Mechanistic Modeling of the Effects of Glucocorticoids and Circadian Rhythms on Adipokine Expression

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ABSTRACT

A mechanism-based model was developed to describe the effects of methylprednisolone (MPL), circadian rhythms, and the glucose/free fatty acid (FFA)/insulin system on leptin and adiponectin expression in white adipose tissue in rats. Fifty-four normal Wistar rats received 50 mg/kg MPL intramuscularly and were sacrificed at various times. An additional set of 54 normal Wistar rats were sacrificed at 18 time points across the 24-h light/dark cycle and served as controls. Measurements included plasma MPL, glucocorticoid receptor (GR) mRNA, leptin mRNA, adiponectin mRNA, plasma leptin, adiponectin, glucose, FFA, and insulin. MPL pharmacokinetics was described by a two-compartment model with two absorption components. All measured plasma markers and mRNA expression exhibited circadian patterns except for adiponectin and were described by Fourier harmonic functions. MPL caused

significant down-regulation in GR mRNA with the nadir occurring at 5 h. MPL disrupted the circadian patterns in plasma glucose and FFA by stimulating their production. Plasma glucose and FFA subsequently caused an increase in plasma insulin. Furthermore, MPL disrupted the circadian patterns in leptin mRNA expression by stimulating its production. This rise was closely followed by an increase in plasma leptin. Both leptin mRNA and plasma leptin peaked at 12 h after MPL and eventually returned back to their circadian baselines. MPL and insulin had opposing effects on adiponectin mRNA expression and plasma adiponectin, which resulted in biphasic pharmacodynamic profiles. This small systems model quantitatively describes, integrates, and provides additional insights into various factors controlling adipokine gene expression.

Introduction

Glucocorticoids are steroid hormones that play a critical role in regulating systemic energy metabolism and modulating immune and inflammatory processes. Most tissues are targets for glucocorticoids and contribute to the wide range of physiological effects of this hormone. Because of their anti-inflammatory properties, synthetic glucocorticoids are used therapeutically for a wide range of both acute and chronic inflammatory and immune disorders including transplant rejection, lupus erythematosus, asthma, and rheumatoid arthritis. However, because of glucocorticoids' physiological effects on systemic energy metabolism, long-term therapy causes adverse effects, leading to metabolic disorders including steroid-induced diabetes, dyslipidemia, muscle atrophy, and metabolic syndrome and limiting their therapeutic usefulness (Bialas and Routledge, 1998; Schäcke et al., 2002).

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White adipose tissue plays an important role in maintaining systemic energy balance by acting as a reservoir for excess energy, and it is an important target for both natural and synthetic glucocorticoids (Ahima and Flier, 2000). In addition, white adipose tissue serves as an endocrine organ, producing hormones called adipokines and immune-modulatory cytokines and chemokines that are released into the systemic circulation. Leptin and adiponectin, the two most important adipokines produced by mature adipocytes, have wide physiological effects mainly on energy metabolism. Leptin is a 16-kDa secreted protein whose concentration in blood is proportional to the amount of fat present in the body (Ahima and Flier, 2000). The most important function of leptin is to suppress appetite by acting on the hypothalamus through its functional receptors. Apart from this function, leptin is also involved in the regulation of energy expenditure by different tissues and hence is involved in regulating many energy-demanding processes including reproduction, hemopoiesis, and angiogenesis (Ahima and Flier, 2000). In addition, leptin-deficient (*ob/ob*) mice, leptin receptor-deficient (*db/db*) mice, and leptin receptor-deficient (*Fa/Fa*) Zucker

ABBREVIATIONS: SCN, suprachiasmatic nucleus; FFA, free fatty acids; GR, glucocorticoid receptor; MPL, methylprednisolone; PK/PD, pharmacokinetics/pharmacodynamics; QRT-PCR, quantitative real-time reverse transcription-polymerase chain reaction; FAM, 5-carboxyfluorescein; CV, coefficient of variation; IDR, indirect response.

rats have been extensively used as rodent models for type 2 diabetes with obesity illustrating its importance in maintaining proper energy balance (Rees and Alcolado, 2005). Adiponectin is another adipokine extensively secreted from mature adipocytes that sensitizes various organs to insulin action by increasing AMP-activated protein kinase phosphorylation (Wang et al., 2008). Furthermore, adiponectin is also involved in regulating lipid metabolism and, in contrast to leptin, is negatively correlated to the amount of body fat present in an organism. Apart from the effects of leptin and adiponectin on systemic energy balance, studies show that these adipokines also have been implicated in the pathophysiology of some inflammatory and immune response processes (Matarese et al., 2005; Fantuzzi, 2008).

Data from the literature suggest that glucocorticoids regulate the expression of these adipokines at both the mRNA and protein levels. Most of these studies are either in vitro or single or limited time points in vivo studies done in humans or rodent models. For example, dexamethasone treatment results in the up-regulation of leptin mRNA expression in isolated rodent adipocytes (Murakami et al., 1995). Likewise, dexamethasone treatment resulted in a sustained increase in plasma leptin concentrations in both normal rats and healthy human volunteers (Miell et al., 1996; Jahng et al., 2008). In addition, both leptin mRNA expression in adipose tissue and plasma leptin concentrations have been found to show circadian oscillations and be controlled directly by the central clock present in the suprachiasmatic nucleus (SCN) (Kalsbeek et al., 2001; Sukumaran et al., 2010b). In contrast to studies on glucocorticoids and leptin expression, studies on the effect of glucocorticoid treatment on adiponectin expression show contradictory results. For example, hydrocortisone treatment of both obese and normal rats was found to down-regulate adiponectin mRNA expression in white adipose tissue, which was also reflected in plasma adiponectin concentrations (Shi et al., 2010). On the other hand, dexamethasone dosing of both normal and hypoxic rats increased plasma adiponectin and similar effects were found in humans (Raff and Bruder, 2006; Jang et al., 2008).

In this study, dynamic changes in leptin and adiponectin mRNA expression in white adipose tissue and plasma protein concentrations were examined in a rich time series animal experiment where normal rats received a single dose of methylprednisolone (MPL), a synthetic corticosteroid. In addition, dynamic changes in important energy metabolism markers including plasma glucose, free fatty acids (FFA), and insulin, which also affect adiponectin expression, were examined along with glucocorticoid receptor (GR) mRNA expression in white adipose tissue. Furthermore, circadian oscillations in the baseline conditions for these biomarkers were characterized to develop a mechanistic PK/PD model that quantitatively describes regulation of these important biomarkers of white adipose tissue by circadian oscillators and their perturbation by glucocorticoid treatment.

Materials and Methods

Animals

This study involves two large rat experiments: circadian nadir and circadian. An extensive description of these animal experiments has been published previously (Yao et al., 2006; Hazra et al., 2008). In brief, both experiments consisted of male Wistar rats that were

acclimatized for 2 to 3 weeks in environments with constant temperature (22°C) and humidity (72%) equipped with 12-h light/dark cycles with free access to standard chow and drinking water. All animals were housed in separate cages to facilitate animal manipulations while minimizing stress to the animals. Although access to food was available at all times, literature data suggest that more than 90% of food intake in rodents occurs during the dark/active period (Yuan, 1993). Our research protocol adheres to the *Principles of Laboratory Animal Care* (National Institutes of Health publication 85-23, revised in 1985) and has been approved by the State University of New York at Buffalo Institutional Animal Care and Use Committee. Rats in the circadian nadir experiment received 50 mg/kg MPL succinate by intramuscular injection in the left hind haunch at the nadir of the circadian patterns of their endogenous corticosterone. Animals were weighed, anesthetized with ketamine/xylazine (80:10 mg/kg), and sacrificed by exsanguinations at 0.25, 0.5, 0.75, 1, 2, 4, 5, 6, 7, 8, 12, 24, 48, 60, 72, 84, and 96 h ($n = 3$ per time point) after drug treatment. In the control circadian experiment, animals ($n = 3$ per time point) were sacrificed on 3 successive days at 0.25, 1, 2, 4, 6, 8, 10, 11, and 11.75 h after lights on for time points in the light period and at 0.25, 1, 2, 4, 6, 8, 10, 11, and 11.75 h after lights off for time points in the dark period. Blood was drawn from the abdominal aortic arteries into syringes with EDTA (4 mM final concentration) as anticoagulant. Plasma was prepared from blood by centrifugation (2000g, 4°C, 15 min), aliquoted, and stored at -80°C. A discrete abdominal fat pad was harvested bilaterally, rapidly frozen in liquid nitrogen, and stored at -80°C.

Plasma Assays

MPL concentrations were determined by a normal-phase high-performance liquid chromatography method with the lower limit of quantification of 10 ng/ml. Commercial enzyme-linked immunosorbent assay kits were used for the quantification of plasma leptin (Rat Leptin TiterZyme EIA; Assay Designs, Ann Arbor, MI) and plasma adiponectin (Rat Adiponectin EIA; ALPCO Diagnostics, Salem, NH) concentrations. Plasma insulin concentrations were measured with a commercial radioimmunoassay kit (RI-13K Rat Insulin RIA Kit; Millipore Corporation, Billerica, MA). All commercial kits were used according to the manufacturer's protocols with standards run in duplicate and samples run in triplicate. Plasma glucose concentrations were measured by the glucose oxidase method (Sigma GAGO-20; Sigma-Aldrich, St. Louis, MO). The manufacturer's instructions were modified such that the assay was carried out in a 1-ml assay volume, and a standard curve consisting of seven concentrations over a 16-fold range was prepared from the glucose standard and run with each experimental set. Experimental samples were run in triplicate. Free fatty acid concentrations in plasma were measured using the nonesterified fatty acids detection kit (Zen-Bio, Research Triangle Park, NC) with standard curves constructed from a commercial standard solution (WAKO NEFA; Wako Pure Chemicals, Osaka, Japan). Intra-assay and interassay coefficient of variation values for all plasma assays were less than 15%.

Quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction Measurements

The quantity of abdominal fat glucocorticoid receptor, leptin, and adiponectin mRNA along with gene-specific in vitro-transcribed cRNA standards was determined by real-time QRT-PCR using TaqMan-based probes. Primer and probe sequences were designed using PrimerExpress software (Applied Biosystems, Foster City, CA) and custom-synthesized by Biosearch Technologies, Inc. (Novato, CA). The QRT-PCR was performed using a Brilliant QRT-PCR Core Reagent Kit, 1-Step (Stratagene, La Jolla, CA) in a Stratagene MX3005P thermocycler according to the manufacturer's instructions. Standard curves were generated using in vitro-transcribed sense cRNA standards. Primer and probe sequences were as follows: glucocorticoid receptor, forward primer 5'-AACATGTTAGGTGGGCGTCAA-3', re-

verse primer 5'-GGTGTAAAGTTTCTCAAGCCTAGTATCG-3' and FAM-labeled probe, 5'-TGATTGCAGCAGTGAAATGGGCAAAG-3'; leptin, forward primer 5'-GGCTTTGGTCCTATCTG-3', reverse primer 5'-GTGTCATCCTGGACTTTG-3' and FAM-labeled probe 5'-TCCTATGTTCAAGCTGTGCCTATCCA-3'; adiponectin, forward primer 5'-GAGACGCAGGTGTTCTTGGT-3', reverse primer 5'-CTTCCGCTCCTGTTCATTTCCA-3', and FAM-labeled probe 5'-CCTAAGGGTGACCCAGGAGATGC-3'. Samples were run in triplicate, and standards were run in duplicate. Additional minus reverse transcriptase controls were run for each RNA sample analyzed to check for genomic DNA contamination; all controls exhibited lack of amplification in RT minus controls. Intra-assay and interassay coefficient of variation values were less than 15%.

Pharmacokinetic and Pharmacodynamic Model

MPL Pharmacokinetics. A two-compartment model with two absorption components from the injection site was used to describe the PK of MPL (Hazra et al., 2008). The PK parameters were fixed with estimates obtained from our previous study as given in Table 1. Equations and initial conditions (IC) are:

$$V_c \frac{dC_{P(IM)}}{dt} = k_{a1} \cdot D_{IM} \cdot F \cdot F_r \cdot e^{-k_{a1} \cdot t} + k_{a2} \cdot D_{IM} \cdot F \cdot (1 - F_r) \cdot e^{-k_{a2} \cdot t} - (k_{el} + k_{12}) \cdot A_{P(IM)} + k_{21} \cdot A_{T(IM)} \quad IC = 0 \quad (1)$$

$$\frac{dA_{T(IM)}}{dt} = k_{12} \cdot A_{P(IM)} - k_{21} \cdot A_{T(IM)} \quad IC = 0 \quad (2)$$

where C , D , and A represent the concentration, dose, and amount of MPL, respectively in the corresponding plasma (P_u) and tissue (T_u) compartments, F_r and $(1 - F_r)$ are fractions of dose absorbed through the absorption pathways described by first-order rate constants k_{a1} and k_{a2} , k_{el} is the elimination rate constant from the central compartment, k_{12} and k_{21} are the first-order distribution rate constants, F is the overall bioavailability of MPL after intramuscular injection, and V_c is the central volume of distribution.

Pharmacodynamics

Figure 1 depicts the integrated PK/PD model that describes the effects of glucocorticoid treatment and circadian oscillations on various plasma markers and mRNA expression in adipose tissue.

Glucocorticoid Receptor Dynamics in White Adipose Tissue. The molecular mechanism governing the genomic effects of glucocorticoid action is considered to be similar in different tissues including white adipose tissue. Because of the lipophilicity of these drugs, they enter into the cells predominantly by passive diffusion. There the drug binds to cytosolic glucocorticoid receptors, causing subsequent conformational changes, phosphorylation, and activation of the receptors (Schaaf and Cidlowski, 2002). The activated drug-receptor complex translocates into the nucleus rapidly, where it binds to specific genomic sequences in the target gene called glucocorticoid response elements (Beato et al., 1989). This causes a change in the rate of transcription of these genes, thereby affecting the mRNA that

are transcribed from these genes. The receptors from the nucleus could either be degraded or recycled back into the cytosol to bind with new drug (Haché et al., 1999). The differential equations and initial conditions (IC) describing this chain of events are:

$$\frac{dR}{dt} = k_{s,GR} \cdot GR_m - k_{d,GR} \cdot R - k_{on} \cdot f_{mpl} \cdot C_{mpl} \cdot R + k_{re} \cdot R_f \cdot DR_n \quad IC = R(0) \quad (3)$$

$$\frac{dDR}{dt} = k_{on} \cdot f_{mpl} \cdot C_{mpl} \cdot R - k_t \cdot DR \quad IC = DR(0) \quad (4)$$

$$\frac{dDR_n}{dt} = k_t \cdot DR - k_{re} \cdot DR_n \quad IC = DR_n(0) \quad (5)$$

where R , DR , and DR_n represent the free cytosolic receptor, cytosolic drug-receptor complex, and nuclear translocated drug-receptor complex concentrations, respectively. The $k_{s,GR}$ and $k_{d,GR}$ are first-order rate constants for the production of free receptor from the translation of GR mRNA (GR_m) and the degradation of the free receptor, respectively, k_{on} is the second-order rate constant for formation of drug-receptor complex (DR) by the binding of free ligand and receptor in the cytosol, and k_t is the first-order rate constant for translocation of the drug-receptor complex from cytosol (DR) into the nucleus (DR_n). Part of DR_n may recycle back to the cytosol controlled by the rate constant $R_f k_{re}$ and the rest gets degraded with a rate constant $(1 - R_f) k_{re}$.

The GR mRNA showed circadian oscillations in white adipose tissue and are described using an IDR model with the mRNA synthesized by a time-dependent synthesis rate [$k_{s,GRm}(t)$], described by two harmonics function and degraded by first-order rate constant $k_{d,GRm}$ (Sukumaran et al., 2010b).

$$\frac{dGR_m}{dt} = k_{s,GRm}(t) - k_{d,GRm} \cdot GR_m \quad IC = GR_m(0) \quad (6)$$

The time-dependent production rate constant was described by a two-harmonics function:

$$k_{s,GRm}(t) = a_{0,GRm} \cdot k_{d,GRm} + \left(a_{1,GRm} \cdot k_{d,GRm} + \frac{2\pi b_{1,GRm}}{24} \right) \cdot \cos\left(\frac{2\pi T}{24}\right) + \left(b_{1,GRm} \cdot k_{d,GRm} + \frac{2\pi a_{1,GRm}}{24} \right) \cdot \sin\left(\frac{2\pi T}{24}\right) + \left(a_{2,GRm} \cdot k_{d,GRm} + \frac{2\pi b_{2,GRm}}{12} \right) \cdot \cos\left(\frac{2\pi T}{12}\right) + \left(b_{2,GRm} \cdot k_{d,GRm} + \frac{2\pi a_{2,GRm}}{12} \right) \cdot \sin\left(\frac{2\pi T}{12}\right) \quad (7)$$

where a_i and b_i are Fourier coefficients associated with the harmonic oscillations. The values for these parameters were obtained by fitting the replicate GR mRNA expression data obtained from the control experiment using the FOURPHARM program (Krzyzanski et al., 2000).

Treatment with exogenous glucocorticoids (MPL in this case) causes an inhibition in GR mRNA expression caused by the transcriptional repression of the GR gene by the activated drug-receptor complex in the nucleus (DR_n) (Oakley and Cidlowski, 1993). The $IC_{50,GRm}$ is the concentration of DR_n at which the synthesis rate of GR mRNA is reduced to 50% of its baseline.

$$\frac{dGR_{m,mpl}}{dt} = k_{s,GRm}(t) \cdot \left(1 - \left(\frac{DR_n}{DR_n + IC_{50,GRm}} \right) \right) - k_{d,GRm} \cdot GR_{m,mpl} \quad IC = GR_{m,mpl}(0) \quad (8)$$

Plasma Glucose, Free Fatty Acid, and Insulin Dynamics. Glucocorticoids are catabolic hormones that increase the production of energy substrates including glucose and free fatty acids used by

TABLE 1
Pharmacokinetic parameters of methylprednisolone
Parameter values were obtained from Hazra et al. (2008).

Parameter	Definition	Value
k_{el} (h^{-1})	Elimination rate constant	5.57
k_{12} (h^{-1})	Distribution rate constant	3.61
k_{21} (h^{-1})	Distribution rate constant	2.84
V_c (ml/kg)	Central volume	718.7
F	Bioavailability	0.214
F_r	Fraction absorbed by k_{a1}	0.725
k_{a1} (h^{-1})	Absorption rate constant	1.255
k_{a2} (h^{-1})	Absorption rate constant	0.219

Fig. 1. Model schematic for the effects of methylprednisolone, circadian rhythms, and glucose/free fatty acids/insulin system on leptin and adiponectin expression. Curved input represents circadian pattern in production, open boxes reflect stimulation, and shaded boxes depict inhibition of production rate of a turnover process. The model is described by eqs. 1 to 25. Definitions of parameters are provided in Tables 1 to 5.

different tissues (Mlinar et al., 2007). For example, they stimulate gluconeogenesis in liver and kidney, which produces and releases glucose into the systemic circulation (Hanson and Reshef, 1997). Likewise, glucocorticoids stimulate lipolysis of stored triglycerides, which releases FFA into the circulation, making them available as energy substrates (Xu et al., 2009). The elevated glucose and FFA concentrations in plasma in turn stimulate the production of insulin from the pancreas, which results in the elevated insulin concentrations (Nicod et al., 2003). Furthermore, plasma glucose, FFA, and insulin show circadian oscillations in their concentrations that are directly controlled by the central circadian oscillators present in the SCN (Boden et al., 1996; La Fleur et al., 1999).

The circadian oscillations in plasma glucose, FFA, and insulin in the circadian experiment were independent of each other because they are directly controlled by the central clock and were modeled using an IDR model with time-dependent synthesis rate described by a one-harmonic function and degraded by first-order rate constants:

$$\frac{dGlu}{dt} = k_{s,Glu}(t) - k_{d,Glu} \cdot Glu \quad IC = Glu(0) \quad (9)$$

$$\frac{dFFA}{dt} = k_{s,FFA}(t) - k_{d,FFA} \cdot FFA \quad IC = FFA(0) \quad (10)$$

$$\frac{dIns}{dt} = k_{s,Ins}(t) - k_{d,Ins} \cdot Ins \quad IC = Ins(0) \quad (11)$$

where $k_s(t)$ and k_d reflect the time-dependent synthesis and first-order degradation, respectively, of plasma glucose (Glu), FFA, and insulin (Ins). The time-dependent production rate constants for these measurements are described by one-harmonic functions:

$$k_{s,Glu}(t) = a_{0,Glu} \cdot k_{d,Glu} + \left(a_{1,Glu} \cdot k_{d,Glu} + \frac{2\pi b_{1,Glu}}{24} \right) \cdot \cos\left(\frac{2\pi T}{24}\right) + \left(b_{1,Glu} \cdot k_{d,Glu} + \frac{2\pi a_{1,Glu}}{24} \right) \cdot \sin\left(\frac{2\pi T}{24}\right) \quad (12)$$

$$k_{s,FFA}(t) = a_{0,FFA} \cdot k_{d,FFA} + \left(a_{1,FFA} \cdot k_{d,FFA} + \frac{2\pi b_{1,FFA}}{24} \right) \cdot \cos\left(\frac{2\pi T}{24}\right) + \left(b_{1,FFA} \cdot k_{d,FFA} + \frac{2\pi a_{1,FFA}}{24} \right) \cdot \sin\left(\frac{2\pi T}{24}\right) \quad (13)$$

$$k_{s,Ins}(t) = a_{0,Ins} \cdot k_{d,Ins} + \left(a_{1,Ins} \cdot k_{d,Ins} + \frac{2\pi b_{1,Ins}}{24} \right) \cdot \cos\left(\frac{2\pi T}{24}\right) + \left(b_{1,Ins} \cdot k_{d,Ins} + \frac{2\pi a_{1,Ins}}{24} \right) \cdot \sin\left(\frac{2\pi T}{24}\right) \quad (14)$$

The values of the Fourier coefficients were obtained by fitting the circadian profiles for these plasma measurements using FOURP-HARM.

The effect of MPL on plasma glucose was modeled as stimulation of its production controlled by parameters $S_{\text{max, mpl}}^{\text{Glu}}$ and $SC_{50, \text{mpl}}^{\text{Glu}}$. Furthermore, stress increases plasma glucose, so to accommodate the stress effect $k_{\text{stress}}^{\text{Glu}}$, a zero-order production rate was added to the model (Arola et al., 1980). This factor existed only for the first 15 min after drug injection, after which it was set to zero. Thus the stimulation of plasma glucose by MPL is:

$$\frac{dGlu_{\text{mpl}}}{dt} = k_{\text{Stress}}^{\text{Glu}} + k_{s, \text{glu}}(t) \cdot \left(1 + \left(\frac{S_{\text{max, mpl}}^{\text{Glu}} \cdot C_{\text{mpl}}}{C_{\text{mpl}} + SC_{50, \text{mpl}}^{\text{Glu}}} \right) \right) - k_{d, \text{glu}} \cdot Glu_{\text{mpl}} \quad IC = Glu_{\text{mpl}}(0) \quad (15)$$

Likewise, the effect of MPL on plasma FFA concentrations was modeled as stimulation of its production controlled by the parameters $S_{\text{max, mpl}}^{\text{FFA}}$ and $SC_{50, \text{mpl}}^{\text{FFA}}$ as:

$$\frac{dFFA_{\text{mpl}}}{dt} = k_{s, \text{FFA}}(t) \cdot \left(1 + \left(\frac{S_{\text{max, mpl}}^{\text{FFA}} \cdot C_{\text{mpl}}}{C_{\text{mpl}} + SC_{50, \text{mpl}}^{\text{FFA}}} \right) \right) - k_{d, \text{FFA}} \cdot FFA_{\text{mpl}} \quad IC = FFA_{\text{mpl}}(0) \quad (16)$$

The effect of MPL on plasma insulin was modeled as stimulation of the production of insulin by both plasma glucose and plasma FFA controlled by stimulation constants $S_{\text{Glu}}^{\text{Ins}}$ and $S_{\text{FFA}}^{\text{Ins}}$. The model assumes that both plasma glucose and FFA stimulate the production of insulin only if glucose and FFA concentrations are above circadian baselines and hence the circadian oscillation in plasma insulin is independent of circadian oscillations in plasma glucose and FFA. Furthermore, stress is found to reduce plasma insulin, which is modeled as inhibition of insulin production by the inhibition constant I_{stress} , which exists only for 4 h after dosing MPL (Arola et al., 1980). These effects of MPL on plasma insulin can be described as:

$$\frac{dIns_{\text{mpl}}}{dt} = k_{s, \text{Ins}}(t) \cdot (1 - I_{\text{Stress}}) \cdot (1 + S_{\text{Glu}}^{\text{Ins}} \cdot (Glu_{\text{mpl}} - Glu) + S_{\text{FFA}}^{\text{Ins}} \cdot (FFA_{\text{mpl}} - FFA)) - k_{d, \text{Ins}} \cdot Ins_{\text{mpl}} \quad IC = Ins_{\text{mpl}}(0) \quad (17)$$

Leptin mRNA and Plasma Leptin Dynamics. Leptin exhibits circadian oscillations in expression, which are directly controlled by the central clock present in the SCN (Kalsbeek et al., 2001). In addition, both in vitro and in vivo studies show that glucocorticoids up-regulate leptin mRNA expression in adipose tissue, which subsequently increases leptin concentrations in plasma (Murakami et al., 1995; Jahng et al., 2008). Adipocytes present in white adipose tissue are the cell types that predominantly produce and release leptin. Although leptin is expressed in other tissues, including heart and testis, it usually acts in an autocrine/paracrine manner in these tissues and its contribution to the plasma is negligible (Karmazyn et al., 2007).

Similar to the circadian oscillations in plasma leptin, the circadian oscillations in leptin mRNA expression in white adipose tissue were modeled using an IDR model with a time-dependent synthesis rate $k_{s, \text{Leptm}}(t)$, described by a one-harmonic function and degraded by a first-order rate constant $k_{d, \text{Leptm}}$ as:

$$\frac{dLept_m}{dt} = k_{s, \text{Leptm}}(t) - k_{d, \text{Leptm}} \cdot Lept_m \quad IC = Lept_m(0) \quad (18)$$

$$k_{s, \text{Leptm}}(t) = a_{0, \text{Leptm}} \cdot k_{d, \text{Leptm}} + \left(a_{1, \text{Leptm}} \cdot k_{d, \text{Leptm}} + \frac{2\pi b_{1, \text{Leptm}}}{24} \right) \cdot \cos\left(\frac{2\pi T}{24}\right) + \left(b_{1, \text{Leptm}} \cdot k_{d, \text{Leptm}} + \frac{2\pi a_{1, \text{Leptm}}}{24} \right) \cdot \sin\left(\frac{2\pi T}{24}\right) \quad (19)$$

The effect of MPL on leptin mRNA expression was modeled as a direct stimulation of the production rate by the activated drug-receptor complex in the nucleus (DR_n) controlled by the stimulation constant $S_{\text{DR}_n}^{\text{leptm}}$ as:

$$\frac{dLept_{m, \text{mpl}}}{dt} = k_{s, \text{Leptm}}(t) \cdot (1 + S_{\text{DR}_n}^{\text{leptm}} \cdot DR_n) - k_{d, \text{Leptm}} \cdot Lept_{m, \text{mpl}} \quad IC = Lept_{m, \text{mpl}}(0) \quad (20)$$

The circadian oscillations in plasma leptin and the effects of MPL were modeled as the direct control of the circadian oscillations and MPL effect on the leptin mRNA expression in white adipose tissue controlled by the first-order production rate constant $k_{s, \text{Lept}}$ and the first-order degradation rate constant $k_{d, \text{Lept}}$ as:

$$\frac{dLept}{dt} = k_{s, \text{Lept}} \cdot Lept_m - k_{d, \text{Lept}} \cdot Lept \quad IC = Lept(0) \quad (21)$$

$$\frac{dLept_{\text{mpl}}}{dt} = k_{s, \text{Lept}} \cdot Lept_{m, \text{mpl}} - k_{d, \text{Lept}} \cdot Lept_{\text{mpl}} \quad IC = Lept_{\text{mpl}}(0) \quad (22)$$

Adiponectin mRNA and Plasma Adiponectin Dynamics.

Data from previous studies on the effect of glucocorticoid treatment on adiponectin mRNA expression in white adipose tissue and plasma adiponectin are contradictory to some studies showing up-regulation and others showing down-regulation in expression (Jang et al., 2008; Shi et al., 2010). However, reports clearly show that insulin down-regulates the expression of adiponectin mRNA in white adipose tissue (Xu et al., 2004). Here, the effect of MPL was modeled in such a way that MPL through its activated receptor directly up-regulates the adiponectin mRNA expression, whereas the plasma insulin concentrations, which are increased by MPL, down-regulates its expression as described by:

$$\begin{aligned} \frac{dAdp_{m, \text{mpl}}}{dt} &= k_{s, \text{Adpm}} \cdot (1 + S_{\text{DR}_n}^{\text{Adpm}} \cdot DR_n) \\ &\times \left(1 - \left(\frac{I_{\text{max, Ins}}^{\text{Adpm}} \cdot (Ins_{\text{mpl}} - Ins)}{(Ins_{\text{mpl}} - Ins) + IC_{50, \text{mpl}}^{\text{Adpm}}} \right) \right) - k_{d, \text{Adpm}} \cdot Adp_{m, \text{mpl}} \\ IC &= Adp_{m, \text{mpl}}(0) \end{aligned} \quad (23)$$

The effect of MPL on plasma adiponectin is controlled by two processes. One is the direct translation of the changes in adiponectin mRNA after MPL and the other is the direct effect of plasma MPL on the secretion of adiponectin from adipocytes into blood:

$$\frac{dAdp_{\text{mpl}}}{dt} = k_{s, \text{Adp}} \cdot (Adp_{m, \text{mpl}})^g - k_{d, \text{Adp}} \cdot Adp_{\text{mpl}} \quad IC = Adp_{\text{mpl}}(0) \quad (24)$$

$$\begin{aligned} \frac{dAdpP_{\text{mpl}}}{dt} &= k_{s, \text{AdpP}} \cdot Adp_{\text{mpl}} \cdot \left(1 + \left(\frac{S_{\text{max, mpl}}^{\text{AdpP}} \cdot C_{\text{mpl}}}{C_{\text{mpl}} + SC_{50, \text{mpl}}^{\text{AdpP}}} \right) \right) \\ &- k_{d, \text{AdpP}} \cdot AdpP_{\text{mpl}} \quad IC = AdpP_{\text{mpl}}(0) \end{aligned} \quad (25)$$

where Adp represents the adiponectin protein concentration inside the adipocytes and $AdpP$ reflects plasma adiponectin, k_s and k_d are the production and degradation rate constants, respectively, for adiponectin present inside the cells and in plasma, and g is an amplification factor controlling the production of multiple proteins by the translation of a single mRNA molecule. The direct effect of plasma MPL on the secretion (or the production rate constant $k_{s, \text{AdpP}}$) of adiponectin into blood is controlled by $S_{\text{max, mpl}}^{\text{AdpP}}$ and $SC_{50, \text{mpl}}^{\text{AdpP}}$.

Data Analysis. ADAPT 5 was used for all data fitting and simulation of model equations (D'Argenio et al., 2009). Replicate data from multiple animals in each of the experiments were pooled, and data from both circadian control and circadian nadir experiments were modeled simultaneously. The goodness of fit was assessed by model convergence, visual inspection of the fitted curves, improved likelihood, and examination of residuals and CV% of the estimated

parameters. Animals in the circadian nadir experiments were given MPL between 1.5 and 3 h after lights on. For simplicity we assumed the treatment time to be at circadian time 2.5 h to compare the data obtained from both the circadian nadir and circadian experiments. Hence, all pharmacodynamic profiles are plotted with respect to circadian time with MPL given at 2.5 h.

Fig. 2. Plasma concentrations versus time after 50 mg/kg i.m. injection of MPL. Solid line represents model simulations (eqs. 1 and 2). Parameter estimates are listed in Table 1. Circles are mean data with error bars indicating 1 S.D. of the mean.

Fig. 3. GR mRNA expression in white adipose tissue in control rats (A) and rats treated with 50 mg/kg i.m. MPL (B). Circles depict mean data with error bars representing 1 S.D. of the mean, and the solid lines show model fitting (parameter estimates given in Table 2). Shaded areas are dark periods, and unshaded areas are light periods.

TABLE 2

Parameter values for GR mRNA expression and receptor dynamics

Parameter	Definition	Estimate	CV%
$a_{0,GRm}$	Fourier coefficient for GR mRNA	2215.9	Fixed
$a_{1,GRm}$	Fourier coefficient for GR mRNA	-273.2	Fixed
$a_{2,GRm}$	Fourier coefficient for GR mRNA	65.91	Fixed
$b_{1,GRm}$	Fourier coefficient for GR mRNA	-10.86	Fixed
$b_{2,GRm}$	Fourier coefficient for GR mRNA	10.08	Fixed
$k_{d,GRm}$ (h^{-1})	Loss rate for GR mRNA	0.3117	26.12
$IC_{50,GRm}$ (nM)	Inhibition of GR mRNA production	15.6	Fixed
$k_{d,GR}$ (h^{-1})	Loss rate for GR	0.05	Fixed
$k_{s,GR}$ (nM/h)(mol/ng) $^{-1}$	Production rate for GR	0.00196	30.78
k_{on} (nM $^{-1} \cdot h^{-1}$)	Association constant	0.016	Fixed
f_{mpl}	Fraction unbound of MPL	0.23	Fixed
k_{re} (h^{-1})	Loss rate constant for DR_n	1.31	Fixed
R_f	Recycling factor for DR_n	0.93	Fixed
k_t (h^{-1})	Translocation rate for DR	58.2	Fixed
$GR_{m,0}$ (mol/ng)	GR mRNA initial concentration (control)	2050	Fixed
$GR_{m,mpl,0}$ (mol/ng)	GR mRNA initial concentration (drug treatment)	2200	Fixed
$R(0)$ (nM)	Free GR initial concentration	86.15	30.78
$DR(0)$ (nM)	DR initial concentration	0	Fixed
$DR_n(0)$ (nM)	DR_n initial concentration	0	Fixed

glucocorticoid receptor mRNA returned to its normal circadian baseline 36 h after MPL, which is well described by the model. Table 2 gives the parameter estimates related to glucocorticoid receptor dynamics. The estimated $K_{d,Grm}$ value of 0.312 h^{-1} (26.1% CV) is higher than the values estimated in liver (0.12 h^{-1}) and muscle (0.17 h^{-1}) (Yao et al., 2006; Hazra et al., 2008). Furthermore, the trough in receptor mRNA expression after MPL is reached quicker in white adipose tissue compared with liver or muscle, which could be attributed to the higher $K_{d,Grm}$ value. In this study the cytosolic receptor density was not measured because of the very low quantity of white adipose tissue obtained from the animals. The parameters governing receptor binding to the ligand and its translocation were obtained from our previous study done in liver from the same animals and were fixed to avoid overparameterization of the model (Hazra et al., 2008). This is a reasonable assumption because the basic molecular mechanism governing the receptor binding and translocation are conserved in these tissues.

Plasma Glucose, FFA, and Insulin Dynamics. Plasma metabolic parameters affecting glucose, FFA, and insulin are altered by MPL and are under direct control of circadian oscillators. The dynamics of circadian oscillations in the concentrations of these markers along with the model fittings are shown in Fig. 4. The plasma glucose peak occurs at 14 to 15 h. Although the model fitting shows a slight difference and predicts the peak at 16 h, in general the data are described quite well. Circadian oscillations in plasma FFA peaked at approximately 6 h, whereas insulin concentrations peaked at 22 h and were captured quite well by the model. The effect of MPL on these plasma markers along with the model fittings are shown in Fig. 5, and the parameter estimates obtained from fitting the data using eqs. 9 to 17 are given in Table 3. Although MPL was given close to the nadir of plasma glucose, the latter were high even at 0.25 h, suggesting a stress effect that was modeled using an additional zero-order production rate K_{stress}^{Glu} ($316 \text{ mg/dl} \cdot \text{h}^{-1}$). Because there are no plasma glucose data available between 0 and 0.25 h after MPL to characterize this rate constant, the value was obtained from the slope of a straight line joining the circadian plasma glucose at 0 h after MPL treatment and the glucose concentration observed at 0.25 h after drug treatment and was fixed in the model. The model prediction shows that plasma glucose comes back to its circadian baseline 20 h after MPL dosing. The drug increased plasma FFA, which peaked at 4 h and then returned to its circadian baseline after 20 h. The model captured both the peak and the return to baseline quite well in spite of the high interindividual variability at the peak plasma FFA. The effect of MPL on plasma insulin shows a biphasic regulation with initial down-regulation followed by a delayed increase in plasma insulin. Previous studies have shown that stress reduces plasma insulin and the initial down-regulation observed in this study is attributed to the stress effect (Arola et al., 1980). Plasma insulin, which reached its trough at 4 h after MPL, was modeled as down-regulation of synthesis of insulin governed by the parameter I_{stress} ($1.046 \text{ ng/ml} \cdot \text{h}^{-1}$, 12.04% CV). Both plasma glucose and FFA were increased by MPL and in turn combined to stimulate insulin production. This caused increased plasma insulin after the initial decline, which eventually went back to its baseline 24 h after MPL. The model does a good job in capturing the initial decline in insulin concentra-

Fig. 4. Circadian patterns in plasma glucose (A), FFA (B), and insulin (C) in control rats. Circles depict the mean data with error bars representing 1 S.D. of the mean and the solid lines showing model fitting (parameter estimates given in Table 3). Shaded areas are dark periods, and unshaded areas are light periods.

tions and the subsequent increase above circadian baseline, but slight differences were observed between the model fitting and the actual data after plasma insulin returned to its circadian baseline. This could be caused by various reasons, ranging from physiological effects of MPL on circadian oscillators that directly control insulin production to animal variability within and between studies. Based on the parameter estimates, the stimulation of plasma insulin production by

Fig. 5. A, C, and D, plasma glucose (A), FFA (C), and insulin (D) in rats given 50 mg/kg i.m. MPL. Circles depict the mean data with error bars representing 1 S.D. of the mean and the solid lines showing model fitting (parameter estimates given in Table 3). B, the first 24 h of plasma glucose in animals treated with 50 mg/kg i.m. MPL (solid line) and control animals (dotted line).

plasma glucose was stronger than that caused by plasma FFA, which is physiologically true.

Leptin mRNA and Plasma Leptin Dynamics. The circadian dynamics of leptin mRNA expression in white adipose tissue and its model fitting are shown in Fig. 6. Circadian expression of leptin peaked at 21 h and was modeled by a single Fourier harmonic function, which did a fair job in capturing the oscillations. Figure 7 shows the effect of MPL on leptin mRNA expression along with the model fittings. MPL caused an up-regulation in leptin expression from a baseline value of 170,000 molecules/ng at 0 h to the peak value of 351,562 molecules/ng, with the peak occurring at 12 h after drug dosing. The magnitude of up-regulation of leptin mRNA expression after MPL treatment was very high compared with the amplitude of its circadian oscillations. The return of leptin mRNA expression to its circadian baseline was very slow and seemed to occur only 72 h after MPL.

The circadian oscillations in the expression of leptin mRNA in white adipose tissue directly controls plasma leptin as shown in Fig. 6. Circadian oscillations in plasma leptin look very similar to oscillations in its mRNA expression with the plasma peak occurring approximately 21 to 22 h (around the same time as its mRNA expression). The effect of MPL on plasma leptin also seems very similar to its mRNA expression in adipose tissue. MPL caused an increase in plasma leptin from its baseline concentration of 25,000 pg/ml at 0 h to the peak of 71,000 pg/ml with the peak occurring at ap-

proximately 12 h. Although leptin mRNA expression did not return back to baseline until 72 h after MPL, the plasma leptin returned to its circadian baseline by 36 h. In general, the model captured the up-regulation of plasma leptin by MPL well, but overestimated plasma leptin at 24 and 36 h after the drug, because the model predicted a slower return of plasma leptin to baseline as controlled by the leptin mRNA expression in adipose tissue. Table 4 lists the estimates of all parameter values associated with leptin mRNA and plasma leptin dynamics.

Adiponectin mRNA and Plasma Adiponectin Dynamics. Adiponectin mRNA expression and plasma adiponectin did not show any circadian oscillations in our study. The dose of MPL produced a biphasic regulation of adiponectin mRNA expression with initial up-regulation followed by a delayed down-regulation, which eventually reached baseline as shown in Fig. 8. In this study, the up-regulation of adiponectin mRNA was modeled as the stimulation of gene expression by DR_n and down-regulation by plasma insulin that were above the circadian baseline. In the biphasic expression of adiponectin mRNA, the initial peak occurred at 2 h after MPL followed by a trough occurring at approximately 15 h, which eventually returned to the baseline.

The effect of MPL on plasma adiponectin was much more complex than the effect on adiponectin mRNA expression in white adipose tissue. Apart from the genomic effect of MPL on plasma adiponectin by increasing its mRNA expression,

TABLE 3
Parameter values for glucose, FFA, and insulin dynamics

Parameter	Definition	Estimate	CV%
$k_{d,Glu}$ (h^{-1})	Loss rate constant for glucose	4.229	83.65
$S_{max,mpl}^{Glu}$	Stimulation of glucose production	0.115	61.42
$SC_{50,mpl}^{Glu}$ (ng/ml)	Stimulation of glucose production	43.19	187.3
k_{stress}^{Glu} (mg/dl)(h^{-1})	Stress induced glucose production	316	Fixed
$Glu(0), Glu_{mpl}(0)$ (mg/dl)	Glucose initial concentration	190	Fixed
$k_{d,FFA}$ (h^{-1})	Loss rate constant for FFA	0.2986	39.92
$S_{max,mpl}^{FFA}$	Stimulation constant for FFA production	0.775	23.23
$SC_{50,mpl}^{FFA}$ (mg/ml)	Stimulation of FFA production	106	50.49
$FFA(0), FFA_{mpl}(0)$ (μM)	FFA initial concentration	120	Fixed
$k_{d,ins}$ (h^{-1})	Loss rate constant for insulin	0.3957	38.4
S_{FFA}^{ins} (μM^{-1})	Stimulation of insulin production by FFA	0.0005	N.E.
S_{Glu}^{ins} (mg/dl)(h^{-1})	Stimulation of insulin production by glucose	0.1467	347.5
I_{stress} (ng/ml)(h^{-1})	Stress inhibition of insulin production	1.046	12.04
$Ins(0), Ins_{mpl}(0)$ (ng/ml)	Insulin initial concentration	2.9,1.8	Fixed
$a_{0,Glu}$	Fourier coefficient for glucose	214.9	Fixed
$a_{1,Glu}$	Fourier coefficient for glucose	-17.64	Fixed
$b_{1,Glu}$	Fourier coefficient for glucose	-32.35	Fixed
$a_{0,FFA}$	Fourier coefficient for FFA	123.4	Fixed
$a_{1,FFA}$	Fourier coefficient for FFA	-3.105	Fixed
$b_{1,FFA}$	Fourier coefficient for FFA	18.79	Fixed
$a_{0,ins}$	Fourier coefficient for insulin	2.11	Fixed
$a_{1,ins}$	Fourier coefficient for insulin	0.904	Fixed
$b_{1,ins}$	Fourier coefficient for insulin	-0.501	Fixed

N.E., not estimated.

MPL also affects secretion of adiponectin from adipocytes (Cabanelas et al., 2010). In this study, the genomic effect of MPL on plasma adiponectin was modeled as the direct production of adiponectin from its mRNA (which is up-regulated by MPL) in the mature adipocytes, which is represented by *Adp*. The effect of MPL on the secretion of adiponectin was modeled as a nongenomic direct effect of MPL in plasma, which stimulates the secretion of adiponectin from adipocytes into blood (*AdpP*). Plasma adiponectin increased after MPL and the peak plasma adiponectin occurred earlier than the peak adiponectin mRNA expression. Figure 8 shows the biphasic pattern in plasma adiponectin with a steeper peak than its mRNA expression occurring at 0.75 h after MPL and nadir occurring at approximately 30 h, which eventually returned to baseline.

Simulation of Driving Forces for PD Effects. Figure 9 shows the simulation of the effects of MPL on free glucocorticoid receptors in cytosol, the drug receptor complex in cytosol, and the complex translocated into the nucleus using eqs. 3 to 8 and parameter estimates from Table 2. The model predicts a very quick, steep decline (within 15 min) in the free cytosolic receptor concentrations after MPL with a biphasic return to its circadian baseline. The initial faster return phase would occur because of recycling of receptors from the drug receptor complex in the nucleus, while the second relatively slower return phase followed the return of the GR mRNA expression to its circadian baseline. The simulated profile of *DR* concentrations in the cytosol shows a quick increase followed by a relatively rapid return to the baseline. The concentrations of *DR_n* present in the nucleus peaked at 30 min after MPL, which follows the increase and return of *DR* complex to its baseline in the cytosol with a slight time

delay. All simulated *GR* dynamic profiles agree with previous in vitro and in vivo studies done in other tissue and cell types (Hazra et al., 2008).

Discussion

White adipose tissue has been strongly linked to the pathophysiology of many metabolic disorders including diabetes, dyslipidemia, and metabolic syndrome, which are also some of the adverse effects of long-term glucocorticoid treatment (Schäcke et al., 2002; Wang et al., 2008). Adipokines play a critical role in the regulation and coordination of adipocyte functioning and hence are related to both physiology and pathophysiology of the tissue (Ahima and Flier, 2000). This study reports the use of rich time series animal experiments and mechanistic PK/PD modeling to examine the effect of synthetic glucocorticoid treatment on adipokine expression in white adipose tissue and quantitatively characterize the relevant physiological factors that are involved in this regulation. Some of the physiological factors considered in this study include the plasma glucose/free fatty acids/insulin system, circadian oscillators, and glucocorticoid receptor dynamics in white adipose tissue, which caused both quantitative and qualitative differences in the regulation of adipokine expression by MPL.

Circadian oscillations in gene expression and plasma biomarkers are important physiological factors that could contribute to quantitative variations and complexity for both pharmacokinetic and pharmacodynamic profiles for many endogenous compounds and drugs (Sukumaran et al., 2010a). All plasma markers and mRNA expression profiles except for adiponectin examined in our study displayed ro-

Fig. 6. Circadian pattern in leptin mRNA expression in white adipose tissue (A) and plasma leptin (B) in control animals. Circles depict the mean data with error bars representing 1 S.D. of the mean and the solid lines showing model fitting (parameter estimates given in Table 4). Shaded areas are dark periods, and unshaded areas are light periods.

bust circadian oscillations. This suggests the importance of taking into account this variation in the baseline conditions that change as a function of time to quantitatively understand both the physiology and pharmacology of drug treatment. Circadian oscillations are important for efficient functioning of an organism and help the animal to anticipate, adapt, and respond to environmental changes (Sukumaran et al., 2010a). For example, as shown in Fig. 4, circadian oscillations in plasma glucose peak during the early dark period when the animal is active and runs on glucose obtained mainly from the digestion of food as its primary energy source. In contrast, the circadian oscillations in plasma FFA peak at the midlight/inactive period when the animal is not feeding and where many tissues use FFA obtained from lipolysis as their main energy source. Likewise, circadian oscillations in plasma leptin, a hormone that suppresses appetite, is controlled directly by the circadian oscillations in its mRNA expression in white adipose tissue and peaks at the late dark period with the nadir occurring at the late light period. This suggests that the lower leptin concentrations at the start of the dark/active period results in minimum inhibition of food intake during the active feeding period and vice versa.

Previous studies have shown that glucocorticoids increase glucose, FFA, and insulin concentrations in plasma, which was confirmed in this study (Mlinar et al., 2007; Jin and

Fig. 7. Leptin mRNA expression in white adipose tissue (A) and plasma leptin (B) in rats given 50 mg/kg i.m. MPL. Circles depict the mean data with error bars representing 1 S.D. of the mean and the solid lines showing model fitting (parameter estimates given in Table 4).

Jusko, 2009). Glucocorticoids increase plasma glucose by stimulating gluconeogenesis in liver, thereby regulating the expression of phosphoenolpyruvate carboxykinase, which is the rate-limiting enzyme in the process (Hanson and Reshef, 1997). Likewise, these agents increase plasma FFA by stimulating the expression of hormone-sensitive lipase and adipose triglyceride lipase, thereby stimulating lipolysis of stored triglycerides (Xu et al., 2009). As shown in Fig. 5, the increase in FFA after MPL is 2-fold higher compared with the amplitude of its circadian baseline oscillations. However, the magnitude of the increase in plasma glucose after MPL was on the same order as its circadian baseline amplitude, which was challenging to model. It was difficult for the model fitting to differentiate the increase in plasma glucose caused by MPL from the peak plasma glucose related to the endogenous circadian rhythms. Simultaneous fitting of both the circadian and drug treatment data helped in differentiating these two processes. In addition, the effect of stress on plasma glucose and insulin further complicated the pharmacodynamic profiles for these biomarkers (Arola et al., 1980). Hence a relatively simple linear model with appropriate stress effects was used to describe the influence of plasma glucose and FFA on plasma insulin. One obvious limitation of this model is that it does not take into account the fact that plasma insulin can affect the disposition of both plasma glucose and FFA by increasing their utilization by different tissues (Nicod et al., 2003). However, inclusion of this cyclic

TABLE 4
Parameter values for leptin mRNA and plasma leptin dynamics

Parameter	Definition	Estimate	CV%
S_{Dr}^{lepm} (nM^{-1})	Stimulation of leptin mRNA production	0.263	16.96
$k_{d,lepm}$ (h^{-1})	Loss rate constant for leptin mRNA	0.0477	11.02
$k_{d,lep}$ (h^{-1})	Loss rate constant for plasma leptin	3.894	296.4
k_{slep} (h^{-1})(pg/ml)	Production rate constant for plasma leptin	0.663	Secondary*
$Lep_m(0)$ (mol/ng)	Leptin mRNA initial concentration (control)	148,000	5.097
$Lep_{m,mpl}(0)$ (mol/ng)	Leptin mRNA initial concentration (drug treatment)	170,000	12.9
$Lep(0)$ (pg/ml)	Plasma leptin initial concentration (control)	25,200	3.72
$Lep_{mpl}(0)$ (pg/ml)	Plasma leptin initial concentration (MPL treatment)	25,000	Fixed

* Secondary parameter.

Fig. 8. Adiponectin mRNA expression in white adipose tissue (A) and plasma adiponectin (B) in rats given 50 mg/kg i.m. MPL. Circles depict the mean data with error bars representing 1 S.D. of the mean and the solid lines showing model fitting (parameter estimates given in Table 5).

regulation into the model resulted in identifiability issues in estimating parameter values. Although many models have been proposed for studying the cyclic regulation of the glucose-insulin system, none of these models took into consideration the circadian oscillations in plasma glucose or insulin concentrations and but rather assumed constant baselines (Landersdorfer and Jusko, 2008).

Dosing MPL caused an up-regulation in leptin mRNA expression in white adipose tissue and plasma leptin with the peak occurring at approximately 12 h with concentrations eventually returning to the circadian baseline. Until now there has been no pharmacodynamic modeling of the effects of any endogenous compounds or drugs on leptin expression in adipose tissue or plasma leptin. However, one study characterized the pharmacokinetics and metabolism of leptin by

injecting radiolabeled leptin tracer into healthy male rats (Zeng et al., 1997). The half-life of plasma leptin was approximately 9.4 min, which agrees with the half-life value from our study of 10.7 min ($k_{dlep} = 3.894 h^{-1}$), giving confidence to our parameter estimates. Furthermore, studies from our group and others have shown that MPL reduces food intake in rats, which could be a direct effect of increased leptin expression after MPL (Jin and Jusko, 2009). The increase in plasma leptin after MPL suppresses appetite by acting on the hypothalamus and suppressing neuropeptide Y production and secretion, thereby suppressing food intake (Wang et al., 1997).

Adiponectin mRNA expression and plasma adiponectin showed a biphasic regulation after MPL with the initial up-regulation caused by the receptor-mediated effects of MPL and the delayed down-regulation caused by the increased plasma insulin induced by the drug. This biphasic regulation might be the cause for discrepancies observed in the literature on the effects of glucocorticoids on adiponectin expression. Because most previous studies were at single time points, depending on the time of measurement of adiponectin mRNA expression or plasma adiponectin after drug dosing, the outcome could vary, ranging from up-regulation to down-regulation or no regulation at all. Furthermore, glucocorticoids increase the secretion of adiponectin independent of the genomic effects that were observed in the plasma adiponectin profile and hence have been incorporated into the model (Cabanelas et al., 2010). This increase in secretion not only increases the peak plasma adiponectin and decreases the time of the peak, but also further decreases adiponectin at the nadir. This is caused by depletion of adiponectin from the precursor compartment consisting of the mature adipocytes (Hazra et al., 2006). Hence the decrease in plasma adiponectin below the baseline is not just a function of insulin regulation, but also occurs because of the depletion of adiponectin from mature adipocytes resulting from increased secretion at early times.

One of the salient features of this study is that the replicate data obtained from both the circadian and MPL experiments were modeled simultaneously to obtain parameter values. However, owing to the high interindividual variability in the biomarkers in the circadian study and the complexity of the model, estimates for some of the parameter values were less precise than desirable, which could potentially be improved by studying the dynamics of the system with different doses and performing either multiple dosing or chronic infusion studies. In addition, this information will be helpful in modifying and evolving the current model, which could be eventually used for understanding disease progression after corticosteroid dosing. This study is one of the first efforts that

TABLE 5

Parameter values for adiponectin mRNA and plasma adiponectin dynamics

Parameter	Definition	Estimate	CV%
$k_{d,adpm}$ (h^{-1})	Loss rate constant for adiponectin mRNA	2.562	Fixed
$S_{D_rn}^{Adpm}$ (nM^{-1})	Stimulation of adiponectin mRNA production	0.0034	Fixed
$I_{max,Ins}^{Adpm}$	Inhibition of adiponectin mRNA production	0.2138	38.49
$IC_{50,mpl}^{Adpm}$	Inhibition of adiponectin mRNA production	0.113	274.3
$k_{d,adp}$ (h^{-1})	Loss rate constant for adiponectin	0.0343	48.09
g	Amplification factor	3.706	48.99
$S_{max,mpl}^{AdpP}$	Stimulation of adiponectin production	0.5683	67.7
$SC_{50,mpl}^{AdpP}$ (ng/ml)	Stimulation of adiponectin production	570.7	177.1
$k_{d,adpp}$ (h^{-1})	Loss rate constant for plasma adiponectin	8.565	119
$Adp_m(0)$ (molecules/ng RNA)	Adiponectin mRNA initial concentration	53,100	Fixed
$Adp(0)$ ($\mu g/ml$)	Adiponectin in adipocytes initial concentration	32,090	N.E.
$AdpP(0)$ ($\mu g/ml$)	Plasma adiponectin initial concentration	6306	Fixed

N.E., not estimated.

Fig. 9. Simulated profiles of free cytosolic receptor (A), drug-receptor complex in cytosol (DR) (B), and nucleus (DR_n) (C) in rats given 50 mg/kg i.m. MPL. The inset in B shows DR truncated at 5 h.

quantitatively describes, integrates, and provides additional insights into the pharmacological effects of glucocorticoids on adipokine production and secretion along with related physiological factors that control these processes.

Authorship Contributions

Participated in research design: Sukumaran, Jusko, DuBois, and Almon.

Conducted experiments: Sukumaran and DuBois.

Performed data analysis: Sukumaran and Jusko.

Wrote or contributed to the writing of the manuscript: Sukumaran, Jusko, DuBois, and Almon.

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