Points to Consider in the Design and Conduct of HPA-Axis Studies
Comparing the Systemic Safety of ICS-Products

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Background & Objectives

- A systematic quality review of published HPA-axis papers strongly suggests widespread failure to translate existing endocrinological knowledge on confounding factors, thereby resulting in highly variable and sometimes inconclusive HPA-axis study outcomes, which may be largely unrelated to the “true” ICS-product performance characteristics;

- For second-entry products aiming to demonstrate PD equivalence by HPA-studies, these confounding factors need to be more carefully addressed and rigorously controlled to accurately quantify ICS-mediated HPA-axis effects and managing the risk of non-product-related study failures;

- The presentation will review and summarize methodological key quality criteria of HPA-axis studies aiming for accurate quantification of systemic HPA-axis effects of ICS-products.

**Results of literature search** (01 Jan 2005 - 30 Jun 2012)

- Citations identified through systematic literature search: n = 80
  - Publications excluded by title and abstract: n = 49
    - paediatric age: 27
    - disease: 12
    - route: 2
  - Full-text articles retrieved for more detailed evaluation: n = 39
  - Studies excluded: n = 8
    - Substance not BD or FP: 4
    - Unclear study details: 2
    - Other: 2
  - Studies evaluated for method: n = 31 *)
    - UFC: 25
    - ACTH test: 4
    - A.M. cortisol: 4
    - CRF test: 1
    - Cortisol-AUC: 6
  - *) studies may deploy several methods

- Studies applying ‘gold’ standard method:
  - Cortisol-AUC: n = 6
Contents of Talk

• General considerations of PD-study outcomes & associated challenges;
• Physiology of HPA-axis regulation;
• Intrinsic and extrinsic factors altering HPA-axis function;
• Methods of assessment (i.e. most sensitive and robust target variables);
• Regulatory requirements (EMA OIP GL as example), incl. critical appraisal of regulatory requirements;
• Considerations regarding study design and conduct:
  – Study population: Screening procedures & subject selection
  – Study conduct: Thorough control of study conditions
  – Selected study design features
    (emphasis on sampling strategy & methodology)
  – Selected data analysis considerations
    (emphasis on outlier issues)
General Challenges of PD Study Outcomes

• Inter-subject variability of PD outcomes is generally larger than variability of PK outcomes;

• Understanding of determinants of PD variability is often incomplete, since the factors affecting PD-response and concentration-response relationships are numerous;

• A considerable part of the variability in PD studies (e.g. hormonal, CNS, cardiovascular) may originate from the characteristics of nonlinear systems with even a few degrees of freedom;

• In addition, the ligand–receptor interaction exhibits nonlinear dynamic behaviour when feedback mechanisms are involved (e.g. HPA-axis);

• Also the ligand-receptor interaction displays considerable inter-subject variability due to various intrinsic (e.g. genetic GCS-receptor-polymorphisms) and extrinsic/acquired (e.g. GCS-receptor resistance due to disease or stress) factors.

Which Read-Out to Select for the Assessment of Systemic Effects of Corticosteroids?

**Pleiotropic Clinical Effects of Glucocorticoids**

- Measures of GCS-mediated adrenal suppression (i.e. inhibition of cortisol secretion) are generally accepted as most sensitive and accessible markers for adverse systemic GCS effects;
- Cortisol secretion most frequently applied quantifiable marker for the assessment of systemic GCS exposure and potency;
- Recommended by EMA OIP GL;
- However, methodological aspects on how to assess HPA-axis function most reliably and sensitive, and important study design aspects are still important matters of debate;
- EMA OIP GL contains misconceptions and provides little useful methodological advice;
- Published HPA-axis papers indicate widespread failure to translate existing endocrinological knowledge into HPA-axis studies of ICS products.

**HPA Axis & Regulation – Important Intrinsic and Extrinsic Factors**

- Complex neuro-endocrine signaling system; mediates internal homoeostasis in response to internal and external stressors;
- Circadian/diurnal rhythmicity, i.e. sleep-wake cycle
- Ultradian pulsatile secretion with negative feedback loop;
- Both negative feedback and diurnal rhythm may be overcome by stress;
- Abrupt shifts of sleep periods associated with profound disruption in the daily cortisol rhythm;
- Cushing’s syndrome, insomnia, depression, chronic liver disease, sleep disordered breathing (SDB), and obesity display HPA-axis hyperactivity;
- Excessive alcohol consumption → increased cortisol levels & altered profiles;
- Genetic or acquired GC-Receptor insensitivity/resistance alters normal HPA-axis regulation (i.e. malfunction of negative feedback-loop).
24-hour Individual Plasma Cortisol Profile

- Pulsatile secretion of ACTH/Cortisol;
- Levels are highest on wakening & decline throughout the day, with nadir values in the evening;
- Higher ACTH pulse frequency in men compared with women (18 vs. 10 pulses/24 hrs);
- Probability to incidentally capture cortisol pulses during 24-hr profiling is also schedule dependent, i.e., increases with increased sample frequency;
- Capturing a single cortisol peak in an individual profile may significantly distort the respective AUC outcome.


Blood sampling and data evaluation strategies required to deal with incidental capturing of cortisol pulses!
Single-centre, randomized, double-blind, double-dummy, 4-period, 4-day repeat-dose crossover study with baseline evaluation comparing two strengths (i.e. 50/100 µg & 50/500 µg) of a SX-FP TEST DPI and Seretide Diskus®

However, capturing of cortisol pulses at the 23 & 24 hr samples of T1?

Substantial impact on AUC outcome!

Low-dose FP R&T treatments well comparable with BL profile;
Overall good dose-separation compared with high-dose treatments, in particular in the onset of circadian rise.

Hardly any dose-separation in the Quiescent Period

Lower daytime cortisol–levels and blunting of circadian rise by high-dose FP R&T treatments
Issue: Incidental Capturing of Cortisol Pulses or “Irregular Profiles”?

Single-centre, randomized, double-blind, double-dummy, 4-period, 4-day repeat-dose crossover study with baseline evaluation comparing two strengths (i.e. 50/100 µg & 50/500 µg) of a SX-FP TEST DPI and Seretide Diskus®

Two irregular profiles with BL heterogeneity, high cortisol levels despite (low-dose) FP treatment & atypical daytime peaks pointing to sleep-shifts or stress exposure?

or

Capturing of cortisol pulses in 2 out of 4 individual profiles?

**Substantial impact on AUC outcomes, depending on sampling schedule!**

**Remedy:**
Collection of 3 samples/time-point in 10-15 minute intervals, with pooling of samples;

or:
Individual quantification of all 3 samples and calculation of median.
Intrinsic and Extrinsic Factors Altering HPA-Axis Function

**Intrinsic Factors**
- Endocrinological diseases (M. Cushing);
- GCR-resistance due to genetic GCR-variants / polymorphisms;
- Psychiatric disorders, i.e. depression;
- Sleeping disorders, i.e. insomnia, sleep-disordered breathing (SDB);
- Chronic liver diseases, e.g. cirrhosis;
- Obesity

**Extrinsic Factors**
- Stress conditions, e.g. job-related (pre)burn out, private distress & conflicts, examinations, etc.;
- Abrupt changes in circadian rhythm such as shift-working or excessive weekend parties, etc.;
- Excessive alcohol consumption;
- Factors altering GCR-responsiveness/sensitivity, e.g. smoking (i.e. acquired GCR-resistance);
- Dietary changes, in particular carbohydrate- (via blood glucose – cortisol axis) and potassium-rich diets;
- Seasonal changes (daylight times).

All Ifs & EFs need to be carefully addressed at subject screening and enrolment;
All EFs need to be rigorously controlled by study design and conduct (e.g. in-house confinement)!

GCR = Glucocorticoid Receptor
GCR-resistance or insensitivity can be either inherited (i.e. genetic variants / polymorphisms) or acquired (e.g. depression, asthma, smoking, etc.);

Describes inter-subject variability in sensitivity to GC feedback suppression;

Variation in GC sensitivity also exists within the normal population with mechanistically unexplained sources (non-suppression in DST-test in 4% to >9% of subjects across studies!);

Most patients with GC resistance display increased plasma ACTH and serum cortisol concentrations, and elevated urinary cortisol secretion; the diurnal rhythm, however, is maintained;

Enrolment of subjects with poor sensitivity to GC feedback suppression into HPA-axis studies aiming for ICS-product comparison should be avoided;

Low-dose (0.25 or 0.5 mg) dexamethasone suppression test (DST) differentiates well between subjects with sensitive or insensitive GC feedback suppression.

van Rossum EFC & Lamberts SWJ. Glucocorticoid resistance syndrome. Best Practice & Research Clinical Endocrinology & Metabolism 2006; 20: 611-26
Different Methods to Assess HPA-Axis Suppression by Corticosteroids: Baseline Values of Various Dose Groups Before Prednisolone Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>2.5 mg baseline</th>
<th>5 mg baseline</th>
<th>7.5 mg baseline</th>
<th>10 mg baseline</th>
<th>15 mg baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cortisol 24-hour C_{av} nmol/L</td>
<td>164 (22%)</td>
<td>177 (14%)</td>
<td>187 (11%)</td>
<td>170 (20%)</td>
<td>180 (16%)</td>
</tr>
<tr>
<td>Plasma cortisol C_{0800} nmol/L</td>
<td>303 (49%)</td>
<td>404 (19%)</td>
<td>394 (18%)</td>
<td>391 (22%)</td>
<td>389 (20%)</td>
</tr>
<tr>
<td>Urinary cortisol Ae, nmol</td>
<td>126 (63%)</td>
<td>115 (34%)</td>
<td>167 (72%)</td>
<td>107 (30%)</td>
<td>88 (24%)</td>
</tr>
<tr>
<td>Serum DHEAS C_{0800}, µmol/L</td>
<td>6.0 (45%)</td>
<td>7.2 (30%)</td>
<td>6.0 (64%)</td>
<td>7.1 (31%)</td>
<td>8.3 (45%)</td>
</tr>
<tr>
<td>Low-dose ACTH test, 30 min, nmol/L</td>
<td>497 (21%)</td>
<td>548 (12%)</td>
<td>533 (11%)</td>
<td>540 (19%)</td>
<td>517 (10%)</td>
</tr>
<tr>
<td>Standard-dose ACTH test, 30 min, nmol/L</td>
<td>527 (16%)</td>
<td>525 (20%)</td>
<td>544 (16%)</td>
<td>554 (17%)</td>
<td>504 (9%)</td>
</tr>
<tr>
<td>Standard-dose ACTH test, 60 min, nmol/L</td>
<td>615 (14%)</td>
<td>602 (24%)</td>
<td>663 (14%)</td>
<td>641 (19%)</td>
<td>606 (8%)</td>
</tr>
<tr>
<td>ACTH after metyrapone test, ng/L</td>
<td>57 (69%)</td>
<td>67 (61%)</td>
<td>90 (88%)</td>
<td>74 (56%)</td>
<td>85 (93%)</td>
</tr>
<tr>
<td>11-deoxycortisol after metyrapone test, ng/L</td>
<td>212 (28%)</td>
<td>207 (41%)</td>
<td>242 (18%)</td>
<td>233 (23%)</td>
<td>233 (22%)</td>
</tr>
</tbody>
</table>

ACTH = adrenocorticotropic hormone, Ae = amount excreted; C_{0800} = concentration at 08:00 hours; CV = coefficient of variation; DHEAS = dehydroepiandrosterone sulfate

Different Methods to Assess HPA-Axis Suppression by Corticosteroids: Treatment Ratio: Mean Dose-Dependent Effects Compared to BL

<table>
<thead>
<tr>
<th>Variable</th>
<th>2.5 mg / BL Mean (95%CI)</th>
<th>5 mg / BL Mean (95%CI)</th>
<th>7.5 mg / BL Mean (95%CI)</th>
<th>10 mg / BL Mean (95%CI)</th>
<th>15 mg / BL Mean (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cortisol 24-hour C&lt;sub&gt;av&lt;/sub&gt;</td>
<td>68 (60-78)</td>
<td>49 (41-57)</td>
<td>31 (24-41)</td>
<td>33 (28-38)</td>
<td>21 (16-27)</td>
</tr>
<tr>
<td>Plasma cortisol C&lt;sub&gt;av&lt;/sub&gt; (08:00, 12:00, 16:00, 20:00, and 08:00 hours)</td>
<td>74 (65-84)</td>
<td>60 (52-69)</td>
<td>48 (37-61)</td>
<td>45 (39-52)</td>
<td>31 (25-40)</td>
</tr>
<tr>
<td>Plasma cortisol C&lt;sub&gt;0800&lt;/sub&gt;</td>
<td>95 (83-107)</td>
<td>85 (73-98)</td>
<td>77 (60-98)</td>
<td>73 (63-84)</td>
<td>50 (39-64)</td>
</tr>
<tr>
<td>Urinary cortisol Ae</td>
<td>45 (34-59)</td>
<td>44 (32-61)</td>
<td>33 (18-61)</td>
<td>23 (17-32)</td>
<td>22 (13-39)</td>
</tr>
<tr>
<td>Serum DHEAS C&lt;sub&gt;0800&lt;/sub&gt;</td>
<td>90 (78-103)</td>
<td>84 (71-100)</td>
<td>66 (50-87)</td>
<td>56 (48-66)</td>
<td>35 (26-46)</td>
</tr>
<tr>
<td>Low-dose ACTH test</td>
<td>92 (85-99)</td>
<td>88 (81-96)</td>
<td>75 (65-86)</td>
<td>71 (65-77)</td>
<td>55 (48-63)</td>
</tr>
<tr>
<td>Standard-dose ACTH test, 30 min</td>
<td>95 (88-102)</td>
<td>91 (83-99)</td>
<td>79 (68-91)</td>
<td>75 (69-82)</td>
<td>59 (51-68)</td>
</tr>
<tr>
<td>Standard-dose ACTH test, 60 min</td>
<td>91 (85-99)</td>
<td>90 (83-99)</td>
<td>74 (63-86)</td>
<td>76 (69-83)</td>
<td>60 (52-70)</td>
</tr>
<tr>
<td>ACTH after metyrapone test</td>
<td>83 (66-105)</td>
<td>97 (74-126)</td>
<td>47 (30-73)</td>
<td>60 (47-78)</td>
<td>38 (25-60)</td>
</tr>
<tr>
<td>11-deoxycortisol after metyrapone test</td>
<td>92 (78-108)</td>
<td>92 (75-112)</td>
<td>75 (54-105)</td>
<td>56 (46-68)</td>
<td>53 (38-73)</td>
</tr>
</tbody>
</table>

ACTH = adrenocorticotropic hormone, Ae = amount excreted; C<sub>0800</sub> = concentration at 08:00 hours; CV = coefficient of variation; DHEAS = dehydroepiandrosterone sulfate

Hermann – HPA-Axis Assessment

Slope of Dose-Response Line and Significance Level of Slope, CV% (inter subject), ED$_{50}$, ED$_{30}$, Inverse Effect Size (Variability of Method/Effect of Increasing Dose), and Relative Sample Size vs. Cortisol 24-hour $C_{av}$ Required to Have the Same Power

<table>
<thead>
<tr>
<th>Variable</th>
<th>Slope</th>
<th>p-value</th>
<th>CV%</th>
<th>ED$_{50}$ (mg) (95% CI)</th>
<th>ED$_{30}$ (mg) (95% CI)</th>
<th>Sensitivity (inverse effect size) (SD of ANOVA/slope)</th>
<th>Relative sample size vs. p-cortisol 24 hour $C_{av}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cortisol 24-hour $C_{av}$</td>
<td>-0.60</td>
<td>&lt;0.001</td>
<td>30</td>
<td>4.3 (2.5-10)</td>
<td>2.5 (1.6-4.9)</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Plasma cortisol $C_{av}$ (08:00, 12:00, 16:00, 20:00, and 08:00 hours)</td>
<td>-0.41</td>
<td>&lt;0.001</td>
<td>28</td>
<td>7.0 (3.1-30)</td>
<td>3.1 (1.6-9.4)</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Plasma cortisol $C_{0800}$</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>27</td>
<td>&gt;15</td>
<td>9.1 (2.7-226)</td>
<td>1.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Urinary cortisol $Ae$</td>
<td>-0.44</td>
<td>0.001</td>
<td>66</td>
<td>2.3 (0.9-71)</td>
<td>&lt;2.5</td>
<td>1.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Urinary cortisol/creatinine $Ae$</td>
<td>-0.43</td>
<td>0.001</td>
<td>63</td>
<td>2.2 (0.9-71)</td>
<td>&lt;2.5</td>
<td>1.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Serum DHEAS $C_{0800}$</td>
<td>-0.41</td>
<td>&lt;0.001</td>
<td>32</td>
<td>12.2 (4.4-85)</td>
<td>5.4 (2.4-26)</td>
<td>0.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Low-dose ACTH test, max response</td>
<td>-0.23</td>
<td>&lt;0.001</td>
<td>16</td>
<td>&gt;15</td>
<td>9.6 (3.9-50)</td>
<td>0.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Standard-dose ACTH test, 30 min</td>
<td>-0.21</td>
<td>&lt;0.001</td>
<td>17</td>
<td>&gt;15</td>
<td>12.3 (4.4-91)</td>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>ACTH after metyrapone test</td>
<td>-0.34</td>
<td>0.002</td>
<td>53</td>
<td>14.8 (2.7-17,873)</td>
<td>5.6 (1.5-1,391)</td>
<td>1.5</td>
<td>8.7</td>
</tr>
<tr>
<td>11-deoxycortisol after metyrapone test</td>
<td>-0.33</td>
<td>&lt;0.001</td>
<td>38</td>
<td>&gt;15</td>
<td>6.9 (2.1-172)</td>
<td>1.1</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Reproducibility of 24-hr Plasma Cortisol Profiles in Controlled Settings

- Study in 31 healthy subjects (20 – 30 yrs), synchronized with a diurnal activity from 08:00 to 23:00 and nocturnal rest;
- Three 24-hour sessions (S1, S2, and S3): S2 two weeks after S1, and S3 four weeks after S2.
- Plasma melatonin and 24 hr cortisol profiles with 3-hr sampling intervals from 08:00 to 20:00 and hourly from 22:00 to 08:00;

Reproducibility vs. Heterogeneity of 24-hr Plasma Cortisol Profiles in Less Controlled (i.e. Semi-Ambulatory) Clinical Settings

Single-centre, randomized, double-blind, double-dummy, 4-period, 4-day repeat-dose crossover study with baseline evaluation comparing two strengths (i.e. 50/100 µg & 50/500 µg) of a SX-FP TEST DPI and Seretide Diskus®

Four cortisol BL-assessments prior to each study period: One 24-hr BL, three 3.5-hr BLs

Subject with low BL Heterogeneity

Subject with high BL Heterogeneity

5:30 a.m.
• **Organ system:** ...PD assessment of systemic effects of ICSs in adults is to assess the effect on the HPA axis;

• **Primary PD read-outs:** Repeated assessment of the change from baseline in 24-hour plasma cortisol as measured by AUC (as the primary variable) and C\text{max};

• **Dose levels:** Inhalation of the maximum recommended total daily dose regimen of the ICS, together with the assessment of a lower dose regimen;

• **Duration of treatment:** Must be justified and must ensure that PK steady state has been reached.

• **Population:** Study should be carried out in patients with asthma;

• **Setting:** Controlled, fully tested environment (i.e. patients should be studied as in-patients on those days when assessments are being carried out).
Requirement of HPA-axis assessment and recommendation of 24-hour plasma cortisol AUC values as the primary outcome variable is in-line with established evidence;

- Requirement of equivalent $C_{\text{max}}$ values as additional outcome variable, does not acknowledge the known pulsatile ACTH/cortisol release characteristics, and hence, appears inappropriate (PK-minded approach to PD-outcomes!);

- Requirement of highest recommended dose-level inconsistent with requirements of “assay sensitivity” (i.e. to assessing PD effects at the steep part of the dose response curve).

**HPA-axis studies at the highest recommended dose-level of ICS are unlikely to be discriminative for product performance characteristics!**
Requirement of **asthma patients as study population** carries many principle and methodological draw-backs, e.g.

- lower (and central) pulmonary deposition,
- inter-occasion variability of airway caliber,
- already compromised HPA-function,
- higher probability of GCR-resistance,
- practical and ethical constraints with wash-out of concomitant asthma medications, in particular in those patients assigned to low-dose treatments groups.

- i.e. *asthma patients represent a highly variable, poorly accessible and hardly-sensitive study population*; risk-benefit appears overall unfavorable;

- Rigorous control of stability of circadian conditions and lifestyle should be observed throughout the entire study and not just at the days of HPA-assessment.
Historically, hardly any HPA-axis specific assessments and screening procedures reported for subject selection in published HPA-axis studies;

However, it is strongly recommended to implement the following assessments:

- Inquire stability of diurnal rhythm/lifestyle and regular sleep habits, i.e. exclude shift-working, travels across time-zones (jet lag), and excessive weekend parties;
- Inquire/exclude current presence of stressful conditions, e.g. job-related (pre)burn out, private distress, exams, etc.;
- Inquire/exclude current presence of sleep disturbances / insomnia;
- Inquire/exclude excessive alcohol consumption and significant liver disease;
- Inquire/exclude presence of depression disorders, e.g. ‘Well Being Five’ Questionnaire [http://www.who-5.org];
- Check for GCR-sensitivity, e.g. by low-dose DST;
- Test for physiological (i.e. “normal”) and reproducible cortisol profiles (i.e. 2 replicate 24-hr cortisol screening assessments 3 days apart), including formal profile analysis prior to enrolment;
What Constitutes Regular 24-hr Cortisol Profiles?

### Structural and quantitative pattern analysis of 24-hr cortisol profiles

The comparative 6-hr period approach
- AUC Period 1 > Period 2 > Period 3
- AUC Period 4 < Period 2

<table>
<thead>
<tr>
<th>6-hr Periods</th>
<th>Time</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4:00 - 10:00 am</td>
<td>Cortisol-AUC should be the highest of all 6-hr periods</td>
</tr>
<tr>
<td>2</td>
<td>10:00 am – 4:00 pm</td>
<td>Cortisol-AUC should be &lt; than that of Period 1, but &gt; than that of Period 3</td>
</tr>
<tr>
<td>3</td>
<td>4:00 - 10:00 pm</td>
<td>Cortisol-AUC should be &lt; than that of Periods 1 and 2</td>
</tr>
<tr>
<td>4</td>
<td>10:00 pm – 4:00 am</td>
<td>Cortisol-AUC may be either lower or higher than that of Period 3, but should be lower than that of Period 2</td>
</tr>
</tbody>
</table>
How Can We Do Better?
Study Conduct: Thorough Control of Study Conditions

- Historically, only few HPA-axis studies were conducted with subjects under controlled diurnal and lifestyle conditions (i.e. in-house confinement);
- However, for a “thorough HPA-axis study” subjects should be confined in-house for the entire study duration, and synchronized for their diurnal activity and nocturnal rest;
- Subjects should be non-smokers and refrain from alcohol throughout the study;
- Subjects should receive standardized meals.
Issues / Problems:

• Historically, simple sampling strategies have been usually applied for cortisol profiling (e.g. sampling at 2-hr intervals);
• Little attention has been obviously paid on issues related to pulsatile secretion of cortisol; i.e. no special strategies for avoidance of confounding cortisol-pulse data in this respect have been reported;
• Little attention has been paid on the existence of more or less sensitive time-periods of the cortisol profile for the assessment of GC-mediated feedback suppression;
Proposed Improvements:

- **Reduce impact of cortisol pulses on AUC:**
  Obtain 3 samples for each collection time-point (~ 10 minutes apart) and calculate median of these 3 samples for each individual timepoint;

- **Avoid major impact of outliers on AUC:**
  Apply close-meshed sampling in the period of most interest (i.e. in the early acrophase; e.g. 60- or even 30-minute intervals from 2:00 to 10 a.m.);

- **Focus on most sensitive time-periods of cortisol-profiles for detection of ICS-mediated suppression:**
  Consider suspension of sampling over the quiescent period (e.g. from 20:00 to 02:00), as this period hardly provides sensitive information on ICS-mediated cortisol suppression; i.e. capture actually 18-hr cortisol profiles (from 02:00 to 20:00) instead of 24-hr profiles.
How Can We Do Better?
Few Data Analysis Considerations

- Consider **calculation of average cortisol concentrations** (Cav) in addition to AUC values:
  - Less impact of outliers (as compared to AUC);
  - puts (schedule dependent) more weight on time-periods of interest (more samples at early morning rise and the entire acrophase);
- Consider **“a priori” definition of “implausible” or “insensitive” profiles** in the study protocol, to allow for exclusion of such profiles from analysis;
- Use consistency of **replicate BL-assessments** (if available) as quality check for compliance with regular sleep and stable lifestyle conditions.

**Implausible / insensitive profiles:**
Increased cortisol profiles vs. BL despite of FP high dose and low dose treatment
Conclusions

• The current practice of HPA-axis studies appears to offer much room for improvement;
• The definition of commonly accepted quality standards and criteria for “thorough HPA-axis studies” (as compared to “thorough QT/QTc studies [ICH E14]) appears worthwhile to be considered;
• A couple of proposals for methodological improvements have been presented herewith and are open for discussion.