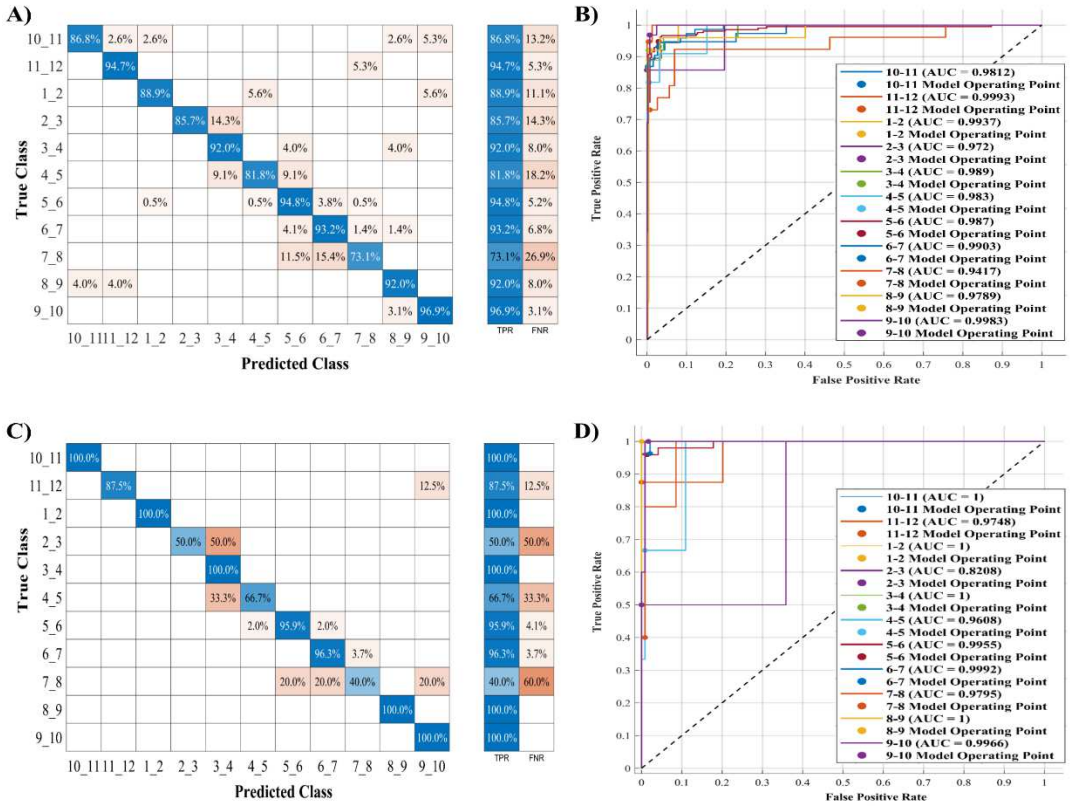


Figure 6. Evaluation of Validation and Test data analysis: The performance of the NN model during 10-fold cross-validation (A & B) and on the test data (C & D) is represented by the confusion matrix and the receiver operating characteristic (ROC) curve. The confusion matrix evaluates the NN model's ability to distinguish classes by reporting specificity, sensitivity, and accuracy. In the confusion matrix, the rows represent the True classes (optimal dose time from TRA), while the columns show the model-predicted classes (optimal time predicted by the NN model) for the set of perturbed cortisol CR scenarios. The predictive and discriminative potential of the NN model during validation and test data analysis is shown by the ROC analysis. In the graphs, class-wise ROC showed minimal overlap and better discrimination potential, further supporting strong separability between optimal and non-optimal time windows.

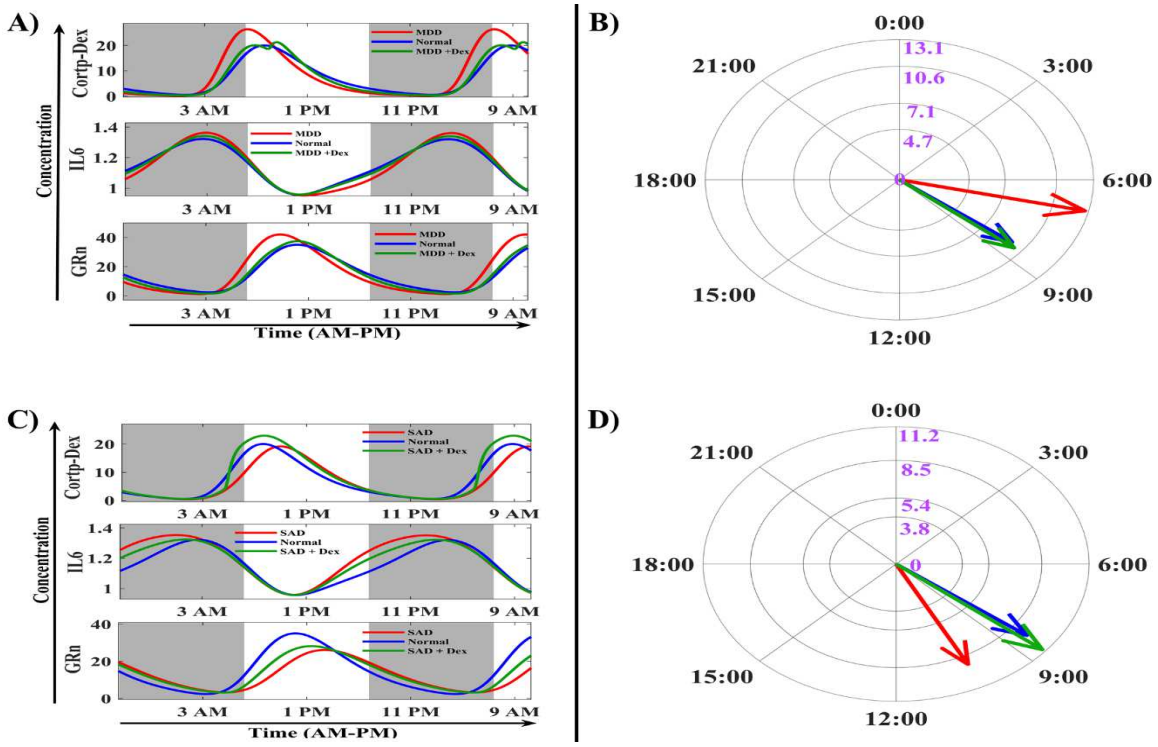


Figure 7. Analysis of Glucocorticoid time-dependent effects on MDD and SAD: (A) represents the circadian dynamics of Cortisol with Dex (Cort-Dex), IL6, and GRn (nuclear GR) in normal, MDD, and Dex-treated MDD (Dex-MDD) states of the system. After administration of Dex (~0.25 mg) at 8 AM, plasma Cort-Dex levels nearly restore the normal physiological profile of IL6 by correcting cortisol and GRn circadian dynamics. (C) Represents the circadian dynamics of Cort-Dex, IL6, and GRn (nuclear GR) in normal, SAD, and Dex-treated SAD (Dex-SAD) states of the system. After administering Dex

(~0.43 mg) at 4 AM, the plasma Cort-Dex levels nearly rescue the normal physiological profile of IL6 by correcting cortisol circadian dynamics. (B & D) show polar plot of cortisol circadian profile (amplitude and phase) in the normal (blue), MDD/SAD (red), and Dex-MDD/SAD (green) conditions of the HPA-Inflammation system. Wherein the angle between the arrows represents the phase difference between each state, and the arrow length shows the amplitude of cortisol in each state of the system. Each concentric circle in the polar plot shows the cortisol concentration (purple marker).and polar plot. In the graph, the gray and white areas represent the Dark (night: 7 PM to 6 AM) and Light (day: 7 AM to 6 PM), respectively. The SI units of Cort-Dex, IL6, and GRn concentration are μg/dL, pg/mL, and nM/mL, respectively. The Blue, Red, and Green lines in the graphs represent the respective states of Normal, MDD, and Dex-treated MDD in the HPA-inflammation system.

Discussion

The persistent exposure to abnormal LD cycles and feeding behavior in the industrial world is known to contribute to circadian dysregulation, resulting in chronic metabolic diseases and inflammation (Bass & Lazar, 2016; Walker et al., 2020). These lifestyle changes alter the cortisol rhythm, which acts as an entrainer for the CRs of peripheral organs, eventually causing internal desynchrony among various biological processes. To understand cortisol regulatory and dysregulatory effects on the peripheral CRs, we used a MM on the HPA-Inflammation system by Somavanshi et al. 2020 (Somvanshi et al., 2020). The model's ODE framework represents GR-based crosstalk between the HPA axis and inflammation by leveraging key biological processes that govern signal transmission among modules. These processes include stressor- and non-stressor–dependent rhythmic HPA axis activation; HPA axis regulation on cortisol secretion and rhythm; formation of the glucocorticoid receptor–cortisol (GC) complex in the cytosol and its subsequent translocation to the nucleus; GC-mediated feedback regulation on HPA axis and GR transcription; LPS–induced phagocytic stimulation of pro- and anti-inflammatory cytokines; GC-driven transactivation of anti-inflammatory cytokines; anti-inflammatory suppression of proinflammatory signaling; and proinflammatory feedback on HPA axis. The reparameterized model replicates similar circadian dynamics of the HPA axis, such as the morning cortisol dynamics following the waking-hour ACTH expression profile (WEITZMAN et al., 1971; Oster et al., 2017). The proinflammatory variable dynamics also showed consistency with the reported findings, such as a ~6 hr delay in the peak of IL6 expression relative to the zenith of cortisol levels under normal conditions (Bauer et al., 1994; Wright et al., 2015). The observed concordance with human circadian physiology confirms the model's ability to reproduce the variables' normal circadian dynamics.

Pathogen recognition in the biological system activates peripheral immune responses primarily through Toll-like receptor 4 (TLR4), which triggers NF-κB and MAPK signaling pathways and induces the release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 (WANG et al., 2013; Smolinska et al., 2011; Kuzmich et al., 2017). However, the magnitude and duration of these peripheral inflammatory processes are tightly regulated around physiological set points by glucocorticoid receptor (GR) signaling. Endogenous glucocorticoids, acting through the GR, counterbalance pro-inflammatory responses by promoting anti-inflammatory pathways (Somvanshi et al., 2020). Upon activation and nuclear translocation, GR attenuates inflammatory signaling by suppressing NF-κB and AP-1 activity, inducing anti-inflammatory mediators such as IL-10 and annexin A1, and limiting the expression of pro-

inflammatory genes (Rhen & Cidlowski, 2005; Barnes, 1998). Through these coordinated mechanisms, GR signaling regulates the trans-activation and inhibition of cytokine production, thereby maintaining and restoring immune homeostasis. Consistent with this physiological regulation, the given model (normal state) reproduced a similar regulatory response pattern across the immune variables after LPS induction. When LPS levels exceed a defined physiological threshold, this triggers an initial rise in pro-inflammatory cytokines (TNF-α and IL-6), followed by an elevation in cortisol levels via the HPA axis **Supplementary Figure 1**. These mechanistic changes promote the controlled release of anti-inflammatory cytokines (TGF-β and IL-10) in the LPS-challenged system, as reported in the studies (Grigoleit et al., 2010). The model simulations reproduce normal physiological responses, supporting its physiological relevance.

Excessive exposure to unnatural light disrupts the regulatory cues of the HPA axis and promotes circadian misalignment in peripheral clocks and their associated processes, including increased pro-inflammatory cytokine levels (Tomatsu, Abbott & Attarian, 2025; Bass & Takahashi, 2010; Bass & Lazar, 2016). As a key circadian entrainer between the master and peripheral clocks, the HPA axis is a potential target for treating circadian dysregulation. Clinicians often prescribe corticosteroids to alleviate pro-inflammatory dysregulation (Paolino, Cutolo & Pizzorni, 2017; Cain & Cidlowski, 2017); however, interindividual heterogeneity in circadian dynamics may lead to suboptimal regulation of the immune system via corticosteroid (Dallmann, Brown & Gachon, 2014; Costello et al., 2016). To understand these variations in circadian dynamics and their impact on drug efficacy, a chrono-based drug therapeutic approach has been proposed (Dallmann, Brown & Gachon, 2014; Smolensky, Lemmer & Reinberg, 2007). This field explores the chrono-pharmacodynamic (PD) and -pharmacokinetic (PK) effects of drug administration during different circadian phase times. Multiple studies reported the usage of MM in studying the effects of drug PK on its PD, which will aid in adjusting the drug therapies in various diseases such as cancer, hypertension (Hesse et al., 2021; Cortés-Ríos & Rodriguez-Fernandez, 2023). One of the tools used in chronotherapy is phase response analysis, which helps to explore the phase shifts in the rhythms of molecular markers, induced by exogenous stimuli (Youngstedt, Elliott & Kripke, 2019). Studies have shown the potential of analyzing phase shifts to identify the optimal timing of drug-induced phase changes in gene expression to alleviate disease physiology (Turek, 1987; Burgess et al., 2010). MPC framework studies have also shown that monitoring phase changes can aid in managing the drug schedule, according to observed phase shifts across different disease states (Abel et al., 2019; Murdoch, Aiello & Doyle, 2023; Abel, Chakrabarty & Doyle III, 2017). In our model-based TRA, Dex

dosing at suboptimal (after 12 o'clock noon) versus optimal (morning hours) timings showed a significant effect on the circadian profiles of pro-inflammatory cytokines and cortisol, relative to the normal state. Suboptimal time points reflect disturbed circadian parameters (amplitude and phase) of cortisol relative to the normal state, which may increase the risk of adrenal insufficiency (Younes & Younes, 2017). The TRA data provide insight into the PK-PD of Dex and its effects on the circadian dynamics of the HPA-Inflammation system, which can be used to filter for the optimal dosing time to increase drug efficacy and reduce side effects.

Precise regulation of inflammatory responses is essential for an efficient immune response during a pathogenic insult. Adrenal insufficiencies, such as hypocortisolemia, alter the regulation of proinflammatory cytokines and alter their levels. The reduction in the plasma cortisol response could be due to several biological factors, including transcortin deficiency, cortisol clearance via reductase activity, GR-mediated negative feedback on the HPA axis, or adrenal injury/atrophy. Long-term exposure to work-life stressors repeatedly activates the HPA axis, leading to a reduced cortisol awakening response due to blunted HPA activation (Siegrist & Li, 2017; Liao, Brunner & Kumari, 2013; Oosterholt et al., 2015). Prolonged hypocortisolemia contributes to a range of systemic changes that lead to chronic inflammation and multi-organ dysfunction. In accordance with previous findings, we simulated the hypocortisolemic condition by reducing the negative feedback sensitivity of glucocorticoid receptors (*Ki_GR1* from 1.2 to 0.6 nM/ml) in the HPA axis. That results in decreased cortisol expression and a corresponding increase in pro-inflammatory responses because of disrupted regulatory signals. In contrast, low-grade inflammation (LGI) can increase IL-6 expression by elevating basal LPS levels without perturbing cortisol levels (Rorato et al., 2017; Rosendo-Silva et al., 2023). High doses of acute LPS strongly stimulate the HPA axis to release cortisol, while very low basal LPS levels (indicating low-grade inflammation) bypass the HPA activation. This leads to a significant rise in circulating IL-6 levels, as seen during disease simulation, with no change in cortisol levels. Factors such as dysbiosis, metaflammation, ongoing infections, and disrupted sleep cycles can often lead to alterations in the baseline levels of LPS in the body. That contribute to the development along with the progression of chronic inflammatory and autoimmune diseases, including rheumatoid arthritis, cardiovascular disease, metabolic disorders, and NAFLD, as well as T2DM (Rosendo-Silva et al., 2023; Rorato et al., 2017; Cifuentes et al., 2025). LGI state was induced in the model by altering the basal LPS expression level, resulting in increased pro-inflammatory cytokine levels without altering cortisol levels. The simulated results for both conditions replicated the reported effects on pro-inflammation and cortisol responses,

highlighting the potential of our model to recapitulate the disease state in line with observed disease physiology.

The effects of Dex administration at different times of day in the perturbed state of the model, as represented by the PRC analysis (**Figure 4A & 5A**), suggested that morning dosing of Dex for hypocortisolemia (~5-7 AM) and LGI (~8-10 AM) would restore normalcy. The TRA-recommended dosing schedule was determined by the levels of Dex and cortisol at specific times, which effectively realign circadian rhythm (both amplitude and phase) in peripheral factors. As in the case of hypocortisolemia, the cortisol level remained low in the morning and was efficiently regulated by an early-morning dose of Dex without further dysregulation of circadian rhythms. A glucocorticoid administration study in patients with Rheumatoid arthritis (RA) also reported that early morning (~ 4-6 AM) is the best time for glucocorticoid administration to overcome adrenal insufficiency and suppress overstimulated pro-inflammatory cytokines (IL6) (Cutolo, 2016; Paolino, Cutolo & Pizzorni, 2017; Kirwan et al., 2010). In the LGI state, cortisol levels remain unperturbed, suggesting mid-morning Dex dosing to reset pro-inflammatory responses. Also, previous studies have reported the efficiency of Dex at a lower dose in the morning hours for the treatment of immune dysregulation in patients (Cutolo, 2016; SMOLENSKY, 2001). The current results indicate that different disease states reflect distinct circadian physiology, and accordingly, glucocorticoid treatment shifts to reset systemic dysregulation and restore normalcy. The study illustrates TRA's potential to identify the best time for glucocorticoid delivery by analyzing dose concentration, phase, and amplitude shifts in the model variables during the disease state.

The computational model-based TRA has the potential to develop NN models to predict optimal chronotherapy. The NN models can be trained to capture the intrinsic patterns of these features in disease settings, potentially helping to predict the optimal timing of glucocorticoid/drug administration (Hesse, Nelson & Relógio, 2024; Sulli et al., 2018). It is difficult to get the clinical data for circadian profiles for HPA-inflammation variables with all possible different combinations of phase and amplitude perturbations in the clinical or experimental setting. In addition, there are insufficient clinical chronotherapeutic studies on glucocorticoids across different diseases, making it difficult to train computational models for chronotherapeutic research. Moreover, the data on chronotherapy for such cases needs to be matched to be able to generate treatment labels for the prediction, which would be another limitation in data availability because of ethical concerns. Therefore, availability of the relevant data for chronotherapy becomes a limitation. Whereas, given a mechanistic model, all possible variations

in the circadian phase and amplitude combinations can be simulated to represent abnormal circadian rhythms. These simulated profiles can further be used in treatment response analysis for each scenario to identify optimal chronotherapy. Synthetic data generation helps by augmenting the lack of circadian data in chronotherapeutic settings (Hesse et al., 2020; Hesse, Nelson & Relógio, 2024). The MM analysis generates human-specific circadian profiles of peripheral and regulatory variables in both normal and disease states, which may facilitate the chronotherapeutic study of drug PK-PD on altered physiology. We used the HPA-Inflammation model to generate synthetic data by recording the circadian features (amplitude and phase differences) of cortisol and IL6 across different sets of perturbed parameters (representing multiple perturbations in different biological properties of the HPA-Inflammation model). Further, the NN model demonstrated higher accuracy, specificity, and sensitivity in predicting the time window for Dex (~0.5 mg) administration during both training and testing, leveraging learning from synthetic data. Together, these results indicate the strong discriminative capacity of the NN model, with good sensitivity and specificity in classifying test data.

Based on the perturbed circadian profile of cortisol, we considered eight scenarios of CR dysregulation that may lead to distinct disease physiologies, as tabulated in **Table 1**. The two selected cortisol profiles, viz., increased amplitude with phase advance in MDD and phase delay with no change in amplitude in SAD, were simulated by perturbing the HPA module parameters. The MDD clinical studies reported increased cortisol expression with advanced zeniths, reflecting consistent hyperactivity of the HPA axis and dysregulated GR feedback activity (Neigh & Nemeroff, 2006). The parameters for cortisol synthesis rate (*V_cort*) and GR negative feedback (*n1*) sensitivity control the circadian profile of cortisol; hence, increasing these parameters will replicate the reported cortisol expression profile (i.e., increased expression along with phase advancement) in the MDD condition (Li et al., 2022; Alesci et al., 2005). The increased n1 leads to a shift (advancement of ~1.5 hr) in the expression of GR nuclear fraction, which causes downregulation of cortisol expression at the normal peak timing (~ 8:30 AM). This shift in negative regulation of cortisol expression induces a zenith-phase advance (~1.7 hr). The perturbed GRn responses lead to inefficient regulation of proinflammatory cytokines (such as IL6), resulting in increased levels. The NN model predicts a morning window (8-9 AM) for Dex (~0.25 mg) administration, as per the observed circadian profiles of cortisol and IL6 in the MDD state. That was further supported by a significant correction in the circadian profile of cortisol and IL-6.

SAD is characterized by alterations in HPA axis activity, leading to changes in the cortisol circadian profile. Clinical studies comparing individuals with SAD to controls indicate a significant delay (~1.5-2 hr) in the cortisol nadir, with no significant changes in overall cortisol secretion (Oren et al., 1996; Thorn et al., 2011). In this model, the GR nuclear recycling rate (*kre*) and the GR negative feedback on GR mRNA synthesis (*km_GR1*) were decreased to simulate the altered circadian cortisol pattern observed in SAD. The decrease in *kre* and *km_GR1* enhances negative GR feedback and limits the replenishment of the nuclear GR fraction, ultimately lowering overall cytoplasmic GR protein levels. This causes HPA axis dysregulation and delays the cortisol response phase. Supporting our findings, fewer studies have documented GR regulatory issues caused by disrupted molecular processes such as GR mRNA synthesis, protein translation, and nuclear GR recycling in depression and affective disorders (Neigh & Nemeroff, 2006; Anacker et al., 2011). The NN model predicted a timing window (2-4 AM) for administering a Dex dose (~0.43 mg), which resulted in significant corrections in the cortisol and IL-6 profiles. This indicates that, at certain times of day within a given SAD profile, a dose of Dex may promote the restoration of normal circadian physiology.

Model Strengths and Limitations:

The proposed modifications to the HPA-Inflammation ODEs explicitly reflect the 24-hour processing of system variables, grounded in underlying mechanistic cross-talks and feedback mechanisms. The calibrated model showed close agreement with reported experimental data and observed human physiology, enabling the HPA-Inflammation model to reproduce system behaviors (at the expression and response levels) in both normal and perturbed (disease) states. In addition to its physiological relevance, the study proposed a TRA method to identify the optimal time window for Dex dosing, based on dexamethasone PK-PD and the disease circadian profile (phase and amplitude) of state variables. This is the first study to introduce a neural network framework for predicting the optimal timing for Dex dosing, using model-derived TRA data. This study proposes the TRA for glucocorticoid chronotherapy and its potential to develop AI tools for managing glucocorticoid dosing. We could ideally generate a large amount of simulated data, but we have restricted it to a range to illustrate a proof of concept on how mechanistic models can be used to generate simulated data (that would mimic n-number of clinical trials) and test treatment effects to inform optimal chronotherapy. Hence we feel that in the chronomedicine settings, such an approach has its value informing treatment decisions or generating clinically testable hypotheses on chronotherapy. Although the model was validated against trends in circadian physiology,

it needs clinical and chronotherapeutic validation in animal studies to improve its generalizability. That helps with more stringent regularization to estimate the parameters and reduce the chances of overfitting. Also, required to consider additional processes, such as zeitgebers (e.g., feeding habit), the peripheral clock system (e.g., Clock/Bmal1 and Per/Cry), the metabolic system (e.g., plasma macronutrient, AMP/ATP levels), and regulatory hormones (e.g., melatonin, serotonin) that significantly affect the cortisol signaling and immune system.

Conclusion:

In conclusion, our study provides a mathematical model of the HPA-Inflammation system that replicates human circadian physiology. The model considers the complex circadian regulation of the immune system by glucocorticoids and helps examine how cortisol imbalance affects the 24-hour expression of inflammatory cytokines. The study also introduces model-based treatment-response analysis to examine the time-dependent effects of exogenous glucocorticoids (Dex) on cortisol and the immune system. The analysis highlights the potential of TRA to identify the optimal timing for Dex dosing in diseased conditions. We also proposed steps for generating synthetic data and NN models for chronotherapeutic predictions. This would be the first study to explain the use of mode-based TRA to develop a protocol for chronotherapeutic dosing of Dex, informed by observed circadian physiology. Overall, we emphasize the importance of chronotherapy in resetting circadian dysregulation of cortisol by adjusting the timing of external glucocorticoid administration.

CRediT authorship contribution statement

Samayaditya Singh: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Conceptualization. **Pramod Rajaram Somvanshi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

Funding

This study is financially supported by the DBT- Junior Research Fellowship Programme (DBT/2021-22/UOH/1640).

Declaration of competing interest

The authors declare that they have no known competing financial/personal interests.

Acknowledgements

The authors acknowledge financial support from the DBT Junior Research Fellowship Program (DBT/2021-22/UOH/1640) and the University of Hyderabad for providing research space.

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