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 peptide 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 diagnosis 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 Heppen study was designed to follow serum levels of iron and hepcidin during the menstrual cycle

Results

Study population
Baseline characteristics of the population are summarised in Table 1:
- Demographic covariates recorded at inclusion
- Baseline value for iron-status variable defined as the concentration measured at the end of cycle visit

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>27.6 (6.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.4 (6.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.8 (9.2)</td>
</tr>
<tr>
<td>BMI (kg.m$^{-2}$)</td>
<td>22.6 (3.0)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.2 (9.6)</td>
</tr>
<tr>
<td>Higham’s score (%)</td>
<td>96.6 (22.6)</td>
</tr>
<tr>
<td>Serum transferrin (g.L$^{-1}$)</td>
<td>53.1 (42.3)</td>
</tr>
<tr>
<td>Serum ferritin (ng.L$^{-1}$)</td>
<td>15.0 (5.0)</td>
</tr>
<tr>
<td>Hemoglobin (g.dL$^{-1}$)</td>
<td>13.4 (1.7)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>83.1 (18.3)</td>
</tr>
</tbody>
</table>

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 the following system of differential equations:

\[
\begin{align*}
\frac{dIr}{dt} &= \text{krelH} (1 + a(y(t) - y_{\text{Ir}})) - \text{koutH}\text{He}(t) + \text{dIr}(t)
\end{align*}
\]

starting from initial conditions

\[
\begin{align*}
Ir(t=0) &= \text{Ir}_0
\end{align*}
\]

Parameter estimation

- Parameter estimation using the SAEM algorithm [2]
- Models 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

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

References

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>90% CI (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>krelH</td>
<td>-2.50</td>
<td>-3.64 to -1.35</td>
</tr>
<tr>
<td>koutH</td>
<td>0.54</td>
<td>0.35 to 0.73</td>
</tr>
<tr>
<td>klossH</td>
<td>0.31</td>
<td>0.19 to 0.44</td>
</tr>
<tr>
<td>krelI</td>
<td>0.52</td>
<td>0.38 to 0.66</td>
</tr>
<tr>
<td>koutI</td>
<td>0.38</td>
<td>0.29 to 0.47</td>
</tr>
<tr>
<td>klossI</td>
<td>0.37</td>
<td>0.28 to 0.47</td>
</tr>
</tbody>
</table>

Figure 1: Evolution of iron (left) and hepcidin (right) concentrations during the study. Each subject is represented by a different colour.

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 decreases the loss of iron
  - Knoll decreases with BMI and increases with hepcidin
  - KsynH and koutH: increase with higham score
  - krelH increases with height

- Parameter estimates reported in Table 2 for the final model
- Large variability for hepcidin
- Similar results with NPDE [3]

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