Reviewer's report

Title: High end of normal ACTH and cortisol ranges are associated with specific cardiovascular risk factors in pediatric obesity: cross-sectional study

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Reviewer: Thomas Remer

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Prodam and colleagues examined the associations of ACTH and total plasma cortisol with several risk factor for CVD in a cross-sectional design in obese children and adolescents.

General:
The questions posed by the authors comprise several unsolved issues and therefore such data and corresponding analyses are important.

However, the language is partly awkward and a number of statements and/or considerations are hard to understand. The paper needs appropriate language editing.

Specific points:

Abstract
1) … cardiovascular risk factors ….and hypertension …can not be “measured” (blood pressure can be measured).

2) What do the author mean by: “Obese children and adolescents …. have high normal morning ACTH and cortisol levels leading to the hypothesis that HPA hyperactivity comes out early in life with an association already present for values in the higher range of normality” ? Do higher ACTH and cortisol levels, that are still in the normal range, represent HPA hyperactivity?

Subjects and methods
3) What were signs suggestive for Cushing’s syndrome, please state criteria.

Statistical analysis
4) Why did the authors use the SEM. This is misleading, they probably did not want to show us (the reader) the uncertainty of how the sample mean represents the population mean. Refer to: http://bja.oxfordjournals.org/content/90/4/514.full

Please provide the SDs for the arithmetic mean values or the median values with (e.g.) the 25th and 75th percentiles.

5) “ACTH and cortisol were also categorized into near tertiles.” What are near tertiles?
Results

6) The authors wrote that 27 subjects were excluded “because they did not satisfy inclusion criteria”. These criteria should be clearly stated.
- What were the criteria for the diagnosis >congenital adrenal hyperplasia<?
- ... urines ... were incomplete in 20 of 31 patients”. Spot samples?, 24-h samples?
- Was the diagnosis “Cushing’s Syndrome” only done in those with “complete” urine collection?

7) I did not understand the sentence: “Analyzing subjects positive to each alteration with respect to the cut-offs chosen, revealed higher ACTH levels in those with ….”

8) Tables and text: why not presenting result in SI units? If there are strong reasons to do so, please provide at least the corresponding conversion factors.

9) A Table with mean values of age, BMI, lipids, hormones etc. given for an overall age range from 4-18 years makes no sense. Provide all parameters according to useful age ranges, Tanner stages, or at least prepuberty vs. puberty (and in that case sex-stratified).

10) It appears to me that the HOMA-IR and Glc0 changes are possibly more pronounced between tertile 1 and tertile 2 (cortisol tertiles, Table 2) compared to tertile 2 vs. tertile 3. Since means of glucose and HOMA are lower in tertile 1 compared to tertile 2, could it not be that a potential positive association between plasma cortisol and HOMA or glucose may exist particularly in the ranges of normal-to-moderately high HOMA or Glc0 levels?

In this range of not so strong insulin increases hepatic CGB production should be less strongly suppressed, implying that more of the Cortisol secreted by the adrenals can circulate as total cortisol CBG-bound.

Please check the corresponding HOMA-IR and Glc0 ranges for their partial correlations with cortisol.

11) Table 3, Title should read: Partial correlations for ACTH (....) and cortisol (....) with cardiovascular risk factors.

Discussion

12) Page 12, “... the link between HPA dysregulation in obesity ...“ How do the authors know that this is a dysregulation? Could it not be a metabolic adaptation?

13) Page 13 “... cardiovascular risk factors remains positive in unselected o b e s e c h i l d a n d / o r o b e s e a d o l e s c e n t populations [9,13].” Please add.

14) Page 13 The authors regarded one of their findings as unexpected, namely the observed lack of an association between BMI and cortisol. They provided as an explanation that the population they examined was a “homogenous population
in terms of obesity“.
- What does this exactly mean for 4-18 years olds?
- How low were the standard deviations or the ranges for body fatness or BMI in the different age groups? [see also SEM vs. SD, (4)]

15) Page 13, Data interpretation:
Similar to the lack of an association between BMI and plasma cortisol in the obese population, a lack of association between BMI or body fatness and urinary free cortisol & free cortisone (in 24-h urines) has been demonstrated in non-obese children [Dimitriou T et al., Am J Clin Nutr. 2003;77:731-6]. However, major glucocorticoid metabolites in 24-h urine samples (reflecting ACTH-driven adrenocortical activity or cortisol secretion) were significantly associated with fatness in these non-obese children [Dimitriou T et al.].

The latter findings together with the present of Prodam et al., strongly suggest that adrenocortical activity (driven by ACTH, i.e., ACTH itself) is related to body composition – during growth – whether children are lean/normal-weight [Dimitriou] or obese [Prodam]. In other words the associations observed by Prodam et al between ACTH and BMI on the one hand and the non-association between circulating cortisol [in the Dimitriou paper: urinary free cortisol and/or cortisone] and BMI on the other hand appear not to be restricted to obese children.

This important issue must not be ignored in the Discussion.

16) Cortisol secretion (even after correcting for body surface area) varies markedly with age and differs between sexes at least after the start of puberty [Wudy SA et al. Am J Physiol Endocrinol Metab. 2007;293:E970-6]. Furthermore, literature data suggest that there is only very little variation in total plasma cortisol levels in lean/normal-weight children with age (this is no contradiction, since metabolic clearance rate of cortisol probably varies correspondingly). However, the question emerges: is this also the case in obese children? Some vague literature hints “say no”. Prodam et al. can contribute to (perhaps) solve this question by showing a scatter of all of their obese childrens’ plasma cortisol measurements on the Y-axis and age on X-axis. Such a Figure would be very interesting and helpful.

17) The authors should shortly discuss or comment on the probable interplay of CBG, total plasma cortisol, free (bioavailable) cortisol, cortisol metabolism/clearance and ACTH responses.

18) Overall discussion is quite long, in parts very speculative, and several conclusions are not clear. Only two (out of several more) examples:
- “The characteristics of this association suggest that ACTH could precede a decreased clearance of steroid hormones, another mechanism discussed as a player in the state of functional hypercortisolism of obesity [8].”
- “However, only ACTH increased the risk to be positive for altered glucose levels. This could be due, as previously discussed, to the fact that in the pediatric
age a transient suppression of cortisol with higher ACTH is an adaptive response of obesity to prevent comorbidities, in particular type 2 diabetes [27]."

Thus, Discussion has to be rewritten and should focus on clearly described hypotheses and argumentations.

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests