Author's response to reviews

Title: Association of serum leptin and ghrelin with depressive symptoms in a Japanese working population

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Author's response to reviews: see over
20 May 2014
Professor Mario Juruena
Associate Editor
BMC Psychiatry
Ref MS: 1729938089112118
Title: Serum leptin and ghrelin are associated with depressive symptoms in Japanese women but not in men

Dear Professor,
Thank you very much for your consideration to the above-referenced manuscript. We revised the manuscript according to the comments by reviewers. Our point-by-point responses to the reviewers’ comments are provided in a separate sheet and coloured in blue the corresponding sentences in text. The page and line numbers referred to our revised manuscript.
We hope that the revision would be satisfactory in addressing issues raised by the reviewers.

Yours sincerely,

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Reviewer #1

Title: Serum leptin and ghrelin are associated with depressive symptoms in Japanese women but not in men

Reviewer: Tobias Hofmann

Reviewer's report:

The article by Akter et al reports on the association of ghrelin and leptin (two hormones primarily known to be involved in the homeostasis of hunger and satiety) with depressive symptoms in a cross-sectional study in a general (thought to be healthy) population of 497 Japanese employees aged 20-68 years. Depressive symptoms were evaluated by the Japanese version of the Center for Epidemiologic Studies Depression (CES-D) scale and peptide levels by multiplex immunoassays.

Study subjects were divided into tertiles based on their leptin or ghrelin levels and due to significant higher circulating levels of ghrelin and leptin in women analyses were conducted for women and men separately.

The multiple logistic regression models were adjusted for several parameters including age and workplace and marital status, job position, occupational physical activity, non-occupational physical activity, smoking, alcohol drinking, energy intake, BMI, ferritin and folate.

Results indicate that higher ghrelin levels are associated with a higher prevalence of depressive symptoms in Japanese women, whereas no association was found for Japanese men. In addition, a trend for higher circulating leptin levels in subjects with less depressive symptoms was observed.

The work is original and contributes to the field of psychoneuroendocrinology of metabolic disturbances, eating and stress and mood disorders since it broadens its basis of human studies where inconsistent findings were observed in the past.

Thank you very much for your thoughtful comments on our manuscript.

Minor Essential Revisions

1. P values < 0.05 were considered to be significant. The title suggests that higher leptin levels are significantly associated with decreased odds for depressive symptoms. Since there is no statistical significance for leptin the title should be changed.

According to the suggestion we changed the title of our manuscript as “Association of serum leptin and ghrelin with depressive symptoms in a Japanese working population”.
2. The articles by Barim et al (2009) (Ghrelin, paraoxonase and arylesterase levels in depressive patients before and after citalopram treatment) reporting on reduced ghrelin levels in depressed patients and Schanze et al (2008) (Ghrelin and eating disturbances in psychiatric disorders) reporting on no association between ghrelin and depression should be cited and discussed in the manuscript. Thus, taken together, a significant positive relationship of depression and ghrelin is not a consistent finding in the literature and the conclusion in the discussion (4th an 5th paragraph) that the “present findings, together with clinical studies, suggest that higher ghrelin levels may be associated with increased prevalence of depressive symptoms” seems too straightforward and results should be discussed more tentative.

We thank the reviewer for his suggestion. We were not aware about the two above mentioned article. In this revision we cited these two references and discussed accordingly (lines 44-46 on page 7 and lines 213-24 on page 16).

3. Methods:

a. Were blood samples taken at a particular time of the day?

Blood samples were obtained in the morning after an overnight fast and particularly taken mostly from 8:30 a.m. to 12:00 a.m. on the day of the health checkup. We mentioned this in methods section (lines 89-89 on page 9).

b. Were protease inhibitors used?

No, protease inhibitors were not used for this study (line 89 on page 9).

4. Statistical analyses: an explanation why models were adjusted to the stated parameters would be helpful. In particular: why did you choose to adjust for ferritin, folate?

The first model was only adjusted for age and workplace and to confirm the association after additional adjustment for lifestyle factors, the second model was further adjusted for other covariates including serum folate and ferritin. We adjusted for a wide range of potential confounding factors, which were a priori selected on the basis of previously reported and well-known factors associated with depression. In addition, in our previous studies from same workplace, we found that serum folate and ferritin is associated with depressive symptoms (Yi et al. 2011; Nanri et al. 2010). Thus, we considered adjusting for ferritin and folate in our analysis. We explained this in statistical analysis section (lines 118-22 on page 11).

5. Tables 1, 2 & 3 and Statistical analyses section: what is meant by workplace A and B? Please explain (tables: in the legends).

As our study is based on two municipal offices, we simply coded these two workplace as workplace A and workplace B. We explained this in Tables and statistical analysis section (lines 121-22 on page 11).
6. Table 3: what does Pinteraction mean?
   We used this term to refer to the p-value for the interaction and in this revision we showed the P for interaction (previously wrote as P_{interaction}) value in a new column of Table 3. P for interaction using the likelihood ratio test was performed to see whether there is any effect modification by sex on the association between leptin, ghrelin, and depressive symptoms. We explain about P for interaction test in statistical analysis section (lines 129-31 on page 11) and also in footnote of Table 3.

7. Table 3: in the legend adjustment for sex was indicated while analyses were conducted separately for men and women.
   Sex was written mistakenly. We deleted sex from footnote in Table 3.

8. Table 3, women, leptin, 2nd tertile: as stated in the discussion, this association was significant which should be stated by a p-value instead of bold characters.
   In this revision we did not use the bold characters. We did not present p-value, because the significant association could be understandable to look at the 95% confidence interval.

9. Table 3: the use of bold characters for significant results should be explained in the legend.
   We refrain highlighting in bold for Table 3.

10. Table 3, 1st column: it should be stated that the odds are indicated for depressive symptoms (e.g.: Odds for depressive symptoms (multivariable adjusted))
    For the first column of Table 3 we changed the writing style.

11. Discussion: is there any explanation that depressive symptoms tend to be higher in the highest leptin tertile in women (which prevents the association of depressive symptoms and leptin levels to become significant in women)?
    We explained this in discussion section (lines 205-12 on pages 15 & 16).

12. Discussion, 2nd paragraph: if there was a threshold for leptin to exert antidepressive effects how would you explain that women (with higher leptin levels than men) display more depressive symptoms than men?
    As we cannot provide plausible explanation for the mentioned sentence “If there was a threshold …” we deleted this sentence from discussion. Instead, we speculate that there is a sex difference on the association between leptin and depressive symptoms (lines 188-92 on pages 14 & 15).

13. Discussion, 3rd paragraph: Does the notion that the positive association of
leptin and depression in the studies by Pasco et al and Milaneschi et al may be due to higher BMIs in their populations (26.8 ± 5.4 in the study be Pasco et al) refer to the leptin resistance in overweight and obese subjects?

Yes, we think that higher BMI linked with leptin resistance is one of the reasons for the positive association between leptin and depression for the studies in Western countries. In this revision we modified these sentences to better understand our views (lines 199-202 on page 15).

Discretionary Revisions

1. I would prefer a figure with several panels instead of table 3 to present the main results. According to the suggestion of another reviewer we also present the findings for CES-D≥19 in Table 3, so, we refrain for presenting the findings in figure.

2. Background, second paragraph: I would insert that leptin is an anorexigenic hormone. We inserted leptin as an anorexigenic hormone (line 10 on page 5).

3. Results, 2nd and 3rd paragraph: indicating that results for CES-D#19 are not shown in any table would be helpful. According to the suggestion of another reviewer we presented the results for CES-D≥ 19 in Table-3. Thus, we did not need to write “results for CES-D≥ 19 are not shown in Table-3”.


Reviewer #2

Title: Serum leptin and ghrelin are associated with depressive symptoms in Japanese women but not in men

Reviewer: Seren Roberts

Reviewer's report:

PEER REVIEW

General comments
This is an interesting paper about the association of specific appetite hormones and depressive symptoms. It is generally well written though some work is need on the discussion section. The study does not offer any insights into the mechanism of effect or possible improvements to treatment or management of depression but provides further insight into the biological mechanisms that may underlie or mediate some depressive symptoms. The study has a number of limitations, some of which are discussed in the paper. The study does not take into account likely treatments for depression which may interfere with the hormones in question and the depression rating scores.

Introduction
The authors make a clear and concise case for the study and report compelling evidence to support their argument. There is little evidence to support their methods or approach. I would also like to have seen a bit more detail about the proposed mechanism of effect. For example, how might these hormones have a possible effect on mood? Also some more discussion about how lifestyle factors in people with depression and antidepressants might influence possible levels for these hormones would be helpful.

The aim of the study is transparent.

Methods
This cross sectional study was undertaken as part of a large study with main methods reported elsewhere. Survey data were collected about demographics, lifestyle and depressive symptoms (using Centre for Epidemiologic Studies Depression Scale) together with a blood sample (after overnight fasting). There is a good description of the procedures for blood testing, data management and analyses. However, the authors need to justify why the hormone values were treated in tertiles (low, medium and high) and not as continuous variables. They also need to specify the value ranges for three tertiles in the text. The authors should also justify why they adjusted for all the additional variables in the second model.

Results
The results are well presented and clear. The authors report an association between higher leptin levels and reduced odds of depressive symptoms in women which is not statistically significant. For me, this should be reported as a trend rather than an associated because the lack of significant result. The authors report similar findings for CES-D #19 but do not provide the results. These should be included in table 3. I assume the results that follow this assertion relate to the depressive symptoms scores CES-D #19 but this needs to be clarified. The clause ‘whereas no clear association was found for men’ (results- end of second paragraph) indicates an association in the preceding clause but again, the association between leptin tertile and depressive symptom in women is not statistically significant (bar the mid tertile). I would suggest rephrasing as this is rather misleading.

Discussion

The first sentence of the discussion again claims leptin is inversely associated with depressive symptom in women with no apparent linear trend. However, this claim is not support by statistical evidence as noted above. The first 2 sentences of the discussion should be removed. First 2 sentences of the second paragraph under discussion also seem repetitive. I suggest starting with …our data are in line….

The discussion overall feels repetitive, covering themes raised in the introduction and results. I would like to seem much more discussion about the authors’ interpretation of the findings, to try to explain some of these findings, particularly exploring how the findings fit with possible models of mechanism of effect (inflammation-depression touched on but not fully explored). Even though this is not a longitudinal study with data to indicate a clear causal relationship, I think it would benefit the paper if the authors made clear their views about their findings and what they think it means. Some discussion about the likely cause and effect relationship might help explain some of the findings in the context of the evidence which conflicts with their findings. The paragraph about the underlying mechanism (pg 13 line 9 - pg 14 line 5) should be included in the introduction. In the discussion, the authors should discuss how their findings fit in with these models. There is no discussion about the differences between the CES-D #19 and the CES-D #16 depression cut off values. I think this warrants further discussion here because the authors suggest in the methods section that the threshold of CES-D #19 is more suitable to Japanese population yet they manly report the CES-D #16 cut off findings.

The brief discussion about the relationship between depression and diet should be included the main body of the discussion. For the limitations section, once they identify the limitation, I suggest the authors propose a study design that might address these issues. E.g. longitudinal
studies with multiple times point assessments, data capture about dietary intake, treatment for depressions, or a study with a clinical sample and so forth. I also think the authors should mention limitations of the analyses e.g. using tertiles etc.

We thank the reviewer for her critical and fruitful comments. Our responses to the specific comments are below:

- Major Compulsory Revisions

1. Include discussion of possible mechanisms of effects in introduction along with a brief discussion about how lifestyle factors in people with depression and antidepressants might influence possible levels for these hormones

In this revision we revised the introduction section and explained possible mechanism along with possible lifestyle factors of depressive patients and their influences on leptin and ghrelin levels (lines 19-25 on page 6, lines 26-39 on pages 6 & 7).

2. justify why the hormone values were treated in tertiles (low, medium and high) and not as continuous variables in results

If we considered serum leptin and ghrelin as a continuous variable we can see only the linear relationship between these hormones and depressive symptoms. But our intention is to see the shape of the relation of leptin and ghrelin with depressive symptoms i.e. whether the association is linear or not. Thus, we choose tertile categories of leptin and ghrelin. And in fact we found a non-linear association between leptin and depression and a linear association between ghrelin and depression in women. We mentioned this issue in statistical analysis section (lines 111-12 on page 10).

3. specify value ranges for three tertiles in the text

We specified the value ranges of leptin and ghrelin for three tertiles in text (lines 140-46 on page 10).

4. justify why they adjusted for all the additional variables in the second model

The first model was only adjusted for age and workplace and to confirm the association after additional adjustment for lifestyle factors, the second model was further adjusted for other covariates including serum folate and ferritin. We adjusted for a range of potential confounding factors, which were a priori selected on the basis of previously reported and well-known factors associated with depression. In addition, in our previous studies from same workplace, we found that serum folate and ferritin is associated with depressive symptoms (Yi et al. 2011; Nanri et al. 2010). Thus, we considered adjusting for ferritin and folate in our analysis. We explain this issue in statistical analysis section (lines 118-22 on page 11).
5. rephrase wording of the association between leptin and depressive symptoms is a trend rather than a statistical association

We corrected these sentences throughout the manuscript according to the suggestion.

6. report findings for CES-D #19

We reported findings for depressive symptoms defined as CES-D ≥ 19 in Table 3. We also discussed about the differences between the CES-D ≥ 19 and the CES-D ≥ 16 depression cut off values as suggested in general comments (lines 224-32 on pages 16 & 17).

7. develop the discussion further by discussing author’s interpretation of the findings and try to explain them, contextualise the findings and how they fit with existing evidence on the mechanism of effect of hormones and make some suggestions about how their findings add to the existing knowledge base.

According to the suggestion of reviewer we re-arranged and added many sentences in discussion section (lines 234-42 on page 17).

8. Propose study designs that might take their research a step further in overcoming some of the limitations of this work.

We stated recommendation of conducting further studies in overcoming some of limitations of our study at the end of the manuscript (lines 259-61 on page 18).

- Minor Essential Revisions

1. Pluralise 'human' at the start of second paragraph in introduction

We corrected this sentence (line 40 on page 7).

- Discretionary Revisions

None
Reviewer # 3
Title: Serum leptin and ghrelin are associated with depressive symptoms in Japanese women but not in men
Reviewer: Martha Payne
Reviewer's report:
BMC Psychiatry
Serum leptin and ghrelin are associated with depressive symptoms in Japanese women but not in men

This important study examines the relationship between ghrelin and leptin levels with depression symptoms in a sample of adults in Japan (which has high prevalence of depression symptoms). The sample size is reasonably large considering the assessments included. Strengths include use of CES-D for depression, and fasting samples for biological measures. This is a good paper overall but a few issues need to be addressed.

Thank you very much for your thoughtful comments on our manuscript.

Major Compulsory Revisions
1. Authors need to be more careful in describing non-significant findings. Their own findings for leptin across tertiles were non-significant for a trend but are discussed as if they were significant. Conclusions should not be drawn from non-significant findings. Similarly the authors present results from a study on page 13, line 5 but then mention that they were not significant.

In this revision we were more careful in describing non-significant findings. We changed the title and conclusion of our manuscript. We deleted the non-significant findings from conclusion in main text. We also made several changes throughout this manuscript.

2. Authors should discuss the finding of protective effect for leptin in 2nd tertile only. Why would 3rd tertile not be significant? Also, authors speculate that lack of association for men was due to lower values, yet 3rd tertile in men was of comparable level to 2nd tertile in women.

Although we found a significant lower odds for depressive symptoms only in the 2nd highest tertile of leptin, a 53% lower odds for depressive symptoms was found for the 3rd highest tertile. In the present revision, we rephrased this finding as trend for lower depressive symptoms for higher levels of leptin rather than an association and discussed this finding accordingly.
In a previous reviewed study Lu et al., suggested that both leptin insufficiency and leptin resistance might contribute to alterations of affective status. This may also partly true in the present study. We explained this in discussion section (lines 205-12 on pages 15 & 16).

As men and women have significant different levels of leptin, we speculate that there may be sex difference for the association between leptin and depression. We discussed this in second paragraph of discussion (lines 188-91 on pages 14 & 15).

3. Results indicate no difference in BMI between groups. This is at odds with most western studies showing a relationship between depression and obesity, and should be discussed.

Our study population is apparently healthy population and have different criteria than clinically depressed patients. Previous study (Pasco et al. 2008), which was based on apparently healthy population also did not found a significant difference in BMI between depressed and non-depressed subjects. We already discussed that our study population is a leaner Japanese population and have different mechanism linking leptin and depression than Westerners (lines 199-202 on page 15).

Minor Essential Revisions

1. Clarify what is meant by ‘technical jobs’ because it does not appear that these jobs would necessarily involve greater physical activity.

We explained about technical jobs in methods section (lines 104-5 on page 10).

Discretionary Revisions

1. None