Reviewer's report

Title: Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone

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Reviewer: Dimitra Argyro Vassiliadi

Reviewer's report:

This is an interesting study aiming to investigate whether measuring cortisol in the saliva or urine provides useful information in assessing individual hydrocortisone dose requirements. Secondary aims included assessment of cortisol levels in plasma, saliva and urine following both physiological and stress hydrocortisone doses and, the inter-individual variability of cortisol pharmacokinetics. Of note the authors analyzed the performance of all currently available tools for the assessment of cortisol levels, that is total plasma cortisol, calculated free cortisol, salivary cortisol and spot urine cortisol. The study is appropriately designed mainly for the secondary aims, and is sufficiently relevant to the specific field, although not particularly novel, apart from the inclusion of spot urine measurements. It highlights the problem rather than providing solutions, except that a measurement of total, salivary or urinary cortisol at 2 hours is more representative of the peak post oral hydrocortisone levels.

I have the following suggestions:

Major Compulsory Revisions

The first blood sampling on the endogenous control day was performed between 0830 and 0900. One cannot be certain that, this is the peak cortisol in all individuals tested since it was not balanced to the awakening time; at least in some individuals the peak cortisol levels may have been missed. The authors should add a comment in the Discussion section.

In the same line, in the figures, it seems more logical to shift the curves of days 2 and 3 to the left, in order to visually compare peak endogenous cortisol levels (time 0 on day 1) to peak cortisol levels after the administration of hydrocortisone and not to the respective time 0, which reflects suppressed post-dexamethasone cortisol levels. It would probably be more appropriate to refer to time 0 on days 2 and 3 as time -30.

Minor Essential Revisions

A limitation of the study is the fairly small number of participants, which does not permit to possibly identify causes of altered hydrocortisone clearance levels and, subsequently cortisol levels, such as high BMI. Also, as the authors already acknowledged another limitation is that the study was performed for only 5 hours and not for 24 hr.

4 mg of dexamethasone were administered at time 2300, that is 9,5 to 10 hours before the beginning of the tests. Can the authors comment on how long one
dexamethasone dose can keep the HPA axis suppressed and whether this would be altered in rapid dexamethasone metabolizers?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests