Published in final edited form as:

Chronobiol Int. 2020 February ; 37(2): 281–296. doi:10.1080/07420528.2019.1660357.

Modeling inter-sex and inter-individual variability in response to chronopharmacological administration of synthetic glucocorticoids

Megerle L. Scherholz^{*,1}, Rohit T. Rao^{*,1}, Ioannis P. Androulakis^{1,2,3}

¹Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, 98 Brett Road, Piscataway, NJ 08854

²Department of Biomedical Engineering, Rutgers The State University of New Jersey, 599 Taylor Road, Piscataway, NJ 08854

Abstract

Endogenous glucocorticoids have diverse physiological effects and are important regulators of metabolism, immunity, cardiovascular function, musculoskeletal health and central nervous system activity. Synthetic glucocorticoids have received widespread attention for their potent anti-inflammatory activity and have become an important class of drugs used to augment endogenous glucocorticoid activity for the treatment of a host of chronic inflammatory conditions. Chronic use of synthetic glucocorticoids is associated with a number of adverse effects as a result of the persistent dysregulation of glucocorticoid sensitive pathways. A failure to consider the pronounced circadian rhythmicity of endogenous glucocorticoids can result in either supraphysiological glucocorticoid exposure or severe suppression of endogenous glucocorticoid secretion, and is thought to be a causal factor in the incidence of adverse effects during chronic glucocorticoid therapy. Furthermore, given that synthetic glucocorticoids have potent feedback effects on the hypothalamic-pituitary-adrenal (HPA) axis, physiological factors which can give rise to individual variability in HPA axis activity such as sex, age, and disease state might also have substantial implications for therapy. We use a semi-mechanistic mathematical model of the rodent HPA axis to study how putative sex differences and individual variability in HPA axis regulation can influence the effects of long-term synthetic exposure on endogenous glucocorticoid circadian rhythms. Model simulations suggest that for the same drug exposure, simulated females exhibit less endogenous suppression than males considering differences in adrenal sensitivity and negative feedback to the hypothalamus and pituitary. Simulations reveal that homeostatic regulatory variability and chronic stress-induced regulatory adaptations in the HPA axis network can result in substantial differences in the effects of synthetic exposure on the circadian rhythm of endogenous glucocorticoids. In general, our results provide insight into how the dosage and exposure profile of synthetic glucocorticoids could be manipulated in a personalized manner to preserve the circadian dynamics of endogenous glucocorticoids during chronic therapy, thus potentially minimizing the incidence of adverse effects associated with long-term use of glucocorticoids.

³Correspondence: I.P. Androulakis, 599 Taylor Road, Biomedical Engineering Department, Rutgers University, Piscataway, NJ 08854, yannis@soe.rutgers.edu, tel: 848-445-6561, fax: 732-445-3753.

*These authors contributed equally to this work.

2 Introduction

Cortisol is a cholesterol-derived hormone and the primary glucocorticoid in humans that is essential to the regulation of metabolic, anti-inflammatory, immunosuppressive and cognitive processes (Arlt & Stewart, 2005; Fietta & Delsante, 2009). Its synthesis is controlled by the hypothalamic-pituitary-adrenal (HPA) axis, which along with the sympathetic nervous system, constitutes the primary stress response mechanism. Secretion of cortisol from the adrenal glands follows a distinct circadian variation with peak activity in the morning hours in humans, early in the active period (Spiga, Walker et al., 2014). Maintenance of cortisol circadian rhythmicity is essential to host survival, enabling the coordination of glucocorticoid activity in peripheral tissues for homeostasis (Smith & Vale, 2006; Nicolaides, Charmandari et al., 2017). The HPA axis is activated in response to emotional and physical stress, leading to increased cortisol secretion, which along with several other mediators, enables an adaptation of the body and brain (McEwen & Kalia, 2010). Homeostasis is thereby maintained by altering the underlying regulatory properties of the system in response to such stressors, a process referred to as allostasis (Juster, McEwen et al., 2010). While successful adaptation maintains normal physiological functioning, allostatic overload is often marked by pathophysiology (Karatsoreos & McEwen, 2011). Glucocorticoid receptors play an important role in mediating glucocorticoid action, and dysregulation of these regulators can influence the transition from allostasis to allostatic overload (Karatsoreos & McEwen, 2011; de Kloet, 2014).

Given the functional role of endogenous cortisol in immunity and pain management, synthetic glucocorticoids have become an important class of drugs used to augment endogenous activity for the treatment of a host of chronic inflammatory and immune conditions such as asthma, rheumatoid arthritis, and Crohn's disease (Buttgereit, Burmester et al., 2005; Liu, Ahmet et al., 2013). Unfortunately, chronic use is often associated with serious adverse effects as a result of disruption of various pathways involved in regular physiological functioning associated with glucocorticoid concentrations outside the normal physiological range. Wide spread adverse effects arise from the pleiotropic influence of glucocorticoids on several tissues including the liver, skeletal muscle, adipose tissue, and pancreas (Fardet & Feve, 2014). As such drug development strives to identify novel dosing regimens that minimize disruption of endogenous cortisol activity, yet preserve the pharmacological benefits of chronic synthetic glucocorticoid therapy (Cutolo, 2016). Several studies have emphasized the advantages of chronopharmacological dosing regimens which minimize the incidence of HPA axis disruption by carefully timing drug administration with endogenous cortisol secretion activity (Alten, Doring et al., 2010; Haus, Sackett-Lundeen et al., 2012; Alten & Wiebe, 2015; Beltrametti, Ianniello et al., 2016; Ravindran & Choy, 2016). However, physiological factors, such as chronotype, sex, age, race or disease state, lead to considerable variability in the pharmacokinetics of synthetic glucocorticoids and in the underlying regulation and responsiveness of the HPA axis (Cutolo, Villaggio et al., 2005; Chrousos, 2010; Kouri, Olkkonen et al., 2013; Hartmanshenn, Scherholz et al., 2016; Ebner & Singewald, 2017; Oster, Challet et al., 2017). Clinical studies have shown that the duration and extent of cortisol suppression is highly variable across patient subgroups (Fisher, Ludwig et al., 1992; Lew, Ludwig et al., 1993; Yehuda, Halligan et al., 2002).

Accounting for such differences in drug exposure and response due to inter-individual differences provides another opportunity to tailor glucocorticoid therapies to improve patient safety (Crown & Lightman, 2005). Furthermore, allostatic regulatory adaptations might result in an altered pharmacodynamic response to synthetic glucocorticoids, which could be clinically meaningful in certain populations. For instance, studies suggest that exposure to psychophysiological stress can alter drug metabolism, pharmacokinetics and pharmacodynamics (Peng & Cheung, 2011; Konstandi, 2013). The importance of such factors is increasingly acknowledged in clinical situations where the levels of endogenous signaling molecules are pharmacologically manipulated, e.g. glucose and insulin concentrations in diabetics (Gonder-Frederick, Grabman et al., 2016). Furthermore, studies have found evidence for altered feedback properties of the HPA axis in stress-related psychiatric disorders such as depression and post-traumatic stress disorder (PTSD). In the case of glucocorticoids, Juruena et al. found different responses to dexamethasone and prednisolone suppression of endogenous HPA activity in the same cohort of depressed patients, suggesting an alteration in drug-specific feedback properties (Juruena, Cleare et al., 2006). Additionally, Yehuda and Seckl have hypothesized that post-traumatic stress disorder (PTSD) is associated with an increase in the negative feedback sensitivity in the HPA axis as well as altered glucocorticoid metabolism (Yehuda & Seckl, 2011). Thus, optimization of glucocorticoid therapy becomes a matter of establishing the right administration time and dose in the target population of interest.

Efforts to establish the relationship between dose and HPA axis suppression has been hindered by this inherent variability in regulatory mechanisms and the complexity of glucocorticoid activity due to the crosstalk between the neuroendocrine and immune systems (Alten & Wiebe, 2015). Mechanistic modeling provides an opportunity to explore the behavior of this dynamic network and to help identify the potential mechanisms leading to marked differences in clinical responses across individuals or patient subgroups. Previously, a semi-mechanistic pharmacokinetic and pharmacodynamic (PKPD) model was used to demonstrate how chronopharmacological dosing can improve patient safety and maintain pharmacological benefits of long-term synthetic glucocorticoid therapy (Rao, Scherholz et al., 2018). These simulations revealed how the circadian features of the endogenous cortisol rhythm, which is critical to host survival and normal physiological function, can be preserved by careful timing of administration and how the pharmacological exposure profile may be manipulated to induce the optimal therapeutic effect. The goal of the current study is to extend this earlier work to male and female populations to elucidate how long-term chronopharmacological dosing of synthetic glucocorticoid influences endogenous glucocorticoid activity and how the dose-response relationship is linked to the underlying regulatory traits of individuals within these populations. Within the context of personalized medicine, this study emphasizes how delivering the ‘right dose’ at the ‘right time’ for the ‘right population’ can minimize the impact to normal biological functioning using our semi-mechanistic PKPD model to simulate basal and high stress conditions.

3 Approach

3.1 Description of the HPA axis model

A nocturnal rat model accounting for the stimulatory and negative feedback mechanisms of the HPA axis was previously established by Rao et al. (Rao & Androulakis, 2017a) to match the endogenous corticosterone rhythmicity in a population of male and proestrous female rats under nominal and high stress conditions. Female rats in the proestrous phase of the estrous cycle, when estrogen levels are the highest, exhibit the most pronounced circadian rhythm in corticosterone compared to other phases of the estrous cycle. This model was extended to account for synthetic glucocorticoid administration to study differences in the response to treatment in male and female populations. Briefly, the primary mediators of the HPA axis, CRH, ACTH and corticosterone (CORT) are described by nonlinear ordinary differential equations (Equations 1–3). The model accounts for the pharmacodynamics of the bound cortisol-receptor complex (Equations 4–7) by describing the following processes (1) transcription of GR mRNA, (2) translation of GR mRNA to protein, (3) binding of cortisol to cytoplasmic GR GR_{bound} , (4) the reversible translocation of the bound receptor complex to the nucleus [$GR_{bound}(N)$] and finally (5), the inhibitory activity of the hormone-receptor complex on GR mRNA expression and secretion of CRH and ACTH from the hypothalamus and pituitary glands, respectively.

A schematic of the model structure is shown in Figure 1 where the key underlying regulatory properties that were adjusted to reflect male and female physiologies under nominal and stressed conditions are emphasized. The individual regulatory properties of the male and female populations were previously identified by Sobol sampling (Rao & Androulakis, 2017a). Briefly, parameter sets were accepted when the simulated corticosterone profiles matched experimental data within ± 1 standard deviation of the scaled experimental cosinor parameters obtained by the original animal study. Three parameters were necessary to describe the male and female corticosterone rhythms: adrenal sensitivity of corticosterone to ACTH (k_{p3}), the glucocorticoid receptor-mediated negative feedback to the hypothalamus (K_{p1}), and the glucocorticoid receptor-mediated negative feedback to the pituitary gland (K_{p2}). All other HPA axis parameters were common to both male and female rats. High stress was simulated by increasing the zero-order CRH synthesis rate constant (k_{p1}) to a chronically stressed value, a method previously used by Siriam et al. (Siriam, Rodriguez-Fernandez et al., 2012; Rao & Androulakis, 2017b). The model input parameters are given in Supplementary Table 1.

Furthermore, the entraining influence of light on the HPA axis via the suprachiasmatic nucleus (SCN) was considered (Equations 8–13). Light is considered to stimulate the hypothalamic suprachiasmatic nuclei (SCN), which induces vasopressin and subsequently influences the HPA axis. Vasopressin is a hormone synthesized in the hypothalamus and serves as an output signal of the SCN with secretion following a circadian rhythm (Kalsbeek, Fliers et al., 2010). Vasopressin induces HPA axis activity in diurnal species and inhibits activity in nocturnal species (Kalsbeek, Verhagen et al., 2008). The model was entrained to a 14-hour light/10-hour dark lighting schedule to stay consistent with the original animal study from which the model was calibrated (Rao & Androulakis, 2017a).

The onset of light-induced effects in the HPA axis ($light_{effect}$) was delayed by 1 to 2 hours after the start of light exposure using a series of transit compartments (Jung, Khalsa et al., 2010). Light exposure was modeled using a step function whereas a Hill function described the phototransduction pathways (Equations 1–6). Simulations were performed in MATLAB® 2017b using MATLAB's built-in ode45 routine.

3.1.1 HPA Axis Mediators

$$\frac{dCRH}{dt} = \frac{k_{p1} \cdot K_{p1}}{K_{p1} + GR_{bound}(N)} - V_{d1} \cdot \frac{CRH \cdot \left(1 + \frac{light_{effect}}{1 + light_{effect}}\right)}{K_{d1} + CRH} \quad 1$$

$$\frac{dACTH}{dt} = \frac{k_{p2} \cdot K_{p2} CRH}{K_{p2} + GR_{bound}(N)} - V_{d2} \cdot \frac{ACTH}{K_{d2} + ACTH} \quad 2$$

$$\frac{dCORT}{dt} = k_{p3} \cdot ACTH - V_{d3} \cdot \frac{CORT}{K_{d3} + CORT} \quad 3$$

3.1.2 Glucocorticoid Receptor Pharmacodynamics

$$\frac{dGR_{mRNA}}{dt} = k_{synGRm} \cdot \left(1 - \frac{GR_{bound}(N)}{IC_{50GRm} + GR_{bound}(N)}\right) - k_{deg} \cdot GR_{mRNA} \quad 4$$

$$\frac{dGR}{dt} = k_{syn,GR} \cdot GR_{mRNA} + r_f \cdot k_{re} \cdot GR_{bound}(N) - k_{on} \cdot (CORT) \cdot GR - k_{deg,GR} \cdot GR \quad 5$$

$$\frac{dGR_{bound}}{dt} = k_{on} \cdot (CORT) \cdot GR - k_T \cdot GR_{bound} \quad 6$$

$$\frac{dGR_{bound}(N)}{dt} = k_T \cdot R - r_f \cdot k_{re} \cdot GR_{bound}(N) \quad 7$$

3.1.3 Light-induced Effects

$$light = \begin{cases} 1 & 7:00 \leq t \leq 21:00 \\ 0 & 21:00 \leq t \leq 7:00 \end{cases} \quad 8$$

$$\frac{dlight_{TCsynth1}}{dt} = k_t(light - light_{TCsynth1}) \quad 9$$

$$\frac{d\text{light}_{TC\text{synth}i}}{dt} = k_i(\text{light}_{TC\text{synth}i-1} - \text{light}_{TC\text{synth}i}), i = \{1,2,3\} \quad 10$$

$$\frac{d\text{light}_{TC\text{deg}1}}{dt} = k_i(\text{light}_{\text{deg}} - \text{light}_{TC\text{deg}1}) \quad 11$$

$$\frac{d\text{light}_{TC\text{deg}i}}{dt} = k_i(\text{light}_{TC\text{deg}i-1} - \text{light}_{TC\text{deg}i}); i = \{1,2,3\} \quad 12$$

$$\frac{d\text{light}_{\text{effect}}}{dt} = k_{us} \frac{\text{light}_{TC\text{synth}i}^n}{K_{M,us}^n + \text{light}_{TC\text{synth}i}^n} - k_{\text{deg},us} \text{light}_{\text{effect}} \left(1 + k_{\text{eff}} \text{light}_{TC\text{deg}i} \right) \quad 13$$

3.2 Description of pharmacokinetic models for synthetic GC administration

The pharmacokinetic models described in Rao et al. (Rao, Scherholz et al., 2018) were used to in this study for once-a-day dosing of intra-venous (IV) or oral synthetic glucocorticoids. Equation 14 describes the disappearance of drug from systemic circulation following IV administration. Equation 15 describes the absorption of drug from the gastrointestinal tract following oral administration. The appearance in systemic circulation for a fast-acting oral is given by Equation 16 and for a slower-acting oral using a series of five transit compartments (TC) in Equations 17–19.

$$\frac{dGC}{dt} = -k_E \cdot GC \quad 14$$

$$\frac{dGC_{GIT}}{dt} = -k_a \cdot GC_{GIT} \quad 15$$

$$\frac{dGC}{dt} = k_a \cdot GC_{GIT} - k_E \cdot GC \quad 16$$

$$dGC_{TC1} = k_{at,1} \cdot (GC_{GIT} - GC_{TC1}) \quad 17$$

$$dGC_{TC,i} = k_{at,i} \cdot (GC_{TC,i-1} - GC_{TC,i}), i = 2,3,4,5 \quad 18$$

$$\frac{dGC}{dt} = k_a \cdot GC_{TC5} - k_E \cdot GC \quad 19$$

3.3 Glucocorticoid Receptor Pharmacodynamics after GC Dosing

The glucocorticoid receptor dynamics were modified to consider the binding of synthetic glucocorticoids to the glucocorticoid receptor in addition to endogenous cortisol.

$$\frac{dGR}{dt} = k_{syn,GR} \cdot GR_{mRNA} + r_f \cdot k_{re} \cdot GR_{bound}(N) - k_{on} \cdot (CORT + GC) \cdot GR - k_{deg,GR} \cdot GR \quad 20$$

$$\frac{dDR}{dt} = k_{on} \cdot (CORT + GC) \cdot GR - k_T \cdot GR_{bound} \quad 21$$

3.4 Dosing Experiments

The chronopharmacology of a daily dose of synthetic glucocorticoid was simulated by varying the administration time, dosing strength, and administration route. Administration time was varied by 4-hour intervals (12:00 AM, 4:00 AM, 8:00 AM, etc.) throughout the simulated day using a dosing interval of 24 hours for once-a-day administration. Doses of 0.1x, 1x, and 10x were evaluated to understand how the strength of negative feedback via the glucocorticoid receptor dynamics influenced pharmacodynamic differences between individuals and sexes. Changes in amplitude and acrophase of the endogenous cortisol rhythm were used to quantify disruption of the HPA axis relative to the baseline conditions without treatment. Amplitude and acrophase were determined when the new stable oscillatory state was reached after chronic once-daily dosing. At that point, we hypothesized that the endogenous corticosterone rhythms adopted new characteristics (amplitude and phase) reflecting the effects of chronic synthetic glucocorticoid treatment. The relative change in amplitude is calculated by Equation 22, where baseline refers to the corticosterone amplitude prior to initiating treatment.

$$Relative\ Amplitude\ (\%) = \frac{Amplitude_{treatment} - Amplitude_{baseline}}{Amplitude_{baseline}} \times 100\% \quad 22$$

4 Results

4.1 Male and female subpopulations used in dosing experiments

For the dosing experiments, an equal number of male and (proestrous) female *in silico* individuals were uniformly sampled from the male and female parameter subspaces previously identified for the three sex-dependent parameters in Rao et al. (Rao & Androulakis, 2017a) [Supplementary Figure 1]. Basal differences in the circadian rhythmicity of endogenous corticosterone are shown in Figure 2, showing that sex-dependent parameterization of the HPA axis model led to unique male and female phenotypes.

4.2 Influence of once-daily chronopharmacological dosing on corticosterone rhythmicity in male and female populations

The pharmacokinetic profiles for synthetic glucocorticoids administered by IV and oral administration are given in Figure 3 for the nominal dose of 1x. Oral administration resulted in a $C_{\max} \approx 40\%$ of the initial dose and $t_{\max} = 2.8$ hours for the faster-acting oral GC and a $C_{\max} \approx 30\%$ of the initial dose and $t_{\max} = 8.4$ hours for the slower-acting oral GC. To ensure the same total exposure across dosage forms for a given dose, the area-under-the-curve (AUC) was maintained across dosage forms such that $AUC_{IV} \approx AUC_{\text{fast}} \approx AUC_{\text{slow}}$.

Following once-a-day administration of synthetic glucocorticoids, the rhythmicity of endogenous corticosterone evolved to new stable oscillations as shown in Figure 4. The amplitude and acrophase of the modified rhythm depended on the time at which the drug was administered, and the dosage form as shown in Figure 5 for males and females. The slower-releasing oral dosage form showed an advance in the dosing times needed to produce a similar change in the amplitude as the IV and fast-releasing oral dosage forms. This difference in time-of-day dependence was attributed to the extended absorption time of the slow-releasing synthetic glucocorticoids before the maximum concentration or effect ($t_{\max} = 8.4$ hours) was achieved. Considering inter-population differences, males had a remarkably greater time-of-day dependence than females, showing larger differences in the suppressive and inductive effects of synthetic glucocorticoids across dosing times.

Once-daily dosing near the normal daily minima of corticosterone levels (~8:00 AM) produced similar modifications to endogenous activity across sexes whereas greater sex differences in response to treatment were observed when dosing near the normal daily maxima (~9:00 PM). In the male population, maximal suppression occurred when drug was administered daily at 4:00 AM by bolus injection, 12:00 AM for the fast-releasing oral dose, and 8:00 PM for the slow-releasing oral dose. In the female population, maximal suppression occurred at administration times of 4:00 AM for the bolus injection and fast-release oral dose, and 12:00 AM for the slow-releasing oral dose. Conversely, induction of the endogenous corticosterone secretion was observed at select dosing times, which largely occurred when drug was administered during the simulated day, or the inactive phase of the nocturnal species. While the time-of-day dependence was qualitatively similar across sexes for the mean phase of the modified rhythms, the male population showed greater inter-individual variability when dosing during the active period (simulated night for nocturnal animals) for all three dosage forms. The female population revealed the inter-individual variability to be more dependent on the dosage form in which maximum inter-individual variability was not restricted to only the simulated active period. Considering amplitude and phase changes, endogenous corticosterone activity would likely be preserved when synthetic glucocorticoids were administered sometime near the start of the simulated daytime (or the start of the inactivate phase in nocturnal species) in both male and female populations, although additional dosing times would be needed to confirm the exact timing.

The circadian characteristics of the modified corticosterone rhythm relative to the individual's sex-dependent regulatory parameters are shown in Figure 6 following a once-daily (bolus) injection at 4:00 AM, the dosing time associated with maximal suppression in both male and female populations, and 4:00 PM, the dosing time associated with maximal

induction in both populations. While stronger receptor-mediated feedback to the pituitary gland (K_{p2}) and increased adrenal sensitivity (k_{p3}) in male rats tended to result in greater disruption of the HPA axis following a 4:00 AM injection, stronger negative feedback to the pituitary gland and increased adrenal sensitivity in females had the opposite effect. Furthermore, females showed an inverse relationship between the strength of receptor-mediated feedback to the hypothalamus and the extent of HPA axis disruption. Despite noticeable sex differences in HPA axis disruption with 4:00 AM injections, similar behavior was observed in both populations following 4:00 PM injections, where stronger negative feedback to the pituitary gland and greater adrenal sensitivity led to higher amplitudes for the modified corticosterone rhythms. The variability in the regulatory mechanisms underlying HPA axis activity produced opposing trends for 4:00 AM injections, but qualitatively behavior after 4:00 PM injections, although larger modifications were observed for the male population at both administration times.

The circadian characteristics of the modified corticosterone rhythm are shown in Supplementary Figure 2 following a once-daily slow-releasing oral dose at 8:00 AM or 8:00 PM, which were the dosing times associated with maximal induction and suppression, respectively, in both the male and female populations. The relationship between once-daily dosing was similar across dosage forms for the administration times with maximal induction effects, that is 4:00 PM for the bolus injection and 8:00 AM for the slow-releasing oral dose. Dosing times with maximal suppressive effects (4:00 AM for the bolus injection and 8:00 PM for the slow-releasing oral dose) also revealed similarities across dosage forms in relationship to the three sex-dependent parameters. These observations demonstrate how the interplay between the underlying regulatory properties of the HPA axis and the timing of peak drug exposure dictates the response of an individual.

4.3 Influence of dosing strength on the corticosterone activity in *in silico* male and female rats

The amplitude of the modified corticosterone rhythm associated with the once-daily bolus injection that resulted in the greatest suppressive and inductive effects on the HPA axis are given in Figure 7 for dosing strengths of 0.1x, 1x, and 10x. The dose-response relationship showed significant differences between the male and female populations, suggesting that female rats were tolerant of higher doses of synthetic glucocorticoids and male populations were more sensitive to HPA axis disruption when differences in pharmacokinetics were not considered. In a similar manner, the slow releasing glucocorticoids had a greater suppressive effect in simulated males compared to simulated females [Supplementary Figure S3]. Interestingly, the slow release glucocorticoids had a stronger suppressive effect and reduced inductive effect in both populations compared to the bolus injection.

4.4 Strength of negative feedback to hypothalamus and pituitary glands

Greater levels of translocated glucocorticoid receptor, $GR_{bound}(N)$, bound to both endogenous corticosterone, are observed in females compared to males prior to dosing as shown in Figure 8. Despite the higher levels of bound receptors, the corticosterone levels were greater in simulated proestrous females, suggesting reduced negative feedback in the female population. This observation may in part explain why females show reduced HPA

axis disruption compared to males and are more tolerant to higher doses of synthetic glucocorticoids. In general, model simulations show that the time at which both maximal induction and maximal suppression occur are delayed by approximately 4 hours for the slow-releasing glucocorticoids in comparison to bolus administration. While the qualitative characteristics of the amplitude response to chronic administration are similar to those after the bolus injection, the extent of suppression is greater for the slow-releasing glucocorticoids than the bolus injection [Supplementary Figure S2–3].

4.5 Chronopharmacological dosing in chronically stressed individuals

Allostatic regulatory adaptations in the sex-dependent parameters enabled the HPA axis to maintain normal corticosterone levels in response to chronic stress (Rao & Androulakis, 2017a). The response to chronopharmacological dosing in these chronically stressed individuals was implemented based on our earlier work. (Rao & Androulakis, 2017a). The selected rats are shown in Supplementary Figure S4 and are well distributed across the male and female parameter subspaces previously identified for the three sex-dependent parameters under chronically stressed conditions.

Dosing in both normal and chronically stressed individuals resulted in similar time-dependent changes in the amplitude and acrophase of the modified corticosterone rhythm as shown in Figure 9. Interestingly, the response in chronically stressed individuals differed from normal conditions as the dose increased to 10x as shown in Figure 10. At the high dose, suppression was observed at all dosing times for the chronically stressed males while induction was observed in females. The response characteristics of the endogenous corticosterone rhythms for the fast-releasing and the slow-releasing oral dosage forms in the high stress simulated individuals were qualitatively similar to the response characteristics in the nominal case [Supplementary Figures 5–7]. Similar to the nominal case, the time at which maximal induction and suppression occur is delayed by ~4hr and the extent of suppression is greater upon exposure to slow-releasing glucocorticoids in comparison to when glucocorticoids are administered as a bolus injection.

5 Discussion

Using a semi-mechanistic representation of the stimulatory and negative feedback processes of the HPA axis, the responsiveness and adaptability of individuals to chronic chronopharmacological glucocorticoid treatment was studied by parameterizing the model to reflect basal differences in endogenous glucocorticoid activity in male and proestrous female populations. This approach enabled an understanding of population differences that explained in part, the susceptibility of some individuals to adverse effects which arise from disruption of endogenous glucocorticoid activity and revealed how chronic treatment may be tailored based on an individual's underlying regulatory behavior. While the circadian characteristics of the modified rhythms after dosing showed similar time-dependent trends across the populations, the magnitude of the observed changes were dependent on the regulatory traits that collectively defined an individual's basal HPA axis activity. The range of administration times in which the endogenous activity would be conserved was consistent across the male and female populations in these simulations, suggesting that the optimal

time of dosing may not require adjustment for sex. Interestingly, the greatest inter-individual variability was observed when the HPA axis was most susceptible to disruption with fewer differences across the population when the HPA axis was more robust to disruption, although the times at which this behavior occurred was dependent on the dosage form.

For the same drug exposure, females were more tolerable to treatment than males considering differences in adrenal sensitivity and negative feedback to the hypothalamus and pituitary glands, given that simulated females exhibited a lower maximal suppressive effect after GC exposure [Figure 7] and more pronounced basal GC rhythms despite having higher GC-receptor complex levels on average [Figure 8]. That is, a higher dose would be needed in females to produce the same response when only sex differences in the hypothesized regulatory mechanisms underlying the HPA axis were considered. By calibrating our mathematical model to proestrous female corticosterone rhythms we implicitly account for the effects of estrogen in the putative sex-specific parameters (K_{p1} , K_{p2} , k_{p3}). Studies in rodents have shown that estrogen and estrogen receptor (ER)- α specific agonists can dampen glucocorticoid-induced negative feedback in the HPA axis, by decreasing the sensitivity to the dexamethasone suppression test (Weiser & Handa, 2009). Moreover, even in humans, women in the luteal phase, when estrogen levels are high, exhibit lower sensitivity to the dexamethasone suppression test (Altemus, Redwine et al., 1997). In agreement with this observation, our simulations suggest that females are also less sensitive to endogenous glucocorticoid suppression upon chronic glucocorticoid administration due to weaker negative feedback and greater adrenal sensitivity.

An extension of this study would consider variability in pharmacokinetic parameters driving differences in drug exposure as well as receptor expression and activity, a scenario that better reflects clinical behavior. By accounting for inter-individual and inter-sex differences in both pharmacokinetics and pharmacodynamics, the putative mechanisms contributing to variability in the dose-exposure-response relationship would enable the separation of individuals based on sensitivity or resistance to glucocorticoid treatment, and further support the design of dosing regimens that result in minimal disruption of endogenous corticosterone activity or the ability to control hormone levels by carefully timing drug exposure.

Chronic-stress related psychiatric disorders like depression, anxiety and PTSD are thought to arise due to allostatic overload (Griffin, Charron et al., 2014). Several studies suggest that individuals presenting symptoms of PTSD and chronic anxiety exhibit lower glucocorticoid levels compared to those without such symptoms (Yehuda, Southwick et al., 1990; Yehuda & Seckl, 2011). These findings have led to the hypothesis that low-dose synthetic glucocorticoid administration might have therapeutic benefits in the management of PTSD symptoms (Yehuda & LeDoux, 2007; de Quervain & Margraf, 2008; Raglan, Schmidt et al., 2017). Supplementing low physiological levels with low-dose cortisol treatment was found to reduce symptom severity in PTSD without adverse effects, particularly by reducing avoidance symptoms and adverse memory retrieval, (Aerni, Traber et al., 2004; de Quervain & Margraf, 2008). These findings suggest that pharmacological manipulation of the allostatic mediators involved in stress-induced psychiatric disorders may provide therapeutic advantages while minimizing the risk of systemic adverse effects associated with synthetic glucocorticoid treatment. However, as discussed previously, prescription of such long-term

use of glucocorticoids is not without risk and has also been found to have long-lasting physical and psychological adverse effects. Simulations using our semi-mechanistic model under high stress conditions revealed how the time- dependent basal activity of the HPA axis may be optimally supplemented with synthetic glucocorticoids at specific dosing times to avoid the risk of further adrenal suppression even upon long-term usage. Model simulations suggest both individual and sex-specific differences in the sensitivity to suppression upon chronic glucocorticoid exposure can have implications for determining whether glucocorticoid use might be an appropriate therapy for individuals with chronic stress-related risk factors. Moreover, simulations reveal how select dosing times induced endogenous cortisol secretion which could purposefully be used to counteract the low physiological levels associated with PTSD symptomology, without explicitly altering the feedback and sensitivity characteristics of the HPA axis.

In general, the population simulations provided insight into how administration time and dose can be selected to maintain endogenous HPA axis activity in personalized manner as needed for successful hormone replacement or other disease states which result in HPA axis dysfunction given some understanding of the basal regulatory properties. Extending the model to consider additional population differences, such as variability in pharmacokinetic parameters, continues the transformation towards a more physiologically meaningful and mechanistic representation of synthetic glucocorticoid action *in silico*. One study reported an increased sensitivity to glucocorticoids in female subjects which was balanced by higher drug clearance, leading to similar overall response to methylprednisolone in male and female subjects (Czock, Keller et al., 2005). Another study suggested both inter-sex and inter-race differences in PKPD, but clinical implication of such differences was limited by the use of a single dose (Magee, Blum et al., 2001). Sex differences in response to glucocorticoid therapies are of particular interest given differences in the underlying regulatory mechanisms of the HPA axis as well as susceptibility to the inflammatory conditions often treated by this class of drugs, such as rheumatoid arthritis (Rao & Androulakis, 2017a). Therefore, future work would successfully incorporate both PK and PD differences for further optimization of chronopharmacological dosing regimens to comprehensively assess patient risk under the ‘right drug-right dose-right time’ paradigm within our integrated *in silico* framework.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge financial support from the National Institutes of Health under award number GM024211 and GM 131800. MLS is supported by a Bristol-Myers Squibb Doctoral Fellowship.

References

- Aerni A, Traber R, Hock C, Roozendaal B, Schelling G, Papassotiropoulos A, Nitsch RM, Schnyder U, de Quervain DJ. (2004). Low-dose cortisol for symptoms of posttraumatic stress disorder. *Am J Psychiatry*. 161:1488–1490. [PubMed: 15285979]

- Altemus M, Redwine L, Leong YM, Yoshikawa T, Yehuda R, Detera-Wadleigh S, Murphy DL. (1997). Reduced sensitivity to glucocorticoid feedback and reduced glucocorticoid receptor mRNA expression in the luteal phase of the menstrual cycle. *Neuropsychopharmacology*. 17:100–109. [PubMed: 9252985]
- Alten R, Doring G, Cutolo M, Gromnica-Ihle E, Witte S, Straub R, Buttgerit F. (2010). Hypothalamus-pituitary-adrenal axis function in patients with rheumatoid arthritis treated with nighttime-release prednisone. *J Rheumatol*. 37:2025–2031. [PubMed: 20682671]
- Alten R, Wiebe E. (2015). Hypothalamic-pituitary-adrenal axis function in patients with rheumatoid arthritis treated with different glucocorticoid approaches. *Neuroimmunomodulation*. 22:83–88. [PubMed: 25228310]
- Arlt W, Stewart PM. (2005). Adrenal corticosteroid biosynthesis, metabolism, and action. *Endocrinol Metab Clin North Am*. 34:293–313, viii. [PubMed: 15850843]
- Beltrametti SP, Ianniello A, Ricci C. (2016). Chronotherapy with low-dose modified-release prednisone for the management of rheumatoid arthritis: a review. *Ther Clin Risk Manag*. 12:1763–1776. [PubMed: 27920546]
- Buttgerit F, Burmester GR, Lipworth BJ. (2005). Optimised glucocorticoid therapy: the sharpening of an old spear. *Lancet*. 365:801–803. [PubMed: 15733723]
- Chrousos GP. (2010). Stress and sex versus immunity and inflammation. *Sci Signal*. 3:pe36. [PubMed: 20940425]
- Crown A, Lightman S. (2005). Why is the management of glucocorticoid deficiency still controversial: a review of the literature. *Clin Endocrinol (Oxf)*. 63:483–492. [PubMed: 16268798]
- Cutolo M (2016). Glucocorticoids and chronotherapy in rheumatoid arthritis. *RMD Open*. 2:e000203. [PubMed: 27042335]
- Cutolo M, Villaggio B, Otsa K, Aakre O, Sulli A, Serio B. (2005). Altered circadian rhythms in rheumatoid arthritis patients play a role in the disease's symptoms. *Autoimmunity Reviews*. 4:497–502. [PubMed: 16214085]
- Czock D, Keller F, Rasche FM, Haussler U. (2005). Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet*. 44:61–98. [PubMed: 15634032]
- de Kloet ER. (2014). From receptor balance to rational glucocorticoid therapy. *Endocrinology*. 155:2754–2769. [PubMed: 24828611]
- de Quervain DJ, Margraf J. (2008). Glucocorticoids for the treatment of post-traumatic stress disorder and phobias: a novel therapeutic approach. *Eur J Pharmacol*. 583:365–371. [PubMed: 18275950]
- Ebner K, Singewald N. (2017). Individual differences in stress susceptibility and stress inhibitory mechanisms. *Current Opinion in Behavioral Sciences*. 14:54–64.
- Fardet L, Feve B. (2014). Systemic glucocorticoid therapy: a review of its metabolic and cardiovascular adverse events. *Drugs*. 74:1731–1745. [PubMed: 25204470]
- Fietta P, Delsante G. (2009). Central nervous system effects of natural and synthetic glucocorticoids. *Psychiatry Clin Neurosci*. 63:613–622. [PubMed: 19788629]
- Fisher LE, Ludwig EA, Wald JA, Sloan RR, Middleton E Jr., Jusko WJ (1992). Pharmacokinetics and pharmacodynamics of methylprednisolone when administered at 8 am versus 4 pm. *Clin Pharmacol Ther*. 51:677–688. [PubMed: 1535301]
- Gonder-Frederick LA, Grabman JH, Kovatchev B, Brown SA, Patek S, Basu A, Pinsker JE, Kudva YC, Wakeman CA, Dassau E, Cobelli C, Zisser HC, Doyle FJ 3rd. (2016). Is Psychological Stress a Factor for Incorporation Into Future Closed-Loop Systems? *J Diabetes Sci Technol*. 10:640–646. [PubMed: 26969142]
- Griffin GD, Charron D, Al-Daccak R. (2014). Post-traumatic stress disorder: revisiting adrenergics, glucocorticoids, immune system effects and homeostasis. *Clin Transl Immunology*. 3:e27.
- Hartmanshenn C, Scherholz M, Androulakis IP. (2016). Physiologically-based pharmacokinetic models: approaches for enabling personalized medicine. *J Pharmacokinet Pharmacodyn*. 43:481–504. [PubMed: 27647273]
- Haus E, Sackett-Lundeen L, Smolensky MH. (2012). Rheumatoid arthritis: circadian rhythms in disease activity, signs and symptoms, and rationale for chronotherapy with corticosteroids and other medications. *Bull NYU Hosp Jt Dis*. 70 Suppl 1:3–10. [PubMed: 23259651]

- Jung CM, Khalsa SB, Scheer FA, Cajochen C, Lockley SW, Czeisler CA, Wright KP Jr. (2010). Acute effects of bright light exposure on cortisol levels. *J Biol Rhythms*. 25:208–216. [PubMed: 20484692]
- Juruena MF, Cleare AJ, Papadopoulos AS, Poon L, Lightman S, Pariante CM. (2006). Different responses to dexamethasone and prednisolone in the same depressed patients. *Psychopharmacology (Berl)*. 189:225–235. [PubMed: 17016711]
- Juster RP, McEwen BS, Lupien SJ. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev*. 35:2–16. [PubMed: 19822172]
- Kalsbeek A, Fliers E, Hofman MA, Swaab DF, Buijs RM. (2010). Vasopressin and the output of the hypothalamic biological clock. *J Neuroendocrinol*. 22:362–372. [PubMed: 20088910]
- Kalsbeek A, Verhagen LA, Schalij I, Foppen E, Saboureaux M, Bothorel B, Buijs RM, Pevet P. (2008). Opposite actions of hypothalamic vasopressin on circadian corticosterone rhythm in nocturnal versus diurnal species. *Eur J Neurosci*. 27:818–827. [PubMed: 18279365]
- Karatsoreos IN, McEwen BS. (2011). Psychobiological allostasis: resistance, resilience and vulnerability. *Trends Cogn Sci*. 15:576–584. [PubMed: 22078931]
- Konstandi M (2013). Psychophysiological stress: a significant parameter in drug pharmacokinetics. *Expert Opin Drug Metab Toxicol*. 9:1317–1334. [PubMed: 23834396]
- Kouri V-P, Olkkonen J, Kaivosoja E, Ainola M, Juhila J, Hovatta I, Kontinen YT, Mandelin J. (2013). Circadian Timekeeping Is Disturbed in Rheumatoid Arthritis at Molecular Level. *PLoS ONE*. 8.
- Lew KH, Ludwig EA, Milad MA, Donovan K, Middleton E Jr., Ferry JJ, Jusko WJ. (1993). Gender-based effects on methylprednisolone pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther*. 54:402–414. [PubMed: 8222483]
- Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A, Kim H. (2013). A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol*. 9:30. [PubMed: 23947590]
- Magee MH, Blum RA, Lates CD, Jusko WJ. (2001). Prednisolone pharmacokinetics and pharmacodynamics in relation to sex and race. *J Clin Pharmacol*. 41:1180–1194. [PubMed: 11697751]
- McEwen BS, Kalia M. (2010). The role of corticosteroids and stress in chronic pain conditions. *Metabolism*. 59 Suppl 1:S9–15. [PubMed: 20837196]
- Nicolaides NC, Charmandari E, Kino T, Chrousos GP. (2017). Stress-Related and Circadian Secretion and Target Tissue Actions of Glucocorticoids: Impact on Health. *Front Endocrinol (Lausanne)*. 8:70. [PubMed: 28503165]
- Oster H, Challet E, Ott V, Arvat E, Ronald de Kloet E, Dijk DJ, Lightman S, Vgontzas A, Van Cauter E. (2017). The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. *Endocr Rev*. 38:3–45. [PubMed: 27749086]
- Peng HT, Cheung B. (2011). A review of psychophysiological stressors on pharmacokinetics. *The Journal of Clinical Pharmacology*. 51:1499–1518. [PubMed: 21257802]
- Raglan GB, Schmidt LA, Schalkin J. (2017). The role of glucocorticoids and corticotropin-releasing hormone regulation on anxiety symptoms and response to treatment. *Endocr Connect*. 6:R1–R7. [PubMed: 28119322]
- Rao R, Androulakis IP. (2019). The physiological significance of the circadian dynamics of the HPA axis: Interplay between circadian rhythms, allostasis and stress resilience. *Hormones and behavior*. 110:77–89. [PubMed: 30862458]
- Rao RT, Androulakis IP. (2017a). Modeling the Sex Differences and Inter-individual Variability in the Activity of the Hypothalamic-Pituitary-Adrenal Axis. *Endocrinology*.
- Rao RT, Androulakis IP. (2017b). Modeling the Sex Differences and Interindividual Variability in the Activity of the Hypothalamic-Pituitary-Adrenal Axis. *Endocrinology*. 158:4017–4037. [PubMed: 28938475]
- Rao RT, Scherholz ML, Androulakis IP. (2018). Modeling the influence of chronopharmacological administration of synthetic glucocorticoids on the hypothalamic-pituitary-adrenal axis. *Chronobiol Int*. 1–18.

- Ravindran V, Choy E. (2016). Modified-release prednisone in rheumatoid arthritis: Rationale for chronotherapy, mechanistic considerations, and clinical implications. *Indian Journal of Rheumatology*. 11:216.
- Smith SM, Vale WW. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci*. 8:383–395. [PubMed: 17290797]
- Spiga F, Walker JJ, Terry JR, Lightman SL. (2014). HPA Axis-Rhythms In Terjung R (Eds). *Comprehensive Physiology*. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp. 1273–1298.
- Sriram K, Rodriguez-Fernandez M, Doyle FJ 3rd. (2012). Modeling cortisol dynamics in the neuroendocrine axis distinguishes normal, depression, and post-traumatic stress disorder (PTSD) in humans. *PLoS Comput Biol*. 8:e1002379. [PubMed: 22359492]
- Weiser MJ, Handa RJ. (2009). Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen receptor alpha within the hypothalamus. *Neuroscience*. 159:883–895. [PubMed: 19166915]
- Yehuda R, Halligan SL, Grossman R, Golier JA, Wong C. (2002). The cortisol and glucocorticoid receptor response to low dose dexamethasone administration in aging combat veterans and holocaust survivors with and without posttraumatic stress disorder. *Biol Psychiatry*. 52:393–403. [PubMed: 12242055]
- Yehuda R, LeDoux J. (2007). Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron*. 56:19–32. [PubMed: 17920012]
- Yehuda R, Seckl J. (2011). Minireview: Stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology*. 152:4496–4503. [PubMed: 21971152]
- Yehuda R, Southwick SM, Nussbaum G, Wahby V, Giller EL Jr., Mason JW. (1990). Low urinary cortisol excretion in patients with posttraumatic stress disorder. *J Nerv Ment Dis*. 178:366–369. [PubMed: 2348190]

Figure 1:

Model Schematic: A schematic of the model depicting the primary stimulatory and negative feedback arms of the hypothalamus-pituitary-adrenal (HPA) axis. Synthetic GCs are administered by either an injection directly into systemic circulation or by oral administration which accounts for the absorption of drug from the gastrointestinal tract. Appearance in systemic circulation following oral administration is indicated by the orange line. The regulatory parameters (K_{p1} , K_{p2} , and K_{p3}) that are necessary to reflect an individual's traits such as male and female physiology or the allostatic adaptation to external stressors are highlighted in red.

Figure 2:

Differences in basal HPA activity between male and female rat populations. The mean corticosterone profiles for the subpopulations of the male and female rats used in the dosing experiments are given in (A) and (B), respectively. The grey shaded areas represent the time at which the system is not exposed to light. Corticosterone concentration is given in arbitrary units (a.u.). The blue and red shaded areas represent the standard deviation.

Figure 3: Pharmacokinetic profiles for a bolus injection, a fast-acting oral dose, and a slow-acting oral dose of synthetic GCs. Representative profiles are shown for a nominal dose of 1x. Plasma concentration is given in arbitrary units (a.u.).

Figure 4:

Modified corticosterone profiles after dosing of synthetic glucocorticoids (GC) by bolus injection at the nominal amount (1x). The corticosterone rhythms with and without dosing are given in (A) for male rats and (B) for female rats. The solid line corresponds to the nominal corticosterone profile based on endogenous HPA axis activity. The modified corticosterone rhythm is indicated by the dotted line. The pharmacokinetic profiles for the bolus injection are indicated by the black line. The grey shaded areas represent the time at which the system is not exposed to light. Corticosterone concentration is given in arbitrary units (a.u.).

Figure 5:

Population mean amplitude and phase of the modified corticosterone rhythm after once-daily chronopharmacological dosing of synthetic glucocorticoids in male rats and female rats. The relative amplitude and difference in the acrophase of the modified corticosterone rhythms after a repeated once-a-day administration of a bolus injection, fast-releasing oral dose, or slow-releasing oral dose are shown in (A) and (C) for male rats and in (B) and (D) for female rats, respectively. The colored shaded areas represent ± 1 standard deviation of the population mean for amplitude and phase changes. The gray shaded areas represent the simulated night. A negative value for phase difference indicates an advance in the acrophase (i.e. peaks earlier in the simulated day) while a positive value indicates a delay in the acrophase (i.e. peaks later in the simulated day).

Figure 6:

Amplitude and phase of the modified corticosterone rhythm after once-daily injection at 4:00 AM and 4:00 PM in male and female rats. The relative amplitude and phase difference for the modified corticosterone rhythms are given for each individual defined by three sex-dependent parameters.

Figure 7:

Influence of dosing strength on the amplitude of the modified corticosterone rhythm following chronic once-daily dosing of a bolus injection in male and female rats. The amplitude is given for the dosing times which resulted in the greatest inductive and suppressive effects at each strength in male rats (A) and female rats (B). The error bars represent ± 1 standard deviation of the population mean for amplitude and phase changes.

Figure 8:

Basal $GR_{bound}(N)$ levels in males and females prior to chronopharmacological dosing. The mean $GR_{bound}(N)$ profiles for the subpopulations of the male and female rats used in the dosing experiments are given in (A) and (B), respectively. The grey shaded areas represent the time at which the system is not exposed to light. $GR_{bound}(N)$ is given in arbitrary units (a.u.). The blue and red shaded areas represent the standard deviation.

Figure 9:

Population mean amplitude and phase of the modified corticosterone rhythm after once-daily chronopharmacological dosing of synthetic glucocorticoids in chronically-stressed males and females. The relative amplitude and difference in the acrophase of the modified corticosterone rhythms after a repeated once-a-day administration of a bolus injection are shown in (A) for male rats and in (B) for female rats, respectively. The colored shaded areas represent ± 1 standard deviation of the population mean for amplitude and phase changes. The gray shaded areas represent the simulated night. A negative value for phase difference indicates an advance in the acrophase (i.e. peaks earlier in the simulated day) while a positive value indicates a delay in the acrophase (i.e. peaks later in the simulated day).

Figure 10:

Influence of dosing strength on the amplitude of the modified corticosterone rhythm following chronic once-daily dosing of a bolus injection in chronically stressed male and female rats. The amplitude is given for the dosing times which resulted in the greatest inductive and suppressive effects at each strength in male rats (A) and female rats (B). The error bars represent ± 1 standard deviation of the population mean for amplitude and phase changes.