

Mechanism-based mathematical modelling as an alternative to animal experiments

Johanne Gudmand-Hoeyer

November 13, 2019



The Novo Nordisk Foundation
Center for Biosustainability

My PhD project was funded by



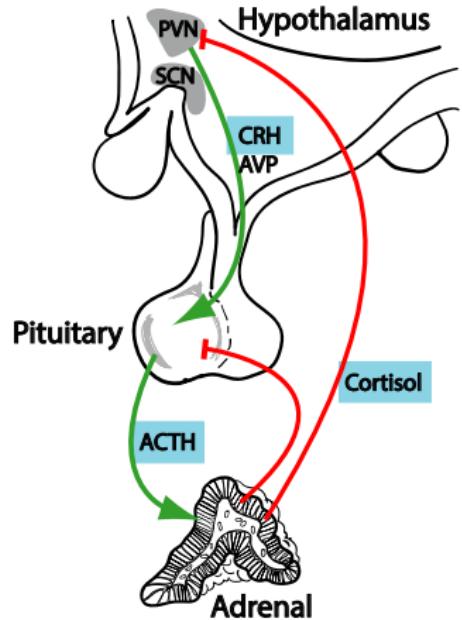
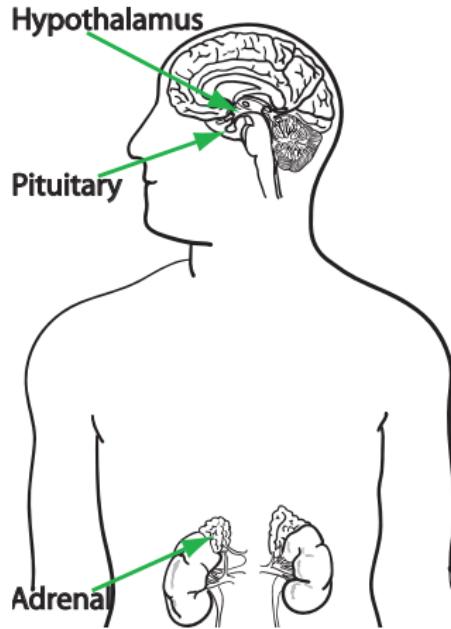
The 3Rs and mechanism-based mathematical modelling

Mathematical models build on current biological knowledge can be used

- in combination with *in vitro* assays instead of some *in vivo* experiments,
- to test biological hypotheses and generate new research questions,
- and to prioritize which experiments are the most important in the further knowledge building.

Mechanism-based modelling refers to the model equations being built on underlying biological mechanisms so that every parameter has a physiological interpretation.

The Hypothalamic-Pituitary-Adrenal (HPA) Axis

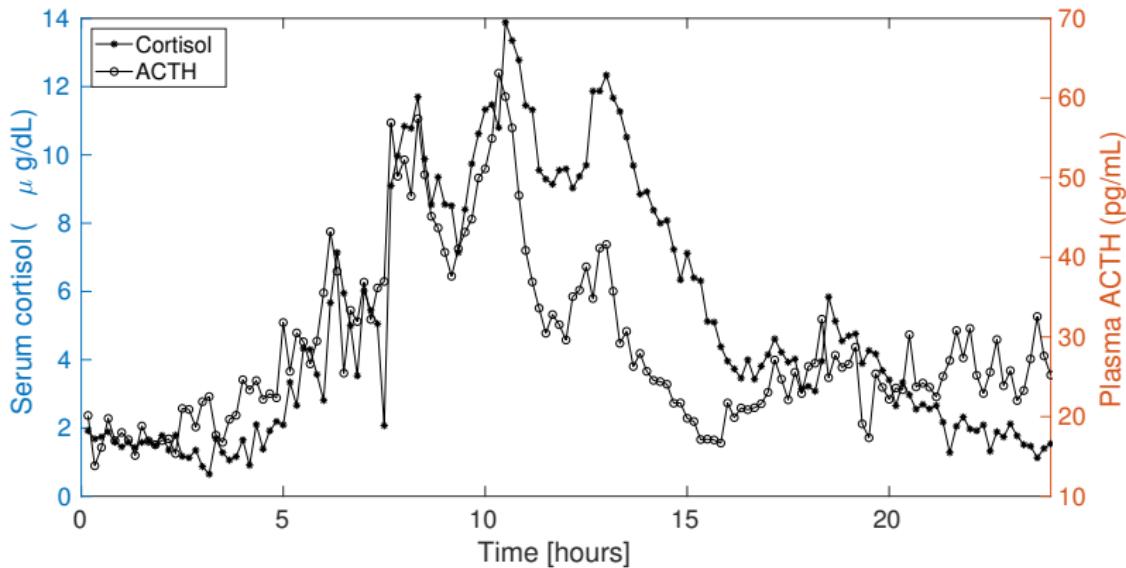


The neuroendocrine HPA axis controls the level of cortisol. The axis consists of feedforward and feedback mechanisms

between the hormones corticotropin-releasing hormone (**CRH**), vasopressin (**AVP**), corticotropin (**ACTH**) and **cortisol**.

Circadian and Ultradian Oscillations

Data from a healthy, young man:

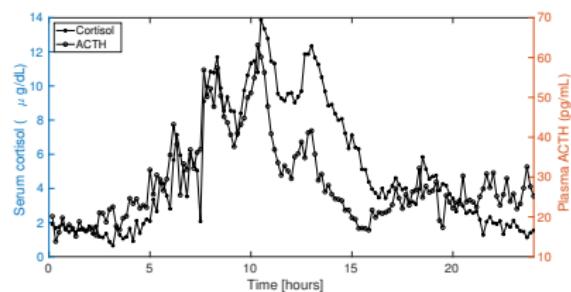


[Data from Carroll and Veldhuis]

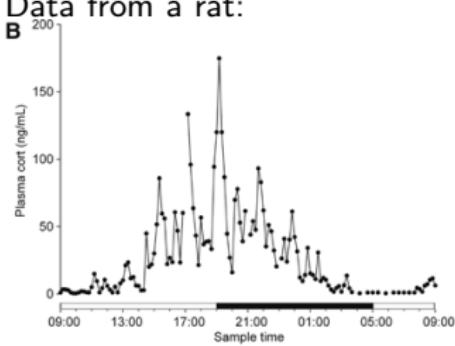
In the levels of the hormones there are a **circadian oscillation** of approximately 24 hours and some faster **ultradian oscillations** of approximately 20-90 minutes periods.

Circadian and Ultradian Oscillations

Data from a healthy, young man:



Data from a rat:

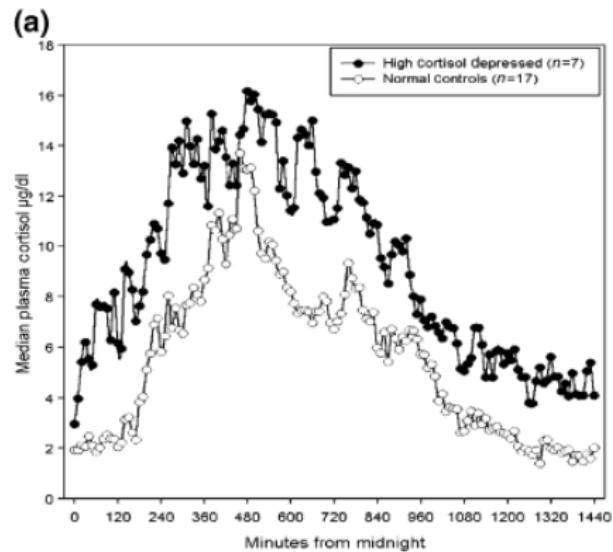


[Waite et al (2012) Eur. J. Neurosci., 36:3142–3150]

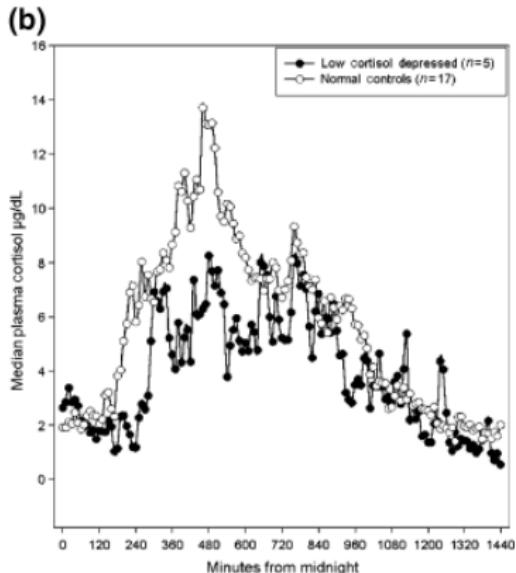
[Data from Carroll and Veldhuis]

Changed hormone concentrations in depressed patients

High cortisol depressed vs. Controls



Low cortisol depressed vs. Controls



[Carroll et al. (2007) Acta Psychiatr Scand, 115 (Suppl. 433), 90–103]

A high percentage of depressed patients have changed plasma concentrations of the HPA axis' hormones.

Animal Models of Depression

Commonly used animal models:

- Learned helplessness
- Forced swim test
- The tail suspension test
- Bilateral bulbectomy
- Social defeat stress
- Chronic mild stress

How to tell whether a mouse is depressed?



[Razafsha et al. (2013) Neuroscience, 240, 204–218]
[McGonigle (2014) Biochemical Pharmacology, 87, 140–149]

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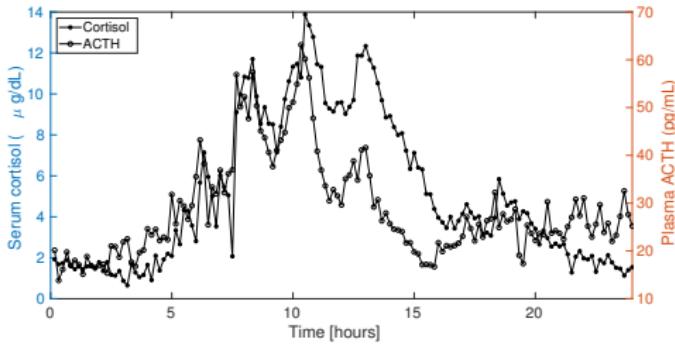
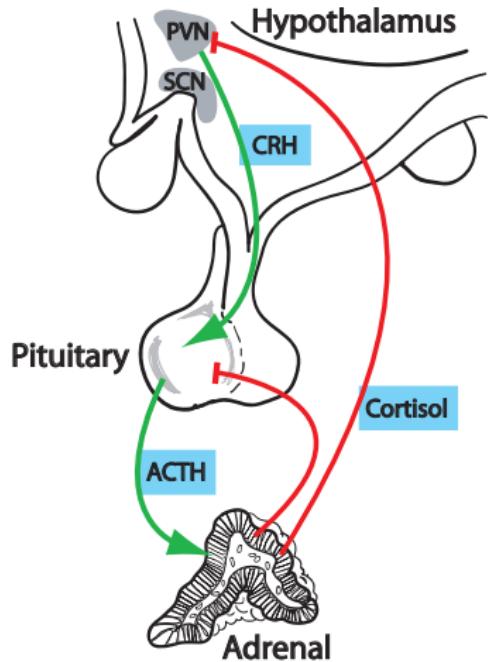
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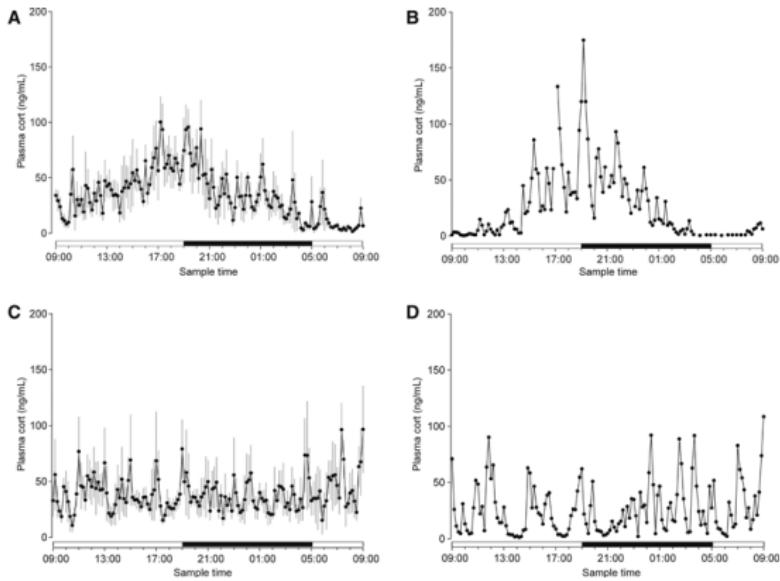
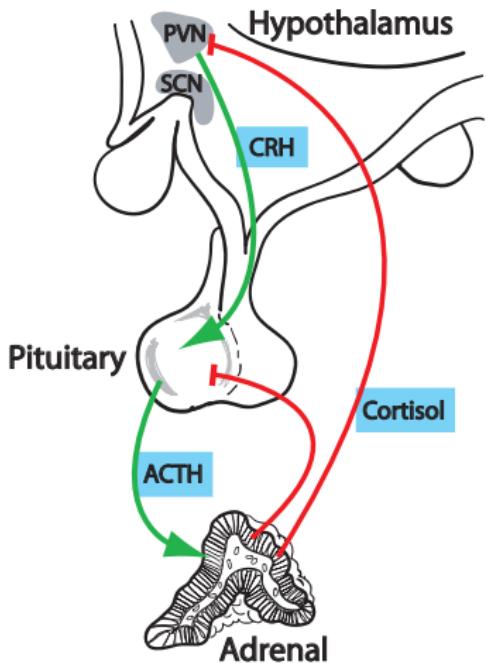
How to tell whether a mouse is depressed?



How to model the HPA axis?

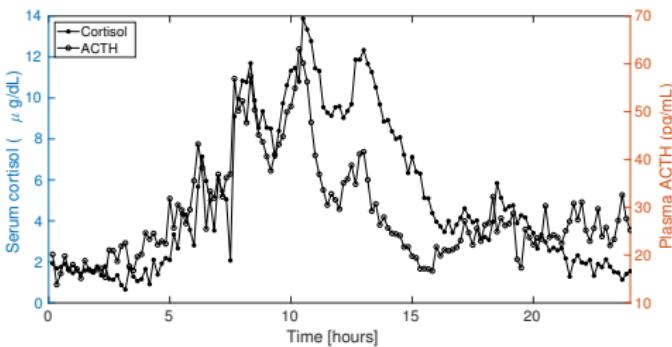
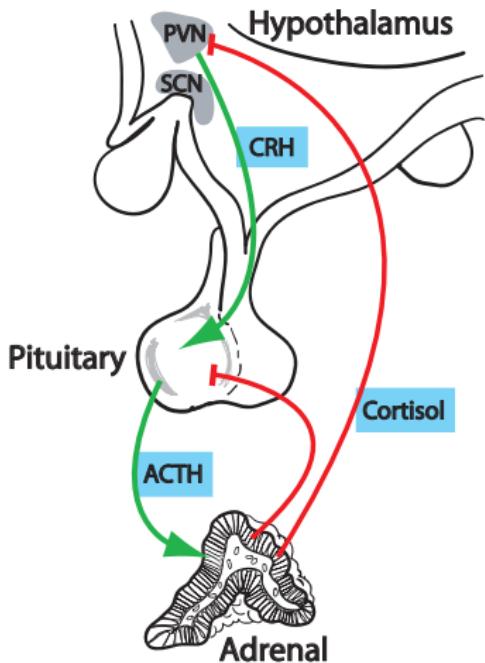


Rats with lesions to the SCN



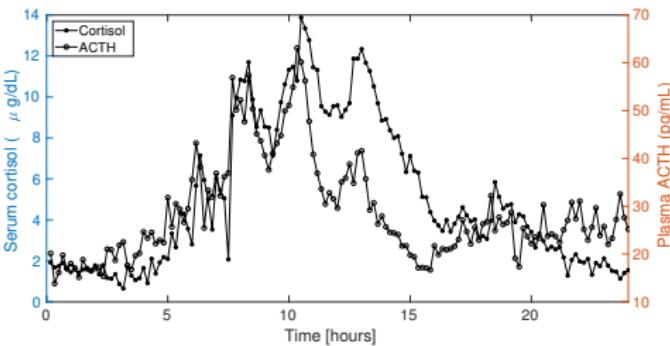
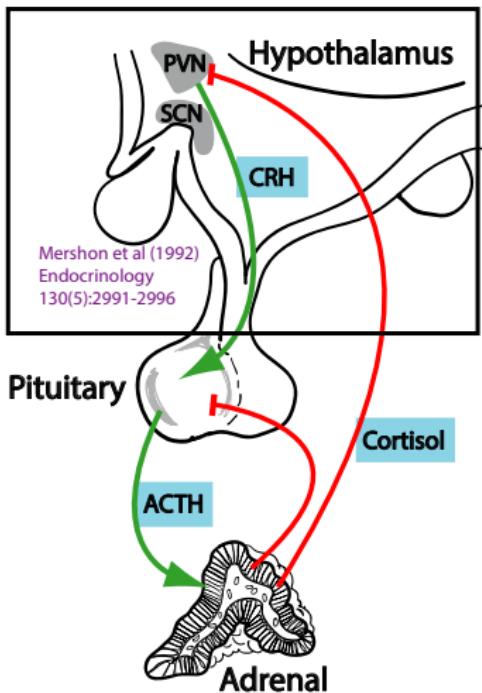
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The Origin of the Oscillations



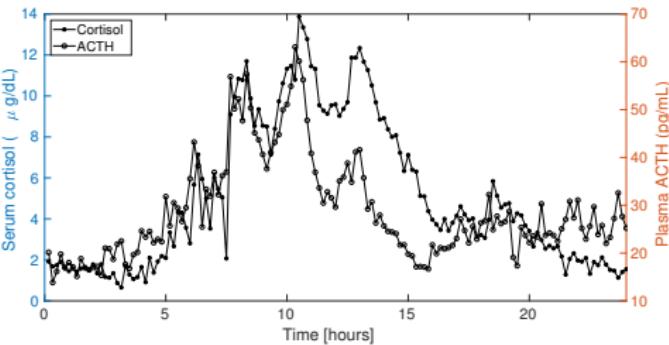
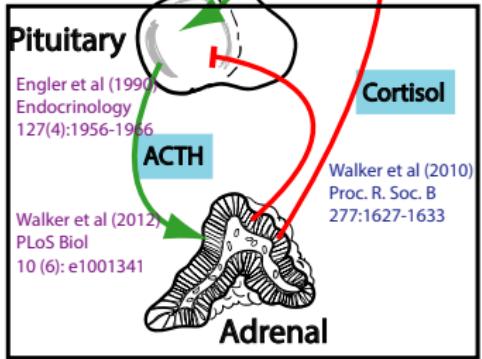
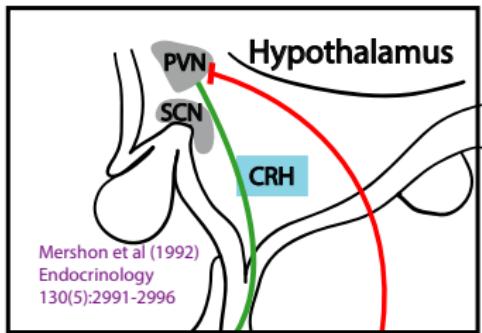
- The **circadian oscillations** enters the HPA axis from the Suprachiasmatic Nucleus (SCN),
- but where does the **ultradian oscillations** origin from?

The Origin of the Oscillations



- The **circadian oscillations** enters the HPA axis from the Suprachiasmatic Nucleus (SCN),
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The Origin of the Oscillations



- The **circadian oscillations** enters the HPA axis from the Suprachiasmatic Nucleus (SCN),
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A Mathematical Model with a Hypothalamic Oscillator

Mathematical Biosciences 257 (2014) 23–32



Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs



Patient-specific modeling of the neuroendocrine HPA-axis and its relation to depression: Ultradian and circadian oscillations



Johanne Gudmand-Hoeyer^a, Stine Timmermann^b, Johnny T. Ottesen^{a,*}

^aDepartment of Science, Systems and Models, Roskilde University, Building 27.1, 4000 Roskilde, Denmark

^bDepartment of Quantitative Pharmacology, H. Lundbeck A/S, 2500 Valby, Denmark

ARTICLE INFO

Article history:

Available online 3 September 2014

Keywords:

Depression

HPA-axis

Patient specific

Non-linear mixed effects ODE model

Parameter estimation

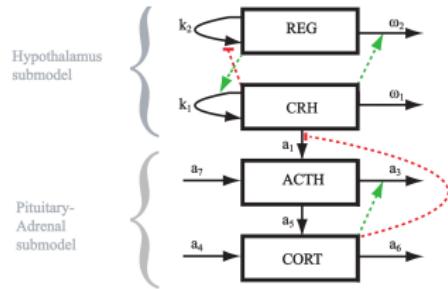
Bio-marker

ABSTRACT

In the Western world approximately 10% of the population experience severe depression at least once in their lifetime and many more experience a mild form of depression. Depression has been associated with malfunctions in the hypothalamus–pituitary–adrenal (HPA) axis. We suggest a novel mechanistic non-linear model capable of showing both circadian as well as ultradian oscillations of the hormone concentrations related to the HPA-axis. The fast ultradian rhythm is assumed to originate from the hippocampus whereas the slower circadian rhythm is assumed to be caused by the circadian clock. The model is able to describe the oscillatory patterns in hormone concentration data from 29 patients and healthy controls. Using non-linear mixed effects modeling with statistical hypothesis testing, three of the model parameters are identified to be related to depression. These parameters represent underlying physiological mechanisms controlling the average levels as well as the ultradian frequencies and amplitudes of the hormones ACTH and cortisol. The results are promising since they point toward an exact etiology for depression. As a consequence new biomarkers and pharmaceutical targets may be identified.

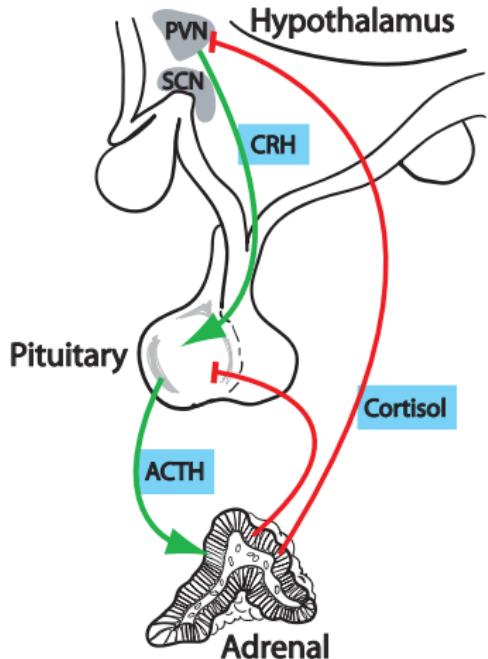
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A Mathematical Model with a Hypothalamic Oscillator

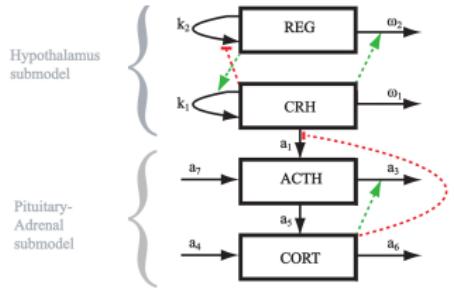


$$\begin{aligned}\frac{dx_1}{dt} &= k_1 \frac{x_2}{(1+x_2)} x_1 - \omega_1 x_1, \\ \frac{dx_2}{dt} &= k_2 \frac{1}{\alpha + x_1} x_2 - \frac{\omega_2 x_1}{\alpha + x_1} x_2, \\ \frac{dx_3}{dt} &= a_7 + a_1 \frac{a_2}{a_2 + x_4} x_1 - a_3 x_3 x_4, \\ \frac{dx_4}{dt} &= a_4 + a_5 x_3 - a_6 x_4.\end{aligned}$$

with $x_1 = [\text{CRH}]$, $x_2 = [\text{REG}]$, $x_3 = [\text{ACTH}]$, and $x_4 = [\text{CORT}]$.



Inclusion of the Circadian Clock



$$\begin{aligned}\frac{dx_1}{dt} &= k_1 \frac{x_2}{(1+x_2)} x_1 - \omega_1 x_1, \\ \frac{dx_2}{dt} &= k_2 \frac{1}{\alpha + x_1} x_2 - \frac{\omega_2 x_1}{\alpha + x_1} x_2, \\ \frac{dx_3}{dt} &= a_7 + a_1 \frac{a_2}{a_2 + x_4} x_1 - a_3 x_3 x_4, \\ \frac{dx_4}{dt} &= a_4 + a_5 x_3 - a_6 x_4.\end{aligned}$$

with $x_1 = [\text{CRH}]$, $x_2 = [\text{REG}]$, $x_3 = [\text{ACTH}]$, and $x_4 = [\text{CORT}]$.

We introduce a circadian rhythm:

$$\Omega(t) = 1 + \sin(\omega_c t + \delta)$$

where t denotes time, $\omega_c = \frac{2\pi}{1440} [\text{min}^{-1}]$ is the circadian clock frequency and δ the circadian phase.

We let four of the previous parameters be dependent upon this:

$$\begin{aligned}a_1 &= p_1 \cdot (2 - \Omega(t)), \\ a_3 &= p_3 + p_4 \cdot \Omega(t), \\ a_5 &= p_6 + p_7 \cdot (2 - \Omega(t)), \\ a_6 &= p_8 - p_9 \cdot (2 - \Omega(t)).\end{aligned}$$

In addition we simplify the model by choosing $\omega = \omega_1 = \omega_2$ and setting $a_4 = 0$

Analysis of the full HPA axis model

- If the circadian sinus-rhythm is substituted by a harmonic oscillator the system can become autonomous
- Existence and uniqueness of solution for any positive initial condition of the full system.
- Existence of a trapping region in R_+^4
- Uniformly persistent
- Small amplitude frequency $\omega_h = \sqrt{\frac{1 - \frac{\omega_1}{k_1}}{1 - \frac{\omega_2}{k_2}} \omega_1 \omega_2}$

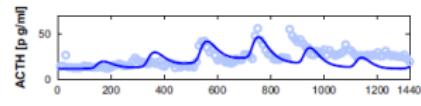
Patient specific parameter estimation

Parameter Estimation conducted with the Shuffled Complex Evolution (SCE) algorithm.

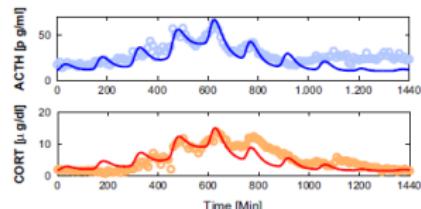
figure a is the data (o) and model output (-) for a hypocortisolemic,

figure b is for a normocortisolemic,

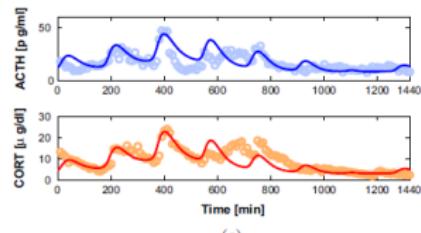
and figure c is for a hypercortisolemic individual.



(a)



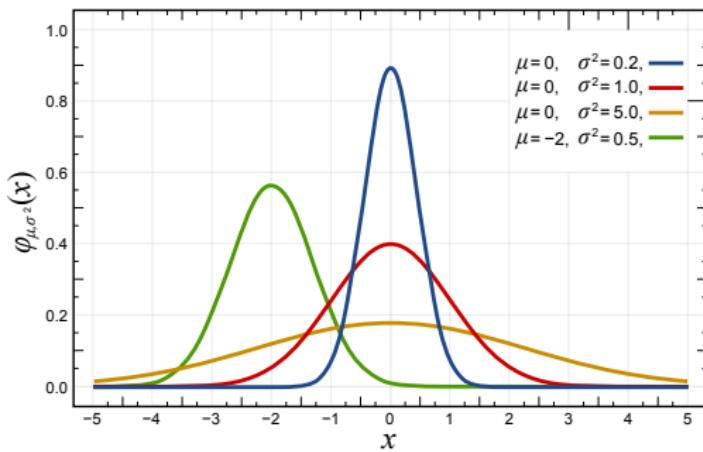
(b)



(c)

Non-Linear Mixed Effects (NLME) modelling

The goal is to decide whether a parameter is coming from a single population distribution or from the three sub-group distributions.



What NLME tells us...

$$\begin{aligned}\frac{dx_1}{dt} &= k_1 \frac{x_2}{(1+x_2)} x_1 - \omega_1 x_1, \\ \frac{dx_2}{dt} &= k_2 \frac{1}{\alpha + x_1} x_2 - \frac{\omega_2 x_1}{\alpha + x_1} x_2, \\ \frac{dx_3}{dt} &= a_7 + a_1 \frac{a_2}{a_2 + x_4} x_1 - a_3 x_3 x_4, \\ \frac{dx_4}{dt} &= a_4 + a_5 x_3 - a_6 x_4.\end{aligned}$$

with $x_1 = [\text{CRH}]$, $x_2 = [\text{REG}]$,
 $x_3 = [\text{ACTH}]$, and $x_4 = [\text{Cortisol}]$.

$$\begin{aligned}\Omega &= 1 + \sin(\omega_c(t + d)), \\ a_1 &= p_1(2 - \Omega), \\ a_3 &= p_3 + p_4\Omega, \\ a_5 &= p_6 + p_7(2 - \Omega), \\ a_6 &= p_8 - p_9(2 - \Omega).\end{aligned}$$

[Gudmand-Hoeyer et al (2014) Math Biosci 257:23–32]

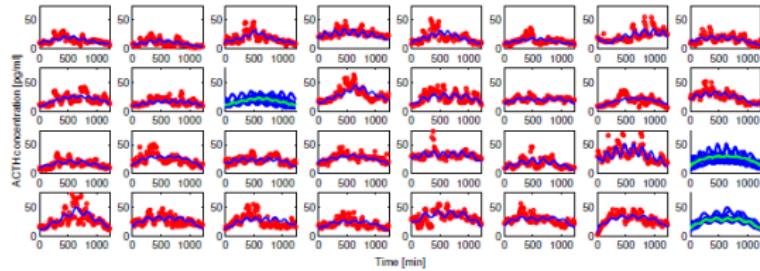
The three parameters p_1 , p_3 and ω describe the deviation between the three groups:

- p_1 is the circadian-dependent rate for how well CRH stimulates the synthesis of ACTH in the low cortisol limit
- p_3 is the circadian-independent component of the elimination of ACTH per cortisol concentration
- ω is the elimination rate for CRH (and REG).

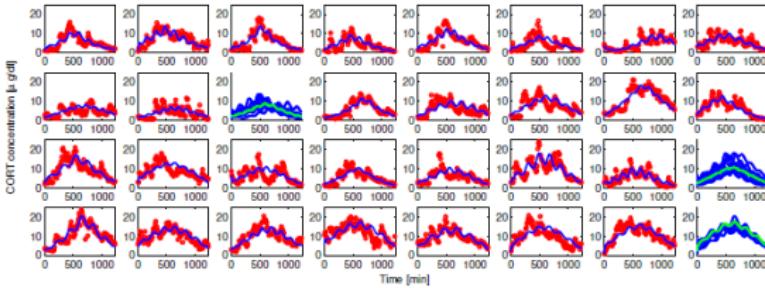
p_3 affects the average levels of ACTH and by that the level of cortisol. However, p_1 and ω have an impact on ultradian frequency and amplitude.

These three parameters are among the sensitive ones, but without being the three most sensitive parameters.

Trellis Plot



(a)



(b)

Above ACTH and below cortisol profiles of first the hypocortisolemic individuals (the 10 first subjects), the normocortisolemic individuals (the next 12 subjects) and then the hypercortisolemic individuals (the last 7 subjects) are shown.

A new equilibrium model for estimating free cortisol

Journal of Steroid Biochemistry and Molecular Biology 181 (2018) 109–124



Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb



Analysis and validation of a new extended method for estimating plasma free cortisol including neutrophil elastase and competition from other steroids



Johanne Gudmand-Hoeyer, Johnny T. Ottesen*

Department of Science and Environment, Roskilde University, Denmark

ARTICLE INFO

Keywords:
Cortisol
Corticosteroid-binding globulin
Mechanism based model
Progesterone
Testosterone
Neutrophil elastase

ABSTRACT

A non-linear mechanistic model for the distribution of cortisol in plasma on free and bound forms is proposed. The influence of progesterone, testosterone and neutrophil elastase on the cortisol distribution in the blood is investigated.

The activity of neutrophil elastase is directly included in the model with the concentration of elastase and the kinetic constants describing the activity of elastase collected in one single input variable. The model is very sensitive towards this input variable and fits data excellently, when it is allowed to be subject specific.

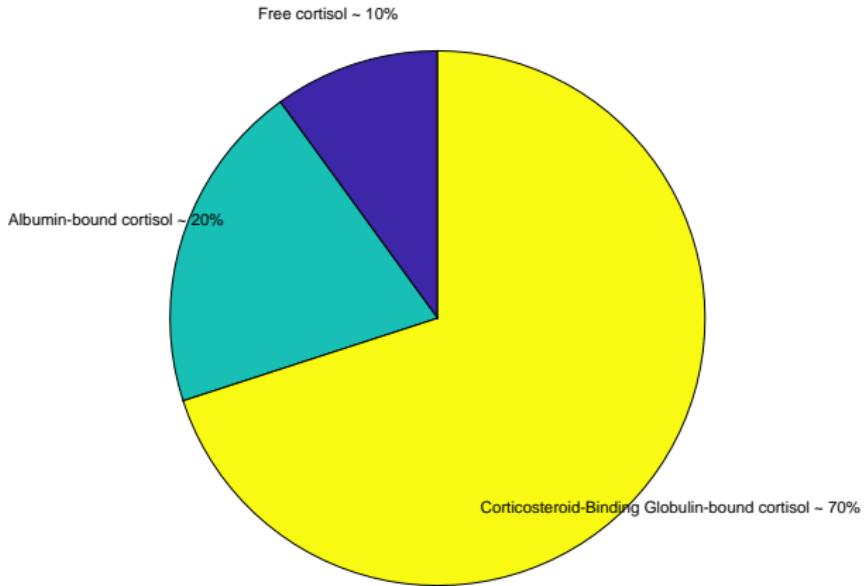
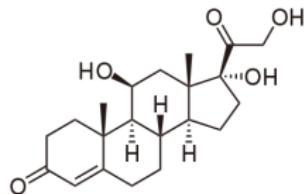
The analysis shows that steroids such as testosterone with low affinity for corticosteroid-binding globulin (CBG) do not significantly influence the concentration of free cortisol. Progesterone has a high affinity for CBG, but low plasma concentrations compared to cortisol. Contrary to expectations, progesterone is shown to impact the distribution of cortisol in plasma both under circumstances with high levels as seen in pregnancy and during the normal menstrual cycle of women.

Comparing the predictions of our model with predictions made with the equilibrium models by Coolens et al. [1], Dorin et al. [2] and Nguyen et al. [3] shows that the models differ considerably not only in their predictions for free cortisol, but also for cortisol on bound forms; i.e. bound to albumin, intact CBG and elastase-cleaved CBG.

Disregarding some of the smallest terms of the model equations a reduced version of the model in form of a fourth order polynomial equation is obtained. The reduced version of the model performs almost identically to the full version and serves as a new formula for calculating the plasma free cortisol concentration.

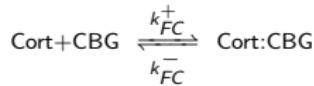
Cortisol in the blood

Cortisol is a steroid hormone.

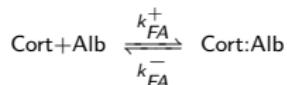


Hence, most of the cortisol in the blood is bound to transport proteins.

Cortisol in the blood - the models by Coolens et al (1987) and Dorin et al (2009)



$X_F = [\text{Cort}]$, $X_C = [\text{CBG}]$, $X_A = [\text{Alb}]$, $X_{FC} = [\text{Cort} : \text{CBG}]$, and $X_{FA} = [\text{Cort} : \text{Alb}]$, while $K_{FC} = \frac{k_{FC}^-}{k_{FC}^+}$ and $K_{FA} = \frac{k_{FA}^-}{k_{FA}^+}$. Assuming equilibrium and conservation of total concentrations (X_{F0} , X_{C0} , X_{A0}):



$$X_F X_C = K_{FC} X_{FC} \quad (1a)$$

$$X_F X_A = K_{FA} X_{FA} \quad (1b)$$

$$X_{F0} = X_F + X_{FA} + X_{FC} \quad (1c)$$

$$X_{C0} = X_C + X_{FA} \quad (1d)$$

$$X_{A0} = X_{FA} + X_A \quad (1e)$$

[Dorin et al. (2009) Clin. Biochem. 42, 64–71] state the model:

$$X_F^3 + d_2 X_F^2 + d_1 X + d_0 = 0 \quad (2a)$$

$$d_2 = X_{A0} + X_{C0} - X_{F0} + K_{FA} + K_{FC},$$

$$d_1 = X_{A0} K_{FC} + X_{C0} K_{FA} - X_{F0} (K_{FA} + K_{FC}) + K_{FA} K_{FC},$$

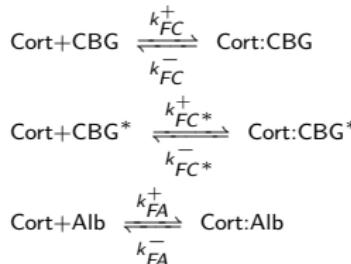
$$d_0 = -K_{FA} K_{FC} X_{F0}.$$

In [Coolens et al. (1987) J. Steroid Biochem., 26 (2), 197–202] albumin is included, but simplified by assuming $N = \frac{X_{FA}}{X_F} = \frac{X_{A0}}{K_{FA} + X_F} \approx \frac{X_{A0}}{K_{FA}} \approx 1.74$,

which results in

$$X_F^2 \cdot K_{FC} (1 + N) + X_F (1 + N + K_{FC} (X_{C0} - X_{F0})) - X_{F0} = 0$$

Cortisol in the blood - the model by Nguyen et al (2014)



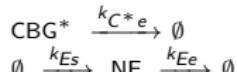
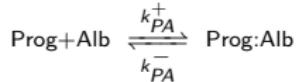
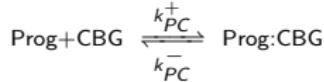
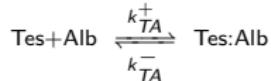
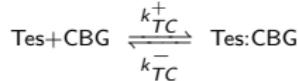
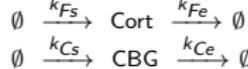
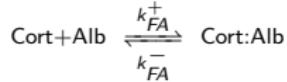
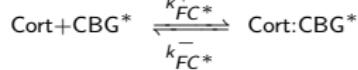
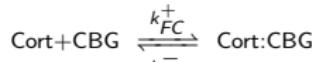
[Nguyen et al. (2014) J. Steroid Biochem. Mol. Biol. 141, 16–25] included the total concentration of cleaved CBG* as an input variable and stated the following model:

$$\begin{aligned} X_F^4 + n_3 X_F^3 + n_2 X_F^2 + n_1 X_F + n_0 &= 0, \\ n_3 &= K_{FA} + K_{FC} + K_{FC^*} + X_{A0} + C_{tot} + C_{tot}^* - X_{F0}, \\ n_2 &= K_{FA} K_{FC} + K_{FA} K_{FC^*} + K_{FC} K_{FC^*} + X_{A0} (K_{FC} + K_{FC^*}) \\ &\quad + C_{tot} (K_{FA} + K_{FC^*}) + C_{tot}^* (K_{FA} + K_{FC}) - X_{F0} (K_{FA} + K_{FC} + K_{FC^*}), \\ n_1 &= K_{FA} K_{FC} K_{FC^*} + X_{A0} K_{FC} K_{FC^*} + C_{tot} K_{FA} K_{FC^*} \\ &\quad + C_{tot}^* K_{FA} K_{FC} - X_{F0} (K_{FA} K_{FC} + K_{FA} K_{FC^*} + K_{FC} K_{FC^*}), \\ n_0 &= -X_{F0} K_{FA} K_{FC} K_{FC^*} \end{aligned} \tag{3a}$$

with the total concentrations of cortisol (X_{F0}), albumin (X_{A0}), native CBG (C_{tot}), cleaved CBG (C_{tot}^*) as input variables, the dissociation equilibrium constants K_{FA} , K_{FC} , K_{FC^*} as parameters, and free cortisol (X_F), cortisol bound to albumin (X_{FA}), cortisol bound to CBG (X_{FC}), and cortisol bound to CBG* (X_{FC^*}) as output.

[Nguyen et al. (2014): J. Steroid Biochem. Mol. Biol. 141, 16–25]

Cortisol in the blood - the new model

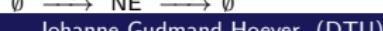
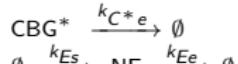
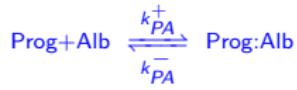
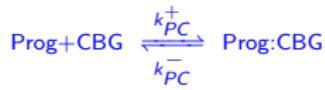
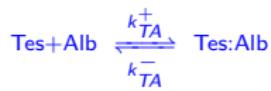
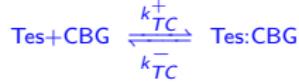
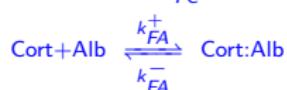
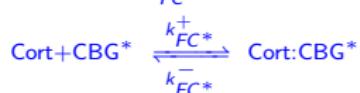
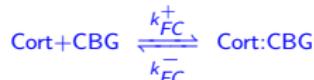


The competitive bindings of **testosterone** (Tes) and **progesterone** (Prog) to CBG and albumin are included.

The enzymatic cleavage of CBG to CBG* by **neutrophil elastase** (NE) is included.

[Gudmand-Hoeyer & Ottesen (2018) J.SteroidBiochem.Mol.Biol. 181: 109–124]

Cortisol in the blood - the new model



Using the law of mass action and assuming equilibrium we obtain ordinary equilibrium relations for the steroid-protein bindings,

$$X_F X_C = K_{FC} X_{FC} \quad (4a)$$

$$X_F X_{C^*} = K_{FC^*} X_{FC^*} \quad (4b)$$

$$X_F X_A = K_{FA} X_{FA} \quad (4c)$$

$$X_T X_C = K_{TC} X_{TC} \quad (4d)$$

$$X_T X_A = K_{TA} X_{TA} \quad (4e)$$

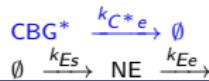
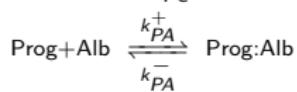
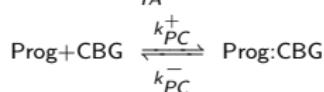
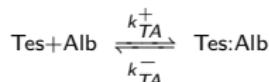
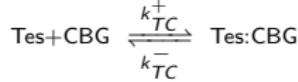
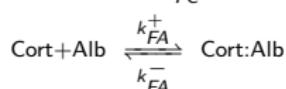
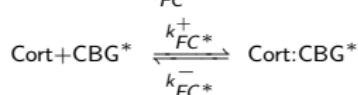
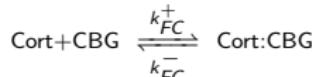
$$X_P X_C = K_{PC} X_{PC} \quad (4f)$$

$$X_P X_A = K_{PA} X_{PA} \quad (4g)$$

with $K_{FC} = \frac{k_{FC}^-}{k_{FC}^+}$, $K_{FC^*} = \frac{k_{FC^*}^-}{k_{FC^*}^+}$, $K_{FA} = \frac{k_{FA}^-}{k_{FA}^+}$, $K_{TC} = \frac{k_{TC}^-}{k_{TC}^+}$, $K_{TA} = \frac{k_{TA}^-}{k_{TA}^+}$, $K_{PC} = \frac{k_{PC}^-}{k_{PC}^+}$, $K_{PA} = \frac{k_{PA}^-}{k_{PA}^+}$ being the equilibrium dissociation constants of the respective reactions.

[Gudmand-Hoeyer & Ottesen (2018): J.SteroidBiochem.Mol.Biol. 181, 109–124]

Cortisol in the blood - the new model



$\emptyset \xrightarrow{k_{Es}} \text{NE} \xrightarrow{k_{Ee}} \emptyset$

Assuming an equilibrium between the elimination and enzymatic synthesis of CBG^* gives,

$$k_{C*}e \cdot X_{C*} = k_{C*}^+ \cdot X_{CE} \quad (5)$$

Making a standard quasi steady state approximation, where the concentration of CBG:NE is assumed constant compared to the changes in the concentrations of other species, gives,

$$X_{CE} = \frac{k_{CE}^+}{k_{CE}^- + k_{C*}^+} \cdot X_E \cdot X_C \quad (6)$$

Combining equation 5 and 6 results in,

$$\frac{X_{C*}}{X_C} = \frac{X_E}{K_{CE}} = n_{CE} \quad (7)$$

with an approximated 'equilibrium dissociation constant'

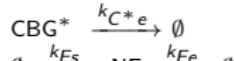
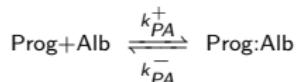
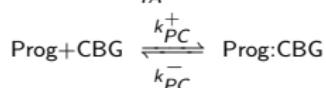
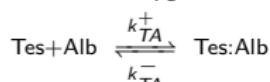
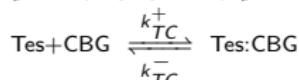
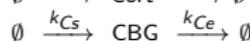
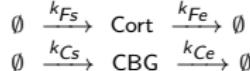
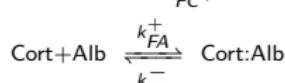
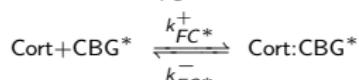
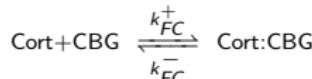
$$K_{CE} = \frac{k_{C*}e}{k_{C*}^+} K_{MCE} \text{ proportional to the Michaelis-Menten constant}$$

$$K_{MCE} = \frac{k_{CE}^- + k_{C*}^+}{k_{CE}^+}.$$

We will regard n_{CE} a measure of elastase activity.

[Gudmand-Hoeyer & Ottesen (2018): J.SteroidBiochem.Mol.Biol. 181, 109–124]

Cortisol in the blood - the new model



Assuming conservation of total concentrations of the involved substances:

$$X_{C0} = X_C + X_{C^*} + X_{FC} + X_{FC^*} + X_{TC} + X_{PC} + X_{CE} \quad (8a)$$

$$= X_C \left(1 + \frac{X_E}{K_{CE}} + \frac{X_E}{K_{M_{CE}}} + \frac{X_F}{K_{FC}} \left(1 + \frac{X_E K_{FC}}{K_{CE} K_{FC^*}} \right) + \frac{X_T}{K_{TC}} + \frac{X_P}{K_{PC}} \right) \quad (8b)$$

$$X_{F0} = X_F + X_{FC} + X_{FC^*} + X_{FA}$$

$$= X_F \left(1 + \frac{X_C}{K_{FC}} \left(1 + \frac{X_E K_{FC}}{K_{CE} K_{FC^*}} \right) + \frac{X_A}{K_{FA}} \right) \quad (8c)$$

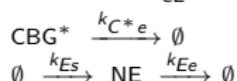
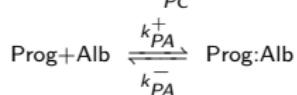
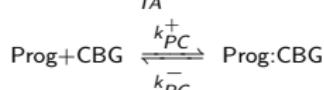
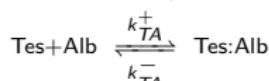
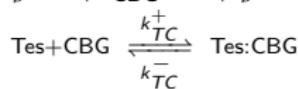
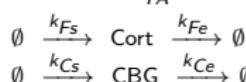
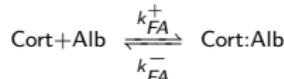
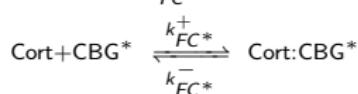
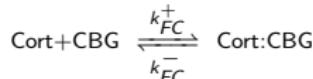
$$X_{T0} = X_T + X_{TC} + X_{TA} = X_T \left(1 + \frac{X_C}{K_{TC}} + \frac{X_A}{K_{TA}} \right) \quad (8d)$$

$$X_{A0} = X_A + X_{FA} + X_{TA} + X_{PA} = X_A \left(1 + \frac{X_F}{K_{FA}} + \frac{X_T}{K_{TA}} + \frac{X_P}{K_{PA}} \right) \quad (8e)$$

$$X_{P0} = X_P + X_{PC} + X_{PA} = X_P \left(1 + \frac{X_C}{K_{PC}} + \frac{X_A}{K_{PA}} \right) \quad (8f)$$

with X_{C0} being the total amount of CBG and CBG*, X_{F0} of cortisol, X_{T0} of testosterone, X_{A0} of albumin, and X_{P0} of progesterone in all forms.
 [Gudmand-Hoeyer & Ottesen (2018): J.SteroidBiochem.Mol.Biol. 181, 109–124]

Cortisol in the blood - the new model



By using relative variables $x_C = X_C/X_{C0}$, $x_F = X_F/X_{F0}$, $x_T = X_T/X_{T0}$, $x_A = X_A/X_{A0}$ and $x_P = X_P/X_{P0}$ and grouping the parameters, the following dimensionless form in five variables and with 13 parameters is obtained,

$$p_0 x_C + p_{CF} x_C x_F + p_{CT} x_C x_T + p_{CP} x_C x_P = 1 \quad (9a)$$

$$x_F + p_{FC} x_F x_C + p_{FA} x_F x_A = 1 \quad (9b)$$

$$x_T + p_{TC} x_T x_C + p_{TA} x_T x_A = 1 \quad (9c)$$

$$x_A + p_{AF} x_A x_F + p_{AT} x_A x_T + p_{AP} x_A x_P = 1 \quad (9d)$$

$$x_P + p_{PC} x_P x_C + p_{PA} x_P x_A = 1 \quad (9e)$$

where $p_0 = 1 + \frac{x_E}{K_{CE}} + \frac{x_E}{K_{MCE}} = 1 + \frac{x_E}{K_{CE}} (1 + \frac{k_{C^*e}}{k_{CE}^+}) \approx 1 + \frac{x_E}{K_{CE}} \approx 2.0$,

$$p_{FC} = \frac{x_{C0}}{K} \approx 19.9, \quad p_{FA} = \frac{x_{A0}}{K_{FA}} \approx 2.0, \quad p_{TC} = \frac{x_{C0}}{K_{TC}} \approx 1.4,$$

$$p_{TA} = \frac{x_{A0}}{K_{TA}} \approx 24.1, \quad p_{AF} = \frac{x_{F0}}{K_{FA}} \approx 6.1 \cdot 10^{-4},$$

$$p_{AT} = \frac{x_{T0}}{K_{TA}} \approx 1.8 \cdot 10^{-4}, \quad p_{CF} = \frac{x_{F0}}{K} \approx 6.6 \quad p_{CT} = \frac{x_{T0}}{K_{TC}} \approx 1.2 \cdot 10^{-2},$$

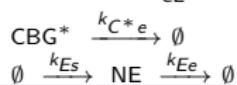
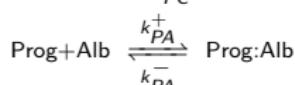
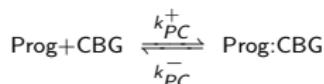
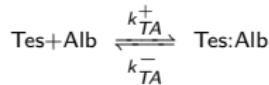
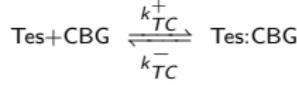
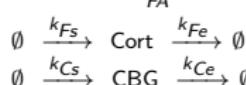
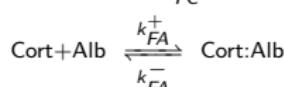
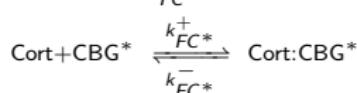
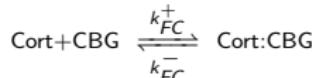
$$p_{CP} = \frac{x_{P0}}{K_{PC}} \approx 0.12, \quad p_{AP} = \frac{x_{P0}}{K_{PA}} \approx 3.0 \cdot 10^{-4}, \quad p_{PC} = \frac{x_{C0}}{K_{PC}} \approx 14.4,$$

$$\text{and } p_{PA} = \frac{x_{A0}}{K_{PA}} \approx 30.1, \text{ with } K = \frac{K_{FC}}{1 + \frac{x_E k_{FC}}{K_{CE} k_{FC^*}}} \approx 30.1.$$

We denote this the static model

[Gudmand-Hoeyer & Ottesen (2018) J.SteroidBiochem.Mol.Biol. 181: 109–124]

Cortisol in the blood - the new model



As the estimated values of the dimensionless parameters describe, the terms involving p_{AF} , p_{AT} and p_{AP} in equation (9d) are much smaller than x_A . This results in $x_A \approx 1$, which may be substituted into the equations 9a-9c and 9e. Hence, solving this system of equations is equivalent to solving

$$a_0 x_C^4 + a_1 x_C^3 + a_2 x_C^2 + a_3 x_C + a_4 = 0 \quad (10a)$$

$$x_F = \frac{1}{1 + p_{FA} + p_{FC} x_C} \quad (10b)$$

$$x_T = \frac{1}{1 + p_{TA} + p_{TC} x_C} \quad (10c)$$

$$x_P = \frac{1}{1 + p_{PA} + p_{PC} x_C} \quad (10d)$$

$$x_A = \frac{1}{1 + p_{AF} x_F + p_{AT} x_T + p_{AP} x_P} \quad (10e)$$

in sequential order, where the constant coefficients a_0 , a_1 , a_2 , a_3 and a_4 are expression in the earlier parameters.

We denote this the reduced static model

[Gudmand-Hoeyer & Ottesen (2018) J.SteroidBiochem.Mol.Biol. 181: 109–124]

Variation in reported equilibrium dissociation constants

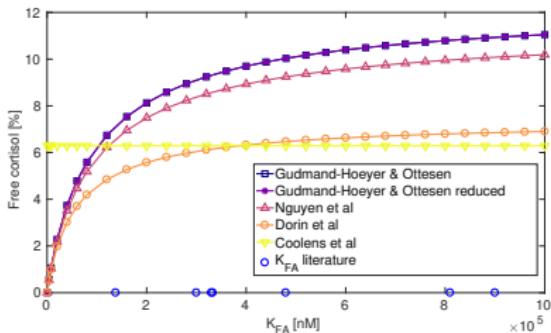
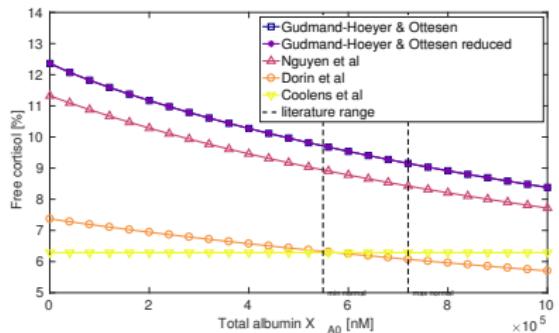
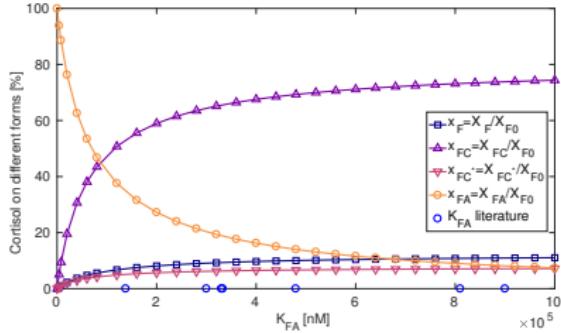
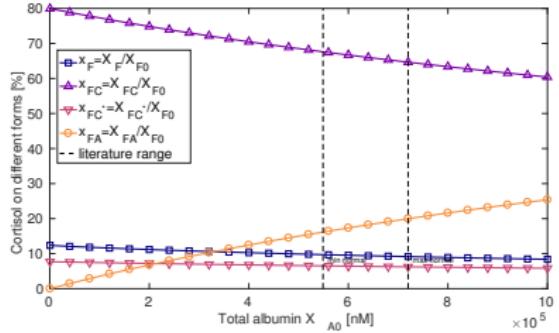
Table 1

Equilibrium dissociation constants.

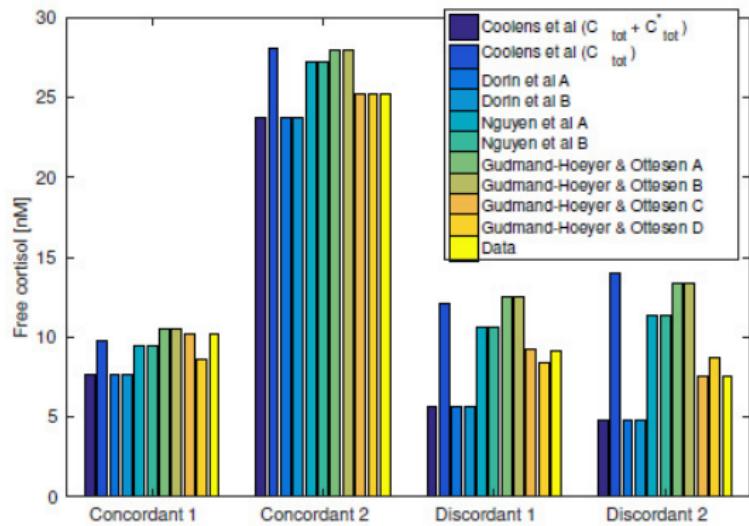
$K_{FC} = \frac{k_{TA}^-}{k_{TA}^+}$ for Cort:CBG	0.71 nM	[24] [#]	$K_{TA} = \frac{k_{TA}^-}{k_{TA}^+}$ for Tes:Alb	2.46×10^4 nM	[37] [#]
	11 nM	[25] [#]		2.50×10^4 nM	[7] [#]
	13 nM	[7,26] [#]		2.78 $\times 10^4$ nM	[38–40] [#]
	18 nM	[23]		2.80×10^4 nM	[27] [#]
	19 nM	[27] [#]		2.9×10^4 – 3.1×10^4 nM	[41] [#]
	21 nM	[28] [#]		4.00×10^4 nM	[42] [#]
	25 nM	[29–31] [#]	$K_{TC} = \frac{k_{TC}^-}{k_{TC}^+}$ for Tes:CBG	189 nM	[7] [#]
	32 nM	[19]		417 nM	[30] [#]
	33 nM	[32]		667 nM	[40] [#]
	39 nM	[33] [#]	$K_{PC} = \frac{k_{PC}^-}{k_{PC}^+}$ for Prog:CBG	11.1 nM	[43] [#]
	240 nM	[34]		38 nM	[31] [#]
	292.2 nM	[35]		41.7 nM	[7,26] [#]
$K_{FC^*} = \frac{k_{TA}^-}{k_{TA}^+}$ for Cort:CBG [*]	6.25 nM	[24] [#]		85 nM	[23]
	292 nM	[19]	$K_{PA} = \frac{k_{PA}^-}{k_{PA}^+}$ for Prog:Alb	1.67 $\times 10^4$ nM	[7] [#]
	1366.0 nM	[35]		9.0×10^4 nM (at pH 7.8)	[23]
	10 · K_{PC}	[3,19,24]		20.0×10^4 nM (at pH 6.8)	[23]
$K_{FA} = \frac{k_{TA}^-}{k_{TA}^+}$ for Cort:Alb	1.378×10^5 nM	[2]			
	3.00×10^5 nM (at pH 7.8)	[23]			
	3.30 $\times 10^5$ nM	[32]			
	3.33×10^5 nM	[7] [#]			
	4.796×10^5 nM	[33] [#]			
	8.10×10^5 nM	[36]			
	9.00×10^5 nM (at pH 6.8)	[23]			

Equilibrium dissociation constants with references. Some dissociation constants are calculated from the corresponding association constant marked by a [#] at the references. Our choice of default parameter values are emphasized in bold.

Albumin



Different models' predictions for four normal individuals



Model predictions compared to data of free cortisol for each of the four subjects Concordant 1, Concordant 2, Discordant 1, and Discordant 2 described in [Lewis & Elder, J. Steroid Biochem. Mol. Biol. 127 (3) (2011) 289–294.]. The brackets after Coolens refer to whether the results were calculated on total CBG ($C_{tot} + C_{tot}^*$) or only intact CBG (C_{tot}). In case A albumin affinity are chosen as $K_{FA}=330,000$ nM and $K_{FA}=137,800$ nM, respectively. Using the ratio of total albumin to its dissociation constant $N = X_{A0}/K_{FA} = 1.74$ of Coolens et al., the total albumin concentrations of case A and B are 574,200 nM and 239,772 nM, respectively. In case C with albumin affinity and concentration as in case A, the activity of neutrophil elastase n_{CE} is varied individually for each of the four subjects. In case D, the parameter k_{nce} is fitted as one value common for the four subjects.

Leaving out the progesterone, testosterone and elastase bound fractions we approximately have

$$C_{tot} \approx X_C \left(1 + \frac{X_F}{K_{FC}}\right)$$

$$C_{tot}^* \approx X_C \frac{X_E}{K_{CE}} \left(1 + \frac{X_F}{K_{FC}^*}\right).$$

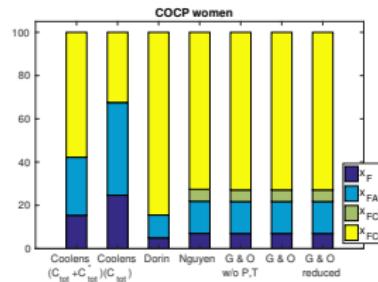
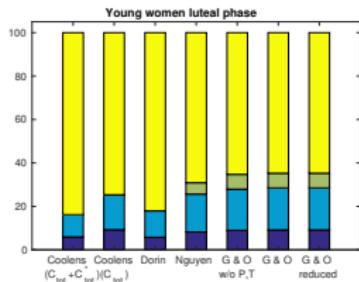
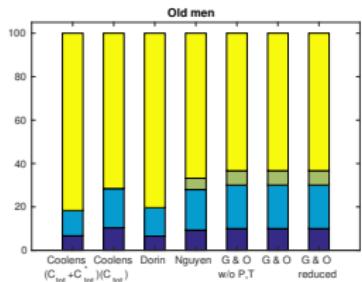
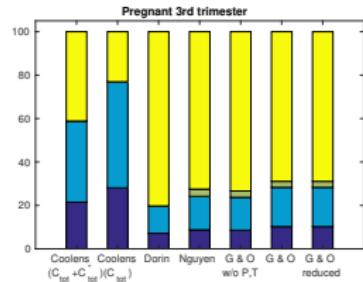
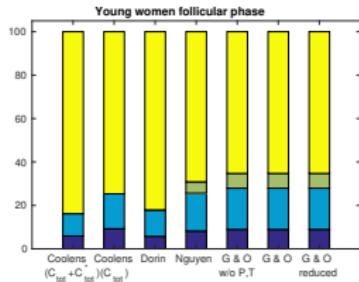
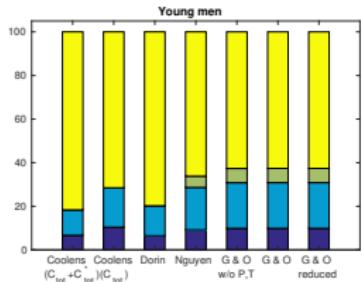
Looking at the fraction of C_{tot}^* to C_{tot} and isolating $n_{CE} = \frac{X_E}{K_{CE}}$ we get:

$$n_{CE} = \frac{X_E}{K_{CE}} = \frac{\left(1 + \frac{X_F}{K_{FC}}\right)}{\left(1 + \frac{X_F}{K_{FC}^*}\right)} \frac{C_{tot}^*}{C_{tot}}$$

$$= k_{nce} \frac{C_{tot}^*}{C_{tot}} = k_{nce} \frac{n_{CC}^*}{1 - n_{CC}^*},$$

$$\text{where } k_{nce} = \frac{\left(1 + \frac{X_F}{K_{FC}}\right)}{\left(1 + \frac{X_F}{K_{FC}^*}\right)}$$

Differences in model predictions across population groups

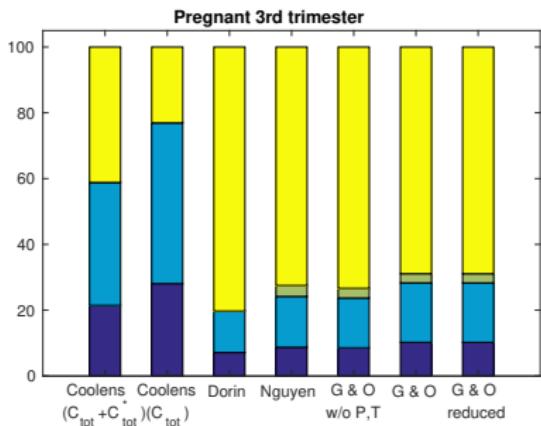
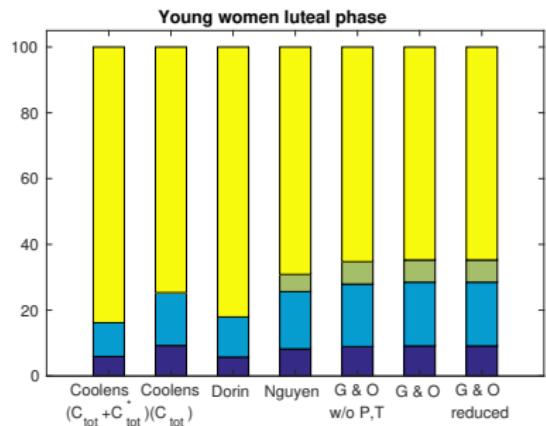


[Cort] (dark blue), [Cort:Alb] (light blue),

[Cort:CBG*] (green), [Cort:CBG] (yellow)

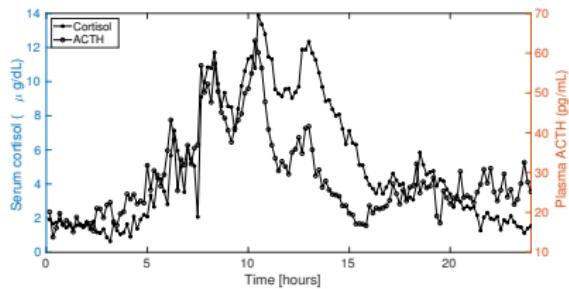
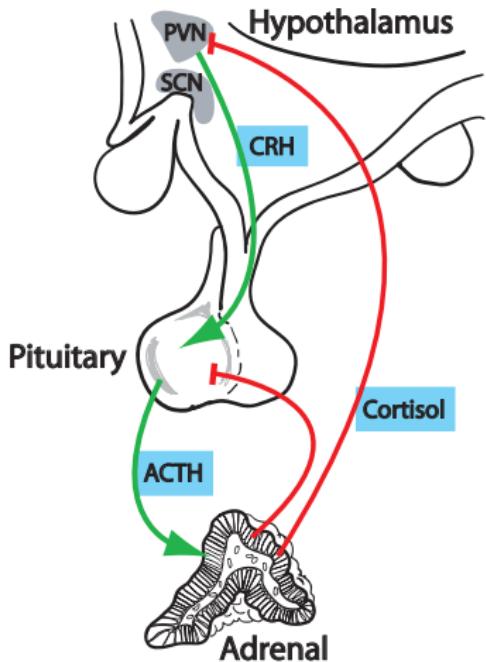
in percentage of total cortisol.

Differences in model predictions across population groups



[Cort] (dark blue), [Cort:Alb] (light blue), [Cort:CBG*] (green),
and [Cort:CBG] (yellow) in percentage of total cortisol.

Mechanism-based modelling



Acknowledgement

Co-authors and great inspiration:

Professor Johnny T Ottesen & PhD Stine Timmermann

Helpful discussions during my stay at Duke University:

Professor Michael C. Reed

Cortisol and ACTH data from:

Dr. Johannes D. Veldhuis & Dr. Bernard J. Carroll

Helpful discussions on parameter values:

Professor Dr. Richard I. Dorin

Thank You for Your Attention!

The PhD project was funded by



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