Author's response to reviews

Title: Are religious beliefs and practices of Buddhism associated with disability and salivary cortisol in office workers with chronic low back pain?

Authors:

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Version: 2  Date: 29 December 2012

Author's response to reviews: see over
Reviewer: Quinette Louw

Thank you very much for your comments. I am confident that they will make the manuscript sound more scientific and also make it easier to read. I have revised the manuscript according to your comments with details given as follows:

1) The introduction should be more specific to the main focus of the manuscript. The first paragraph is a very general introduction to LBP and should be revised to introduce the main focus of the paper at an earlier stage.

In paragraph 1, the authors refer to the incidence rate as a percentage while it is usually expressed as the number of new cases per population.

The second sentence of paragraph one requires grammatical correction.

Response: The first paragraph has been omitted as suggested.

2) Paragraph 2
In the last sentence the authors suggest that “the epidemiological literature suggest…….” This should be revised as one paper cannot be representative of the epi literature unless it is a systematic review. Perhaps the authors should search for a systematic review to reference. This particular paper was also published almost 7 years ago. The reference that the authors refer to is published. This sentence referred to above is the crux of the paper and should thus be well justified.

Response: The old reference has been replaced with the new one (a systematic review paper) (see Page 5 Lines 6-7).

3) Paragraph 3
The aspects of religion should be introduced earlier in the introduction. The study by Abraido was published in Spanish and it is not certain whether they have truly established a causal relationship. The authors should confirm that and possible also reference another study since this is a main justification for their paper. The last sentence states that the authors’ hypothesis that religion may reduce disability:
• I do not think that the authors can test this hypothesis in this cross-sectional study
• It is unclear if they are referring to the hypothesis of this study as another hypothesis is stated at the end of the intro (which is appropriate).

Response: Since the first paragraph has been omitted, the aspect of religion is introduced earlier in the introduction (the 2\textsuperscript{nd} paragraph).

The study by Abraido et al (2004) was published in English. However, the study employed a cross-sectional study design which only allowed the association between exposure and outcome to be examined. It is not possible to establish a causal relationship between exposure and outcome, although the authors claimed such. The sentence has been revised to correctly reflect the notion (see Page 5 Lines 12-14).
The last sentence of the paragraph was our hypothesis based on the literature review. Since the main idea of the paragraph was presented in the first sentence of the paragraph, the last sentence has been omitted to lessen confusion.

4) Paragraph 4
The last sentence is important but clarification and further explanation is required. The authors state that mindfulness treatments are associated with improved physical function. This should be explained in more detail.

Response: An explanation of why mindfulness treatments are associated with improved physical function has been added (see Page 6 Lines 7-12).

5) Salivary cortisol is measured in this study, but not mentioned in the intro although the authors conclude that it adds to the strength of the study.

Response: The introduction about salivary cortisol has been added (see Page 6 Lines 19-23).

Methodology (Major revisions)

6) State the type of office workers? How were they recruited?

Response: The manuscript has been revised as suggested (see Page 8 Lines 15-19).

7) Why were office workers deemed appropriate for a study of this nature? Could the association be different in another working population?

Response: The main reason for choosing office workers as a study population is because the 1-year prevalence (34–51%) and incidence (23%) rate of low back pain is quite high among office workers A significant portion of patients with LBP has developed chronicity and disability and the economic consequences of treating chronic and disabling LBP are substantial. Effective prevention of low back pain in office workers would be significantly beneficial to the society, which is our main research interest. Disability due to low back pain in different occupations is unlikely to be identical because the physical requirements for different occupations are different. Thus, the association between the religious beliefs and practices of Buddhism and disability could be different among different working populations. Generalization of the results from this study to other populations should be made with caution. The notion has been added as another limitation of the study (see Page 19 Lines 19-23).

8) How was the screening done for the exclusion criteria?

Response: The sentence ‘Inclusion and exclusion criteria were determined by using a self-reported questionnaire’ has been added into the revised manuscript (see Page 8 Lines 12-13).
9) How were subjects assured of anonymity, particularly since they were asked about their religious beliefs and practices?

Response: To ensure anonymity of subjects, an identification number was used in the self-administered questionnaire. Personal information, including their name, for each identification number was kept in a separate document.

10) The main questionnaire is the Thai Buddhism questionnaire. The authors reference a national research document but there is no indication where readers can access this document. Since this is the main exposure, the authors should provide a reference of a published paper to be transparent about the validation process of the paper. An inappropriate questionnaire could have profound effects on the findings of this cross-sectional study.

Furthermore, the authors only refer to the internal consistency of the questionnaire and the other psychometric properties of the questionnaire should also be mentioned.

Response: Unfortunately, the researchers who developed the questionnaire have not published their study in an English-language journal. We agree with the reviewer that this circumstance can adversely affect our study. However, prior to conducting the present study, we thoroughly examined the methodology of their study to ensure its appropriateness.

The manuscript has been revised by adding some more information about the psychometric properties of the questionnaire (see Page 9 Lines 13-15, Page 9 Lines 20-21, Page 10 Lines 4-5). However, for the Buddhist lifestyle subscale, construct validity could not be established because there was no gold-standard group for comparison.

11) Why was the Roland Morris selected???

Response: We used the Roland-Morris Disability Questionnaire because of two main reasons. First, it is a specific tool used to assess disability level associated with low back pain. Second, it is available in a Thai version.

12) Salivary cortisol – which “Wednesday” are the authors referring to?

Response: We collected the salivary sample on the Wednesday of the week to reduce day-to-day variation in salivary cortisol. The manuscript has been revised to clarify the issue (see Page 11 Line 23).

13) How was the randomization done?

Response: The manuscript has been revised to include the information as suggested (see Page 8 Line 22).
14) The last sentence of the results should be part of the discussion.

Response: We are not sure whether the reviewer refers to the sentence ‘The religious beliefs and practices of Buddhism accounted for an additional and significant 6% of variance in psychological stress, after controlling for confounder variables’. This sentence is simply the finding of the study from the reading of Table 3.

15) Statistical analysis / Results
I would recommend statistical review of this paper, particularly with reference to the explanation of the statistics- large sections of the results should be in the data analysis sections. Could confidence intervals be calculated for correlations?

Response: The manuscript has been revised as suggested (see Page 13 Lines 10-20). We prefer not to show the results of correlational analyses among the religious beliefs and practices of Buddhism and each variable because it would make the manuscript very long. However, the variables chosen for inclusion in the regression analyses were those significantly correlated with the RDQ score and AUCG based on correlational analysis (p<0.05).

16) Which were the significant confounders? (refer to relevant tables in subsequent sections)

Response: The manuscript has been revised by adding the significant confounders (see Page 15 Lines 6-9 and Page 15 Lines 18-19).

17) It will be good to include another table to describe the findings of the religion questionnaire to illustrate the variability of the data as I suspect that many of the participants would probably have reported high commitment to their religion and it is also reflected in the table on page 27. It will be good to know about the distribution of this data and how it was applied in the regression model.

Response: Table 1 has been revised to include the mean scores of three subscales of the religion questionnaire (see Table 1).

18) Discussion (Minor revisions)
The first paragraph states the outcome with respect to Buddhistm and stress- this was not the main outcome according to the hypothesis which appears to present disability as the main outcome. This should be checked and revised.

Response: The manuscript has been revised as suggested (see Page 16 Lines 2-5).

19) Page 18: second last sentence- authors should explain this possible mechanism in more detail.

Response: The manuscript has been revised as suggested (see Page 18 Lines 21-23).
20) Explain why a homogeneous sample is a strength as this is the case in most project.

Response: Disability due to low back pain and psychological stress level in different occupations is unlikely to be identical because different occupations have different work characteristics. Several previous studies recruited subjects with no consideration of their occupation. In this study, only office workers with chronic low back pain were included and we considered that the inclusion of subjects of the same occupation increased the homogeneity of the sample. However, we have decided to omit the homogeneity of the subjects as a strength of the study to lessen any confusion.

21) A further limitation is the effect of reporting on socially undesirable behaviour which could introduce biased associations. This should be included and explained in the limitations sections as this would be a significant threat in a society where more than 97% of the population is Buddhist.

Response: The manuscript has been revised as suggested (see Page 19 Line 23).
Reviewer: Anette Harris

Thank you very much for your comments. I am confident that they will make the manuscript sound more scientific and also make it easier to read. I have revised the manuscript according to your comments with details given as follows:

Compulsory revision:
1) Response rate should be reported for the whole sample. Not only those who respond to the invitation letters. How many were eligible and how many participated? How many were invited to collect saliva cortisol?

Response: The manuscript has been revised to include information as suggested (see Page 14 Lines 8-12).

2) Cortisol analysis:
   - What was the intra- and inter-assay variability?

Response: We employed the commercially available kit manufactured by IBL-America for detecting the free cortisol level in saliva. This particular kit has been proved reliable. The kit is based on salivary ELISA and has been widely used as previously reported in several publications. The manufacturer has demonstrated the information regarding intra and inter assay variations as shown below:

Intra-Assay Variation
The intra-assay variation was determined by 20 replicate measurements of 4 saliva samples. The within assay variability is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>4.52</td>
<td>0.94</td>
<td>12.79</td>
<td>17.50</td>
</tr>
<tr>
<td>SD (ng/mL)</td>
<td>0.120</td>
<td>0.042</td>
<td>0.230</td>
<td>0.258</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.65</td>
<td>4.52</td>
<td>1.80</td>
<td>1.47</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Inter-Assay Variation
The inter-assay (between-run) variation was determined by quadruplicate measurements of commercial control samples in three different days runs. The between-assay variability is shown below:

<table>
<thead>
<tr>
<th>Control</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>24.29</td>
<td>40.85</td>
</tr>
<tr>
<td>SD (ng/mL)</td>
<td>1.81</td>
<td>2.38</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.47</td>
<td>5.82</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>
**Inter-Lot Variation**

The Inter-Lot (between-lot) variation was determined by duplicate measurements of five saliva samples in three different kit lots. The between-lot variability is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>1.22</td>
<td>12.69</td>
<td>15.81</td>
<td>4.16</td>
<td>4.53</td>
</tr>
<tr>
<td>SD (ng/mL)</td>
<td>0.07</td>
<td>0.35</td>
<td>0.70</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.97</td>
<td>2.73</td>
<td>4.43</td>
<td>2.35</td>
<td>2.72</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

- Why do you only calculate area under the curve (AUC) with respect to ground not the increase (AUCI)?

**Response:** Based on a systematic review paper by Chida and Steptoe (2009), AUCG positively correlates to AUCI. AUCG has been found to be a slightly more reliable marker in terms of individual stability than AUCI (Clow et al 2004). We then calculated AUCG to quantify the cortisol awakening response of each subject (Chida and Steptoe 2009 and Pruessner et al 2003).

**References**


Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH: Two for mulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003,28:916-931.

- Was the cortisol data normally distributed or did you perform any log transformation before the analysis?

**Response:** All data are normally distributed, except cortisol at 0 min.

**Tests of Normality**

<table>
<thead>
<tr>
<th></th>
<th>Kolmogorov-Smirnova</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>df</td>
</tr>
<tr>
<td>AUCG</td>
<td>.057</td>
<td>96</td>
</tr>
<tr>
<td>Cortisol_0 min</td>
<td>.126</td>
<td>96</td>
</tr>
<tr>
<td>Cortisol_30 min</td>
<td>.062</td>
<td>96</td>
</tr>
</tbody>
</table>

a Lilliefors Significance Correction

* This is a lower bound of the true significance.
It is worth mentioning here that cortisol at 0 min may be affected by each individual's factors such as age, gender, light, awaking time and participant adherence as previously reported by Clow et al (2004). If the measurement is done to assay the single point of salivary cortisol, it may not properly reflect the life stress that we would like to assess. So, in the current study, we measured the cortisol levels not only at 0 min, but also at 30 min post-awakening time as well.

By the way, we also performed the log transformation. X-axis (concentration) values were transformed into log before plot in the standard curve. The 4 Parameter Logistic or 4PL nonlinear regression model is commonly used for curve-fitting analysis in bioassays or immunoassays such as ELISAs or dose-response curves. The 4 parameter logistic (4PL) curve describes ELISA assays much better than linear curve fits. There are many reasons but basically the 4PL model equation has a maximum and a minimum built into the model which is more reasonable to describe biological systems. There is no biological system that will increase or decrease forever as the curve goes to infinity (basically what a linear curve fit does).

- The cortisol levels at awakening and 30 minutes later should be presented (with mean and 95% CI), not only the AUCG. The dynamics of the cortisol curve is important.

Response: Table 1 has been revised to include the mean cortisol levels at awakening and 30 min after awakening (see Table 1).

- Since the major results were that religious beliefs and practices of Buddhism have a significant effect on psychosocial stress, measured with cortisol, I would like to see a cortisol figure, split into two groups; those who have religious beliefs and practices of Buddhism and those who not. Are they lower at awakening or do they have less cortisol response after awakening? If there is not enough space for a figure I would like to have the levels presented in a text.

Response: The score of the questionnaire ranges from 30 to 180, with higher scores indicating more spirituality or religiousness. The questionnaire does not have a cut-off point to indicate whether a person has the religious beliefs and practices of Buddhism. However, the manuscript has been revised to include cortisol levels at awakening and 30 min after awakening for those who had total scores higher and lower than the mean total score of the study population (see Page 14 Lines 16-21).

Discretionary revision:
1) Most limitation are clearly stated but saliva cortisol sample, based on one day only, should not be characterized as strength; we know that there is a huge day-to-day variation (page 19, first line)(see Hellhammer 2007).
Response: The manuscript has been revised by omitting the use of salivary cortisol as a biomarker for psychological stress as a strength of the study.

2) Relevant empirical data (Chida) are sited but I miss a theoretical point of view. Do we all agree that high cortisol levels after awakening are harmful? According to allostatic load (McEween) this is the right hypothesis, but according to cognitive activation theory of stress (Ursin and Eriksen) it's not.

Response: The function of cortisol awakening response (CAR) should be further elucidated, but it has been suggested to be associated with a stress-related preparation in regard to the upcoming day by the hippocampus. One hypothesis is: "that the cortisol with an elevated level after awakening may accompany an activation of prospective memory representations at awakening enabling individual's orientation about the self in time and space as well as anticipation of demands of the upcoming day. It is tempting to speculate that for the CAR, anticipation of these upcoming demands may be essential in regulating the CAR magnitude for the particular day. The hippocampus is, besides its established role in long-term memory consolidation, involved in the formation of a cohesive construct and representation of the outside world within the central nervous system processing information about space, time and relationships of environmental cues. This puts the hippocampus in a pivotal position for the regulation of the CAR."
Therefore, in our current study, the context is in accordant with the reports of McEwen (2006) and Chida and Steptoe (2009).

Ursin and Murison (1983) actually agreed that sustained arousal, particularly sustained high levels of cortisol, vagal discharges, and thyroxin may produce somatic pathology.

According to the report of Ersin and Eriken (2004), for the HPA axis, an increased baseline levels of cortisol was emphasized, but attenuated responses to ‘stress’ stimuli, to negative or inconsistent expectancies, and to depression. Sustained arousal may eventually lead to a hyporesponsive HPA axis. We think that this cognitive activation theory of stress may be convinced if the feedback (or negative) regulation is still functioning. There have been well documented that the sustained arousals rather disrupt the feed back regulation of HPA axis, especially when pathology has occurred.

References