

Objective: to describe the evolution of hepcidin and iron serum concentrations during the menstrual cycle in healthy women.

Context

- ★ Iron is a key element for the organism
 - limiting digestive absorption
 - transported by ferroportin, a cellular iron-exporter located on macrophages, hepatocytes and enterocytes
 - serum levels regulated through sophisticated storage mechanisms
 - ★ Hepcidin is a peptidic hormone synthesized by the hepatocytes
 - regulates iron storage and release by interacting with ferroportin to prevent iron from being released into the general circulation
 - increasingly used for the differential diagnostic of iron-related disorders such as anemia or iron overload
 - ★ Significant variations of iron-status variables during the menstrual cycle [1]
 - ★ Loss of iron during menses triggers compensating regulation mechanisms
 - hepcidin also shown to exhibit circadian variations
 - different baseline values according to gender and, for women, to age
 - no study investigated possible variations of hepcidin during the menstrual cycle
- ⇒ The Hepmen study was designed to follow serum levels of hepcidin and iron during the menstrual cycle

Methods

Experimental design

- ★ 90 healthy women aged 19 to 44 years recruited in 4 participating centers
- ★ Inclusion visit
 - one blood sample collected to verify inclusion criteria
- ★ Follow-up during one menstrual cycle
 - 6 fasting blood samples taken in the morning throughout the cycle, starting on the second day of menses
 - determination of iron status variables in serum (iron, hepcidin, hemoglobin, ferritin, transferrin, saturation)

Statistical model Non-linear mixed effect model for the j^{th} observation y_{ij} in subject i ($1 \leq i \leq N$, $1 \leq j \leq n_i$):

$$y_{ij} = f(\theta_i, t_{ij}, z_i) + \epsilon_{ij} \quad (1)$$

- f denotes the structural model
- z_i denotes the covariates for subject i
- θ_i denotes the individual parameters for i , modelled through a log-normal distribution:

$$\theta_{ik} = \mu_k e^{b_{ik}} \quad (2)$$
 where μ is a population fixed effect and b_i is the random effect for subject i
- ϵ_{ij} denotes the measurement error

Joint modelling

- ★ General pattern for both hepcidin and iron:
 - initial decrease during menses, followed by a rebound higher than baseline
 - stabilisation during the second half of the cycle
- ★ Considerable fluctuations observed
- ★ Final model of iron (Ir) and hepcidin (He) described by

$$\begin{aligned} \frac{dIr(t)}{dt} &= k_{synI}(t) - k_{outI}.Ir(t) \\ \frac{dHe(t)}{dt} &= k_{synH}(t)(1 + \alpha(Ir(t) - Ir_0)) - k_{outH}.He(t) \end{aligned} \quad (3)$$

starting from initial conditions

$$\begin{aligned} Ir(t=0) &= \frac{k_{synI_0}}{k_{outI_0}} \\ He(t=0) &= \frac{k_{synH_0}}{k_{outH_0}} \end{aligned} \quad (4)$$

- ★ Time-dependent synthesis and elimination rate constants

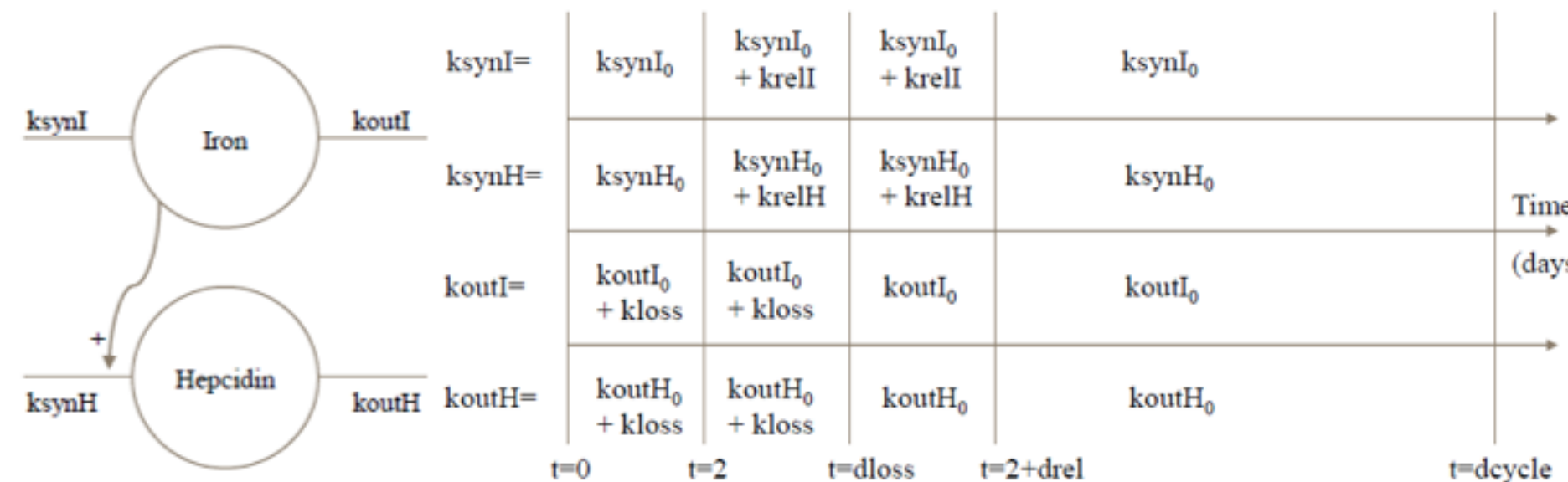


Figure 2: Joint model for iron and hepcidin in non menopausal women (left), and values of the parameters controlling the turnover of both molecules according to the time in menstrual cycle (right).

- Effect of demographic and biological covariates:
 - usage of contraception decrease the loss of iron
 - krelH: decreases with BMI and increases with hemoglobin
 - ksynH and koutH: increase with Higham's score
 - koutH and krelH: decrease with ferritin
 - krelH: increases with height
- Parameter estimates reported in Table 2 for the final model
- large variability for hepcidin

When should we dose hepcidin ?

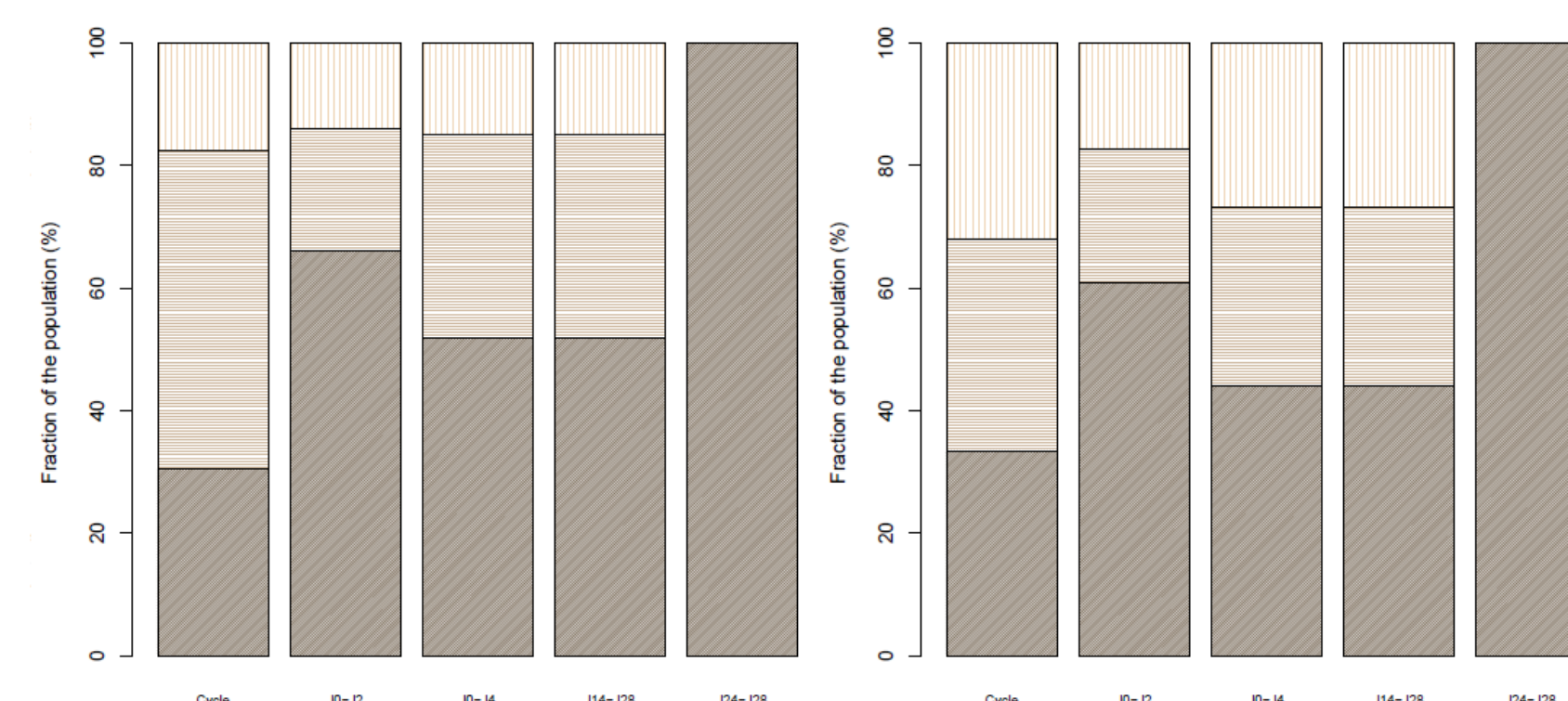


Figure 5: Variations of iron (left) and hepcidin (right) over different time periods during the cycle (dark: <20%, medium: 20-50%, light: over 50%).

- ★ Over the entire cycle, large variations in both iron and hepcidin concentrations in most women
- ★ Last days of the cycle: most stable period
 - ⇒ recommended time to measure both iron and hepcidin
- ★ However, significant residual variability (not taken into account in the simulation)

Parameter estimation

- ★ Parameter estimation using the SAEM algorithm [2]
 - Monolix version 4.3.2
 - models written in MLXTRAN
- ★ Model building
 - iron and hepcidin were modelled first separately then simultaneously with a link function
 - structural and variability model selection
 - log-likelihood ratio test for nested models
 - Bayesian criterion information for non-nested models

Covariate model building

- ★ First step: covariate exploration for each parameter
 - model with all available covariates
 - removal of covariates for which the Wald test had a p-value higher than 0.2
 - backward elimination of remaining non-significant covariates one by one, starting from the one with highest p-value, with a threshold of p=0.05
 ⇒ covariate model for each parameter
- ★ Second step: stepwise model building
 - full model including all covariate models found for each parameter
 - backward elimination of remaining non-significant covariates one by one (p=0.05)
- ★ Elimination of non-significant variances

Parameter	Estimate (RSE%)	IIV (RSE%)
ksynI ($L^{-1}.\mu\text{mol.d}$)	6.87 (5)	0.16 (14)
koutI (d^{-1})	0.38 (5)	0 (-)
kloss (d^{-1})	0.083 (22)	1.22 (16)
krelI ($L^{-1}.\mu\text{mol.d}$)	2.50 (12)	0.54 (21)
drel (d)	5.60 (6)	0 (-)
ksynH ($L^{-1}.\mu\text{mol.d}$)	1.00 (6)	0.29 (15)
koutH (d^{-1})	0.33 (8)	0.47 (12)
krelH ($L^{-1}.\mu\text{mol.d}$)	0.15 (24)	1.15 (17)
α	0.015 (45)	0.92 (49)
$b_{Iron} (a_{Iron})$	0.29(≈0)	
$b_{Hep} (a_{Hep})$	0.43(0.21)	
BIC	5396.41	
-2LL	5271.36	

Table 2: Estimates of the parameters and their variability for the final model.

Model evaluation

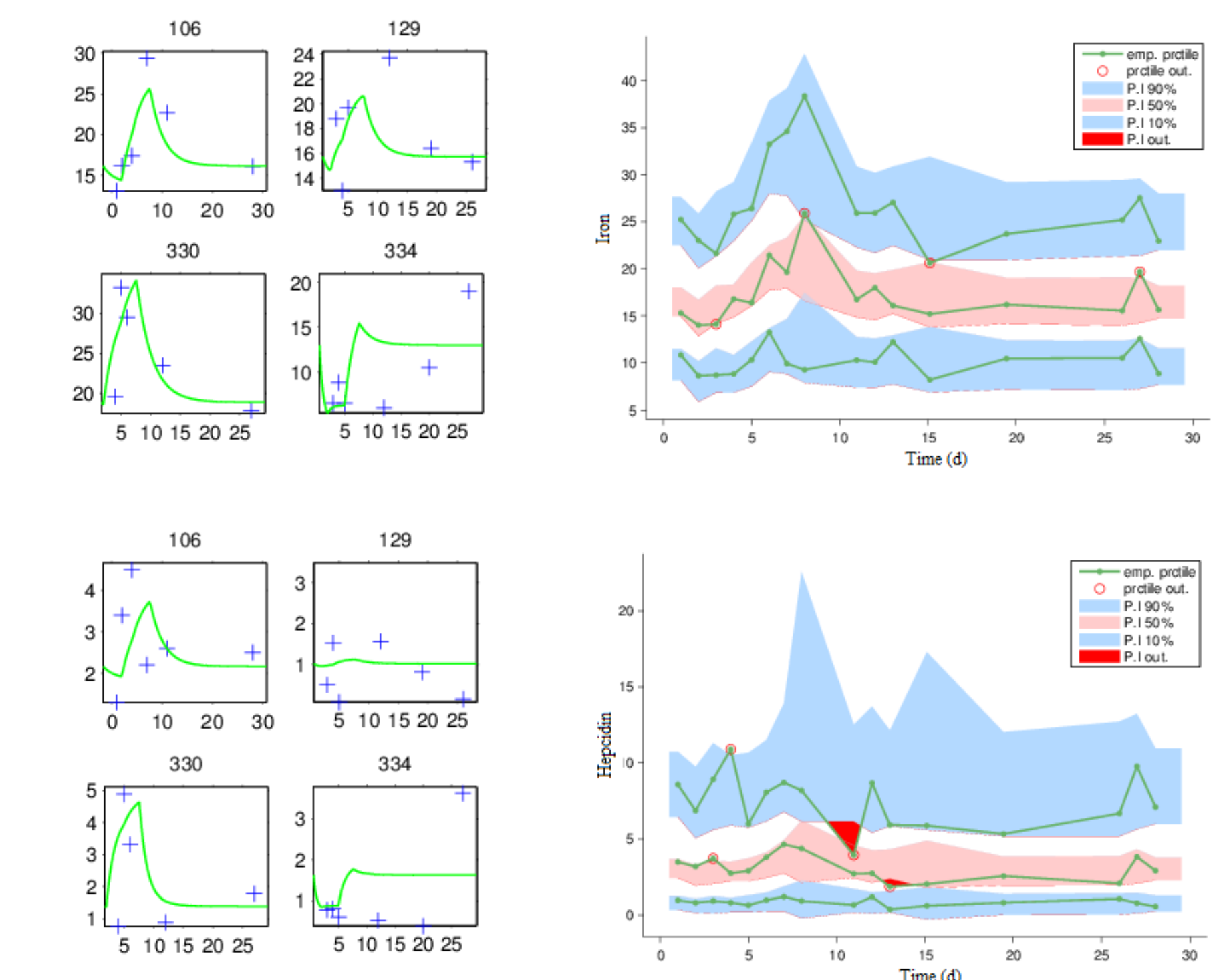


Figure 3: Individual fits for 4 subjects (left) and Visual Predictive Check (VPC, right) for the final model (top: iron, bottom: hepcidin).

- ★ Similar results with NPDE [3]

Simulations

Evolution during menstrual cycle

- ★ Joint model used to simulate full profiles of iron and hepcidin over the entire cycle
 - 200 simulated datasets with the same covariate distribution
 - stratification for method of contraception and age

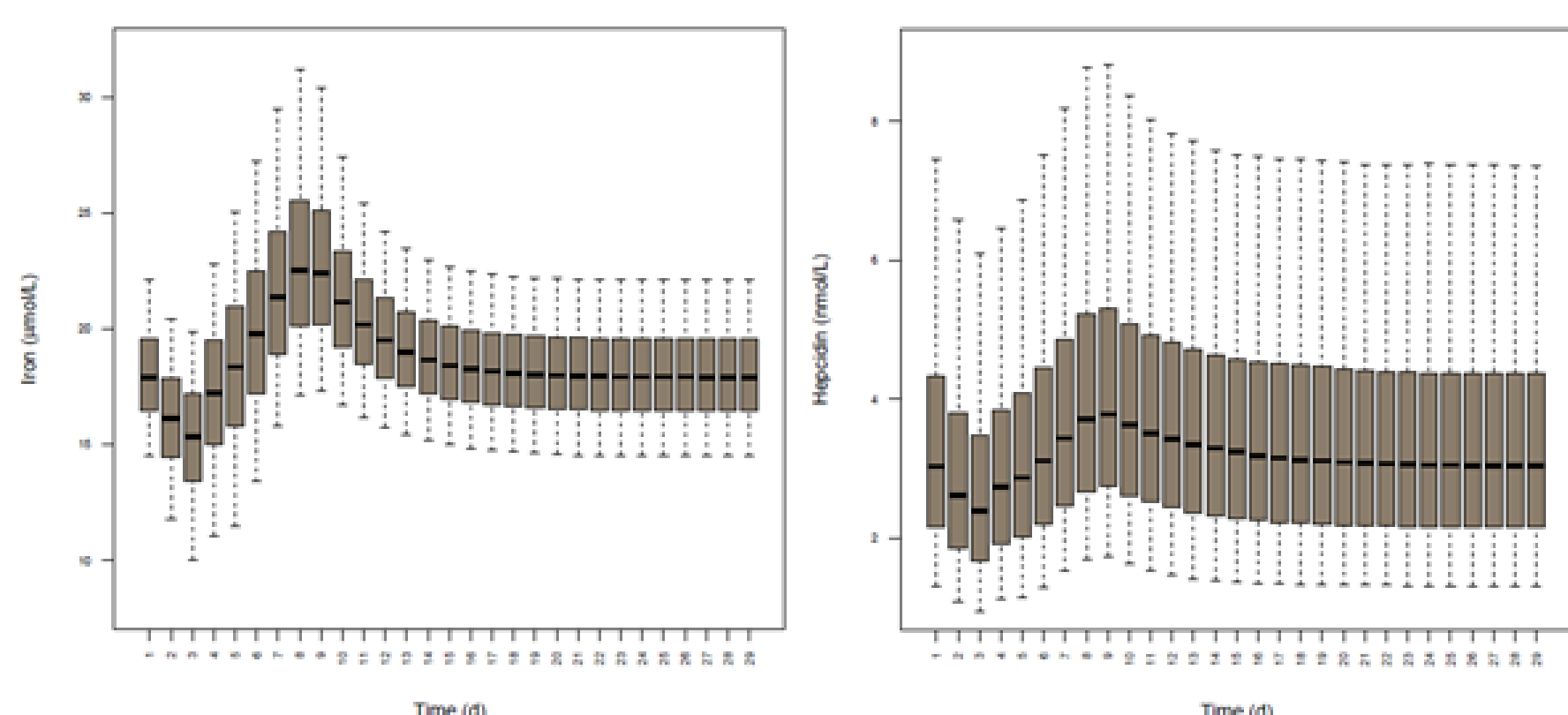


Figure 4: Simulated evolution of iron (left) and hepcidin (right) over one cycle (in women using contraception).

References

[1] I Kim, EA Yetley, and MS Calvo. Variations in iron-status measures during the menstrual cycle. *The American Journal of Clinical Nutrition*, 58:705–9, 1993.

[2] M Lavielle. Monolix (modèles non linéaires à effets mixtes). inria, orsay, france. URL <http://www.lixoft.eu/top-content/resources/docs/UsersGuide.pdf>, 2014.

[3] E Comets, K Brendel, and F Mentré. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for r. *Computer Methods and Programs in Biomedicine*, 90:154–66, 2008.