Author's response to reviews

Title: Depression, Smoking, Physical Inactivity and Season independently associated with Midnight Salivary Cortisol in Type 1 Diabetes

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Author's response to reviews: see over
To BMC Endocrine Disorders, 21/07/2014.

Dear professor Timothy Shipley, editor-in-chief of BMC Endocrine Disorders,

We are glad you have shown interest in our manuscript and are thankful for the valuable advice we have received.

We hereby resubmit our manuscript (1888199225130058) titled

“Depression, Smoking, Physical Inactivity and Season independently associated with Midnight Salivary Cortisol in Type 1 Diabetes” by authors Eva O. Melin, Maria Thunander, Mona Landin-Olsson, Magnus Hillman, Hans O. Thulesius.

First we want to answer the questions from the editor.

(1) "Please provide more information about the trial, including its full name, purpose, interventions, outcome measures, and current status. Also provide references to results that have been published."

Answer: Our current article “Depression. Smoking, Physical Inactivity and Season independently associated with Midnight Salivary Cortisol in Type 1 Diabetes” is part of a RCT with Affect School and Script Analysis compared with Basic Body Awareness Therapy for patients with diabetes, psychological symptoms and inadequate glycemic control. We have previously published one manuscript [1] describing the current population. We found that depression, obesity and smoking were independently associated with inadequate glycemic control in patients with type 1 diabetes. We also found that alexithymia and anxiety were associated with depression but not with HbA1c.

We are now just about to terminate a manuscript, which we have not yet sent to any medical journal “Affect School and Script Analyses (ASSA) compared with Basic Body Awareness Therapy (BBAT) in patients with diabetes, high HbA1C and psychological symptoms – a randomized controlled trial”. Primary outcome is depression; secondary outcomes are improved HbA1c and self-image, and reduced alexithymia and anxiety (ClinicalTrials.gov NCT01714986). Intervention is terminated for the current population, but recruitment will continue for patients with type 2 diabetes in 2015. The Affect School is described in an article about an intervention for patients with chronic benign pain [2].


Second we want to answer questions from Nicholas Mezitis

**Reviewer's report:**
The HPA axis and cortisol secretion express a circadian rhythm entrained to the
local environment through a variety of local cues (‘zeitgebers’), the most important of which is daylight. Artificial light of specific wavelengths, supratentorial perturbations, chemical stimulants and medications also influence cortisol secretion.

The present study addresses cortisol (MSC) levels of patients with diabetes mellitus in the context of specific behaviors. Additional conclusions are drawn based on the season during which samples were collected.

The following points should be clarified:

1. The total number of patients screened (196 + 85) should be stated, with an explanation for why such a large number ‘dropped out’ of the study after having presumably signed informed consent for participation.

   **Answer:** Patients had signed informed consent for participation, which included blood tests, MSC sampling and participation in a randomized controlled trial, but several did not deliver MSC.

   We wrote in the previous ms: “A group of 85 eligible patients who failed to deliver MSC properly”, we have changed this into “There were 62 patients who chose not to deliver MSC samples and 23 who failed to deliver proper samples” (rows 112-113).

   Altogether there were 23 failures with handling the MSC samples for the following reasons: patients did not write date and time for sampling; the date and time for arrival at the laboratory was not written; the pre-addressed envelopes were mixed up by the patients; some samples did not contain enough saliva; and in some cases MSC was sampled too long after assessments with the psychological instruments.

   For the rest, 62 patients, we assume they thought it was inconvenient to collect samples at midnight. Anyway we have thoroughly analyzed whether these 85 patients differed in any aspect from those who did deliver MSC samples. We have followed your advice and added “clinical psychiatric diagnoses” and use of antidepressants to the analysis (rows 114 -116).

2. How many of the 85 ‘dropouts’ were on antidepressants or had a psychiatric diagnosis?

   **Answer:** Nine patients (11%) of the dropouts were on antidepressants compared to 13 (7%) among the participants, P=0.33. Thirteen (15%) of the dropouts had a psychiatric diagnosis compared to 27 (14%) among the participants, P=0.71. We have added these P-values at the rows 114-116 in the article.

3. Patients receiving antidepressants (13) were grouped with those identified as depressed based on HADS-D questionnaire, who were not taking medication for this problem (7). It is important to distinguish MSC levels corresponding to these two groups separately, as antidepressant ...

   **Answer:** First, in the introduction section we added “Antidepressants are also associated with alterations of the HPA axis” (rows 82-83).

   Median (q₁; q₃) MSC was for five patients with self-reported depression using antidepressants 8.7 (3.3; 18.0); for 15 patients with self-reported depression who were not using antidepressants it was 6.7 (5.1; 13.0), P=0.76. For 8 patients without self-reported depression but using antidepressants median (q₁; q₃) MSC was 4.4 (3.1; 8.6) and for 168 patients without self-reported depression who were not using antidepressants it was 4.8 (3.0; 7.1), P=0.90. This information is added in Table 2 in the article. We also wrote on rows 213-215” Median MSC did not differ between users and non-users of antidepressants in patients...
with self-reported depression (P = 0.76), and not in patients without self-reported depression (P = 0.90) (Table 2)."

4. MSC levels were collected once for each participant, but these collection dates varied over the course of the year. Were subjects with specific characteristics (e.g. smoking, physically active etc.) clustered in particular seasons for their sampling?

Answer: In Table 3 it is shown that subjects with specific characteristics were not clustered in particular seasons. The highest prevalence of physical inactivity was found in summer (P=0.21); the highest prevalence rates of self-reported depression (P=0.41) and smoking (P=0.32) were found in spring. We added information about age and gender in Table 3: Median age in spring was 45 years, in summer 40 years, and in autumn/winter 44 years (P=0.090). Finally, the logistic regression analysis shows that season, smoking, physical inactivity, depression, and age were independently associated with high MSC (Table 4). Also we added sub analyses of the 137 patients who were non-depressed, non-smoking and physically active, and for these patients there were no difference in age between autumn and spring, and in this population the association between MSC ≥9.3 nmol/L and spring was significant (AOR 7.9) with autumn/winter as reference (AOR 1). See rows 224-234.

5. The hours of daylight in the Nordic countries vary widely according to season and in a study on cortisol this issue should be addressed in the discussion at least.

Answer: We have added in the introduction at rows 75-79: “Seasonal changes in depressive symptoms are considered to be the result of a failure to adapt to the shift in day length that accompanies seasonal change. Light is the most important time-marker for entraining the circadian rhythms in physiology, and the hours of daylight in Sweden vary widely according to season.”

We have added in the discussion section at rows 298-301: “Spring is in Sweden characterized by rapidly increasing light intensity and longer day light periods, which might influence cortisol secretion as light is an important time-marker for cortisol secretion.”

6. Reference studies on MSC collection allow for 5-6 hrs of fasting prior to sampling. The present study considered 30 minutes an adequate interval before sampling, yet subjects were advised not to brush their teeth 60 minutes before sampling. Does this imply that food remnants could have been in the oral cavity during sampling?

Answer: Big meals late at night are not the cultural habits in Sweden as in the Mediterranean countries, so the likelihood for that is low. To interfere with mealtimes in patients with type 1 diabetes is potentially hazardous due to the risk for hypoglycemia attacks, which also might affect MSC levels. Therefore we consider that a shorter fasting period in patients with type 1 diabetes is preferable. We also assume that severe restrictions on what to do or not to do 5-6 hours prior to sampling would have resulted in a lower participation rate. Brushing the teeth is associated with an increased risk for bleeding in the mouth which could interfere with the saliva concentration of cortisol; this is why we recommended avoiding brushing the teeth one hour prior to sampling. To avoid bleeding is probably more important than food remnants in the mouth. A high degree of physical activity late at night is also less probable in patients with type 1 diabetes. There are obviously different instructions in literature for how long the
fasting period should be. We have now written at rows 315-321: “We chose a 30 minutes (60 minutes for brushing the teeth) restriction period of eating etc before MSC sampling. A variety of restriction periods before salivary cortisol sampling are found in the literature: 15 minutes [14, 22], 30 minutes [20, 31, 32], 2 hours [8], and 3 hours [28]. How much a shorter or longer restriction period would affect the results is difficult to say, but a long restriction period might negatively affect the participation rate, and for patients with type 1 diabetes it is preferable not to interfere with ordinary mealtimes in order to avoid hypoglycemia episodes”.

7. How was the diagnosis of Type 1 diabetes mellitus confirmed?

Answer: The patients achieved their diagnoses by specialists in paediatrics, endocrinology or diabetology on clinical grounds. C-peptide or specific antibodies were not controlled.

8. The frequency of hypoglycemia episodes was not addressed, yet this is a major factor generating both a stress response and depression in this population.

Answer: Only 9 participants reported severe hypoglycemia attacks and they did not have higher median MSC (P=0.96). We have included this information in Table 2. We have also presented a definition for severe hypoglycemia attacks at the rows 179-180. Those who reported severe hypoglycemia attacks were not more depressed than patients without hypoglycemia attacks (P=0.23), the last we did not include in the article.

9. The stratification of groups is confusing with one group (34) featuring all risk factors, a reference cohort (137) without risk factors, and mention made of a third (?) group (45) with at least one risk factor.

Answer: We have in the results section on rows 202-205 added “There were 137 (70%) non-depressed, non-smoking and physically active patients; 45 (23%) were either depressed (self-reported), smokers or physically inactive, or had combinations of these variables; and 14 (7%) were non-depressed but with lacking data regarding lifestyle factors”. First we describe the results for all patients and at rows 220-222 we write “Thirty four patients (17%) had MSC ≥9.3 nmol/L which was associated with smoking (AOR 5.5), spring (AOR 4.3), physical inactivity (AOR 3.9), self-reported depression (AOR 3.1), and older age (per year) (AOR 1.08). We have now removed the descriptions of the subgroups of these 34 patients with high MSC. We have changed the headline at the row 224 into “MSC in non-depressed (self-reported), non-smoking and physically active patients”. Here we now only present results for the 137 non-depressed, non-smoking and physically active patients. We do not mention the 45 with at least one of these features anymore and we do not present any picture.

10. Waist circumference was measured, but MSC results are not reported in relationship to abdominal obesity.

Answer: We have now added median MSC values for patients with and without abdominal obesity for men and women separately in Table 2. We have also calculated CORs for the associations between abdominal obesity and MSC ≥9.3 nmol/L for men (AOR 0.7, P = 0.76) and women (AOR 0.9, P = 0.92) (Table 4).

In summary, the subject is complex and although the topic and the information
collected are important, the text of this submission must be revised with focus on clarity and brevity.


Third, we want to answer questions from Margaret Grey

Reviewer's report:
Major compulsory revisions
The major problem with this manuscript is that while the analyses were cross-sectional, causation is implied throughout the discussion. Given that the hypotheses were not supported, implying that cortisol causes depression is quite problematic and goes far beyond the data reported. Further the statement, that ‘treatment for depression would lead to decreased complications’ is not supported at all by these data. That may be a hypothesis to examine in an RCT, but it was not tested in this paper.

Answer: We have now withdrawn the discussions of causation you mentioned and the statement that treatment for depression would lead to decreased complications. You are right, we can’t draw these conclusions from our data. This hypothesis will be tried in a randomized controlled trial (registered with ClinicalTrials.gov (NCT01714986) with reduced depression as primary outcome and in a long time follow up we will also investigate incidence of diabetes complications and mortality.

Discretionary Revisions
More minor concerns include the need to add a brief explanation about how complications were defined. It is not sufficient to say that these were explained
else where.

Answer: We had presented these definitions in our first study of this population [1], and as we only wanted to present the prevalence of diabetes complications as a sort of background information we wrote it in this way as we tried to keep the manuscript short. We now think it is better to exclude the information regarding diabetes complications as we do not use the information in any way.

While the background section identifies the variables to be studied, there is no coherent model to explain the hypothesized interactions.

Answer: As disturbances of the circadian rhythm of cortisol secretion are associated with depression, coronary calcification, and higher all-cause and cardiovascular mortality, we wanted to investigate whether a disturbance of the circadian rhythm manifested as a high MSC was associated with depression and glycemic control in patients with type 1 diabetes. As several environmental and intra individual factors have impact on cortisol levels we wanted to control for these factors. We have changed in the manuscript as follows at rows 28-29: Disturbances of the circadian rhythm of cortisol secretion are associated with depression, coronary calcification, and higher all-cause and cardiovascular mortality. At rows 87-95 we write: “The main hypothesis of this study was that a disturbed circadian rhythm manifested by high midnight cortisol is associated with depression and with impaired glycemic control in patients with type 1 diabetes. The primary aim of this study was to test the associations between midnight salivary cortisol (MSC), depression and HbA1c, and control for behavioural, environmental and intra individual factors with possible impact on cortisol secretion like smoking, physical inactivity, season, medication, age and gender in patients with type 1 diabetes. Secondary aims were to present MSC levels for a reference group of non-depressed type 1 diabetes patients with a healthy life style (physically active and non-smoking), and to explore seasonal variations.”

Seasonality was found, but winter was not included in the data collected. This omission loses the potential to see SAD. The discussion about this aspect of the study also suggests the MSC causes suicidal ideation, which again, was not possible to determine in this correlational study.

Answer: Our original purpose was not to identify SAD; in that case we would have tried to recruit patients during the whole year. Patients are controlled bi-annually and with a recruitment period of 9 months, we thought we would have a good chance to reach most patients at the clinic. But as we know that there are seasonal variations of depressive symptoms in SAD (and also in bipolar disorders) and in suicide incidence, we wanted to investigate if there were seasonal variations of depressive symptoms and of MSC levels. We have now written at rows 273-276: “Third, there was no data from the middle of January until the end of March which makes it impossible to exclude seasonality in depressive symptoms, though we did not find any. Fourth, to confirm the seasonality of MSC levels there is a need for repeated measurements throughout the year“.

We have also omitted the discussion regarding high MSC and suicide ideation.

**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.

Reference.


Fourth, we want to answer questions from Jessica Markowitz:

Reviewer's report:
Major Compulsory Revisions
This is an interesting study which appears to be novel. I have a few concerns I would like the authors to address, listed below.

Abstract
• Is there research to back up the first sentence? It appears to me that in this manuscript, you are testing this idea and correlation does not equal causation. Perhaps be more tentative in that first statement, or provide evidence in the introduction that this is the case.

Answer: We have changed the first sentence in the abstract as follows: Disturbances of the circadian rhythm of cortisol secretion are associated with depression, coronary calcification, and higher all-cause and cardiovascular mortality.

• In results section of abstract, clarify that older age was associated with high MSC.

Answer: We have now written in the results section: Thirty four patients (17%) had MSC $\geq 9.3$ nmol/L, which was associated with smoking (AOR 5.5), spring season (AOR 4.3), physical inactivity (AOR 3.9), self-reported depression (AOR 3.1), and older age (per year) (AOR 1.08) and we have added this information in the conclusion section.

Methods
• In the sentence discussing “somatic comorbidities” please check the parentheses in this sentence, as I think you are missing one – I do not think you mean to include social blindness in this category.
• What is social blindness? Please define.

Answer: We have corrected the parentheses. We have replaced social blindness with “visual impairment to such a degree that reading the questionnaires was impossible” which is no longer included in the category “somatic comorbidities”.

Metabolic variables
• Please define WC at its first use.

Answer: We have done so.
Diabetes complications
• It this section necessary?
Answer: Actually not. We have omitted diabetes complications.

Validation of the HADS-D
• Could some of the 9 patients be on antidepressants? Or was there no overlap and there were 14 patients with either self-reported depression or on antidepressants?

Answer: To clarify we have made the following changes:
First, under the methods section at rows 153-155 we wrote: Positive associations between self-reported depression and clinical psychiatric diagnosis with and without use of antidepressants confirmed the validity of the HADS-D.

Second, under the results section we have added at rows 205-206: Clinical psychiatric diagnoses were established in 27 (14%) patients and 13 used antidepressants.

Third, at rows 245-249 we wrote:
Validation of the HADS-D
The associations (COR (CI), P) were significant between self-reported depression and “clinical psychiatric diagnosis and use of antidepressants” (9.0 (2.5-32.1), 0.001 (n=13)), and between self-reported depression and “clinical psychiatric diagnosis without use of antidepressants” (5.7 (1.5-21.3), 0.009 (n= 14)), with no “clinical psychiatric diagnosis or antidepressants” as reference (n=169).

Fourth, at rows 269-270: Yet, clinical psychiatric diagnoses, both for those using and not using antidepressants, were clearly associated with self-reported depression.

We have also in Table 2 clarified how many patients with and without self-reported depression that used antidepressants.

Discussion
• Page 13, line 262, “MSC was associated with self-reported depression in patients with type 1 diabetes, which indicates a disturbance of the circadian rhythm” – why does it indicate this? Can you please explain a bit more in the manuscript?

Answer: We wrote at rows 277-282: A normal circadian rhythm of cortisol is characterized by maximum levels in the morning and minimum levels at midnight [8]. In this study we chose to use MSC ≥9.3 nmol/L as cut-off, a very high level that was recently used to differentiate pseudo-Cushing’s syndrome from true Cushing’s disease [8]. The association between this very high level of midnight cortisol and self-reported depression indicates a disturbance of the circadian rhythm in depressed patients with type 1 diabetes.

• Why do you think some of your findings differ from previous research? Please address this in this section.

Answer: we wrote at rows 288-298: We found the highest midnight cortisol levels in spring and the lowest in autumn/spring, which is a new finding. First, we have not found any previous study where seasonal variations of midnight salivary cortisol are analyzed;
measurements have been performed during daytime and in late evening. Second, according to a review of circannual hormonal changes, basal levels of circulating glucocorticoids seem to be lower during the spring and summer and peak during the autumn and winter. However, one research group reported the highest cortisol concentrations at daytime in February, March, and April (the high levels in March and April are findings quite consistent with ours), and the lowest concentrations in July and August which do differ from our findings. Another research group found that the cortisol awakening response was attenuated in persons with SAD during winter months but did not find any seasonal variations of cortisol secretion in healthy individuals.

- You theorize that salivary cortisol may be used in future research to evaluate if a person recovers from a depressive episode. Why would this be preferable to the person meeting with a doctor or therapist to assess this? What is the benefit? Also, I am not sure this section fits into the rest of the manuscript.

  Answer: We meant that measuring cortisol could be done in addition to meeting a doctor, as sometimes it is difficult both to patients and doctors to understand if a person has recovered from depression (particularly in alexithymic patients). Anyway we have now omitted the suggestion that MSC can be used to evaluate if a person recovers from a depressive episode.

Conclusions
- “Routine systematic depression evaluation at diabetes control visits, and necessary treatment...would eventually bring down long term diabetes complications and the comorbidity associated increased mortality” I do not think you can conclude this.

  Answer: We have now written at rows 329-332 “Normalized cortisol levels have been observed after resolution of depressive symptoms, but if recovery from depressive symptoms will lead to decreased MSC levels in patients with type 1 diabetes is a subject for future research”.

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

Sincerely,

Eva Melin, MD, PhD student (defending PhD later this year).

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