Title: Salivary cortisol differs with age and sex and shows inverse associations with waist-hip ratio in Swedish women: a cross-sectional study

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Author's response to reviews: see over
Dear editor!

Thank you for considering our manuscript “Salivary cortisol differs with age and sex and shows inverse associations with WHR in women”. We truly appreciate the reviewers’ insightful comments and have now carefully considered them. The paper has been accordingly revised and we addressed all comments in due order below.

Sincerely,

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Response to Reviewer 1:

Reviewer's report:
The manuscript is general well written and well structured. However, I have some minor queries for the methods and results and I would prefer a shorter title.
The manuscript aimed to study diurnal variation of cortisol and explore its association with abdominal obesity in a large unselected population gender stratified. The topic is relevant and within the scope of the journal. The question posed by the authors is well defined. Methods are appropriate and well described. The manuscript is general well written and well structured. However, I have some minor queries for the methods and results.
Discretionary revision: I would prefer a shorter title.

We have shortened the title to “Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: a cross-sectional study

Minor Essential Revisions:
Methods
Did you use cotton, polyester or cortisol Salivette®? Please include.

We used cotton, which has now been added on row 7 under “procedures” in the methods section.
Did you record time of sampling? Did you include time of sampling in you statistical analysis? Please clarify and if yes please include in the statistical analysis.

Unfortunately we did not record actual time of sampling and could thus not account for it either. We have added that as one of the limitations on row 7 under “methodological considerations”. In addition, on row 9 under “procedures” in the methods section we have added that participants were instructed to collect their saliva sample with a maximum of 30 minutes time shift from 0800 h and 2200 h.

Did you adjust for time of awakening? Please clarify and if yes please include in the statistical analysis.

As already stated under “methodological consideration” we did not account for time of awakening. To clarify we have changed the word “standardization” to “adjustment” on row 7.

Results
Figure 1-3 are a repetition of results in tables 3-5. Please only present data one time.

Figure 1-3 does indeed repeat parts of the contents from tables 3-5. However, we think that the figures are important as they present the results in a more pedagogical way and the tables are “only “ supplementary information, not to appear in the actual journal.

Response to Reviewer 2:

Reviewer's report:
In this cross sectional study the authors investigated the relationship between morning and evening cortisol (in saliva) and fat distribution.

Major Compulsory Revisions
Although this is a large study, nonetheless there are several limitations: (i) this study was probably designed for other aims, and the investigation of the cortisol in saliva appears to be a secondary aim;

The main purpose of the Skaraborg project has been to study the early development of hypertension, type 2 diabetes, and obesity in an adult population focusing on the interaction between genes, lifestyle, and environment. Stress is an important common background factor and the analyses of saliva cortisol were thus included in the study protocol as a biological marker at an early stage when designing the study.

(ii) many more salivary samples should be collected to properly investigate HPA dynamics throughout the day;

We agree and have already acknowledged that under “methodological
considerations” in the discussion.

(iii) several limitations have been discussed by the authors themselves. Nevertheless, the data are of interest, and some findings are new whereas others confirm previous researches.

Minor Essential Revisions
- Please define whether morning cortisol was collected just before work-up or at random, provided all subjects were in the fasting state. Moreover, were evening sample collected after dinner?

As stated in the method section the participants were instructed to abstain from exertion 1 hour before the saliva collection. However, subjects who got up very early might have “started up the system” a lot more than those who woke up shortly before the saliva collection and time of awakening should therefore preferably have been accounted for. Still, we have already acknowledged this limitation under “methodological considerations”.

- The tables reports some significant differences in the morning and evening cortisol in relation to sex and age. However, both modify body composition and BMI values, but associations between cortisol and fat distribution were not adjusted for BMI. In the literature there are several examples of association between BMI and/or WHR (or waist).

This is a valid point and we have now added that the results remains unchanged when also adjusting for BMI on row 14 under “associations between salivary cortisol and abdominal obesity” in the result section.

- The authors found an association between cortisol and WHR in women. However, the potential confounding effect of estrogen compounds was taken into account in the multiple regression analysis. I’m wondering what happens if those women taking estrogens were excluded from the analysis. Estrogens can in fact modify body composition, fat distribution and, additionally, cortisol metabolic clearance rate. I’m not convinced that adjusting for these confounding compounds can definitely exclude potential bias.

While this is a fair point the pattern actually remains in stratified analyses of estrogan supplementation, which we have now added on row 18-19 under “methodological considerations”.

- In table 1 the authors distinguished males and females under 50y and over 50y. I think that this classification is unnecessary and eventually misleading. The authors have cortisol data in different decades of age. Therefore, age effects can be observed looking at these data. Finally, in the evaluation of male and female cortisol values, the authors used age 30-39 as the reference for further analysis. This is completely arbitrary. If all decades are included in an appropriate analysis, (for trend, etc), the overall impact of age can be fully appreciated without any reference, as young age cannot be per se considered as a reference (in addition, the authors do not have cortisol values in younger
subjects (i.e. 20-29y).

One of the reasons for stratifying at 50 years of age was that this cut-off is considered the most appropriate to distinguish post-menopausal women (Morabia A, 1992;21:222, Wirfåldt E, 2005;53:135). When it comes to using the youngest age group as reference this choice might be regarded as arbitrary, still, using the highest or the lowest age group as reference is a common approach when exploring age effects and the results are valid whichever reference group you choose. However, on row 3-5 under “age-differences” in the result section we have now added information on tests for trend, which mainly confirms the analyses where a reference group was used.