Author's response to reviews

Title: Self-perceived psychological stress is associated with parameters of adiposity and subclinical atherosclerosis disease in Mexican population. The GEA Study.

Authors:

Janinne Ortega-Montiel MD (janis_boly@hotmail.com)
Rosalinda Posadas-Sanchez MSc (rossy_posadas_s@yahoo.it)
Carlos Posadas-Romero MD (cposadasr@yahoo.com)
Wendy A Ocampo-Arcos BSc (mayte1190@yahoo.com.mx)
Aida X Medina-Urrutia MSc (aidaxm@yahoo.com)
Guillermo C Cardoso-Saldaña PhD (gccardosos@yahoo.com)
Esteban Jorge-Galarza MSc (esjoga@yahoo.com.mx)

Version: 2 Date: 20 March 2015

Author's response to reviews: see over
March 20, 2015.

Natalie Pafatis  
Executive Editor, BioMed Central

Please find enclosed the revised Manuscript MS: 1443412822119931 of the Original Article entitled “Self-perceived psychological stress is associated with parameters of adiposity and subclinical atherosclerosis disease in Mexican population. The GEA Study.” which we submit for you to review and consider its publication in your journal. We have followed all your suggestions and all the changes have been made. We have also answered the Reviewers comments and have listed a reply for each one of their concerns. We hope that you will find the revised manuscript suitable for publication.

I thank you for your attention and courtesy.

Sincerely,

Rosalinda Posadas-Sánchez, MSc.  
Departamento de Endocrinología  
Instituto Nacional de Cardiología “Ignacio Chávez”  
Address: Juan Badiano No. 1 col. Sección XVI, Tlalpan, C.P. 14080. Mexico, D.F., Mexico.  
Tel: +(52)55732911 Ext. 1272 Fax: +(52)55734687  
E-mail: rossy_posadas_s@yahoo.it
Major Compulsory Revisions

Why did not the authors evaluate the psychological status by standardized questionnaire?

GEA Study, was designed to investigate genetic risk factors associated with premature coronary heart disease, subclinical atherosclerosis and other coronary risk factors in Mexican mestizo population. The evaluation of the psychological status was not the main objective of the study. In spite of, a single-item question to evaluated psychological stress was included in the questionnaire in order to know the prevalence of self-perceived psychological stress in the studied population. This measurement has been shown to predict overall stroke as well as myocardial infarction in prospective studies (Am J Cardiol 1991, 68:1171-1175., Stroke 1990, 21:223-229), and to correlate with other constructs representing psychological stress including financial stress, stressful life events and locus of control (Lancet 2004, 364:953-962). Taken together, these results indicate a validity of the single-item question in investigations of cardiovascular risk.

The authors should also give the treatment. Did the treatment impact in the IMT?

Because of the retrospective assessment of the data and, because the main objective was not to evaluate the effect of treatment on the IMT, we did not give any treatment.

The authors should give some details about the machine wich they have used to examine the patients.

Details on the ultrasound device used in the measurement of the IMT are now given on page 8 in the first paragraph.

How did they calculate IMT?

The details are now given on page 8, first paragraph: “The common carotid artery, bulb and internal carotid artery were bilaterally scanned over a length of 10 mm. The mean combined outcome of the six segments was reported.”
Reply to comments by Reviewer 2

Discretionary Revisions

(1) Table 1 and 2 could be combined.

We have combined table 1 and 2 with the principal finding of both.

(2) Discussion: “Alterations in the control and action of the hypothalamic-pituitary-adrenal axis play a major role in this context, with the participation of the sympathetic nervous system, inflammation, or an interaction between genetic predisposition and stress” – Alterations in parasympathetic activity also play a role and may be mentioned here.

We have included the parasympathetic activity as a pathological mechanism in the discussion part of the manuscript.

Minor Essential Revisions

(1) The authors should point out the clinical meaning of increased visceral fat accumulation and increased IMT, e.g., previous reports on associations with cardiovascular morbidity and mortality.

Now in the background text, we have mentioned the clinical importance of visceral and subcutaneous abdominal fat accumulation in the pathogenesis of atherosclerosis disease, on page 4.

(2) Means and p-values should not be doubled in the text body and tables.

We have supreme the means and p-values in the text.

(3) The abbreviation “HOMA” is not explained in the legend of table 2.

The abbreviation “HOMA” is now explained in table’s 1 legend

Major Compulsory Revisions

General remarks:
(1) The language used in the manuscript contains a number of misspellings and incorrect expressions which I will not completely list here. I recommend that the manuscript is corrected by a native speaker.

The misspelling and incorrect expressions have been corrected.

(2) The reader gets confused because the authors mix the terms obesity, central obesity, abdominal obesity, adiposity, visceral fat etc. These terms need to be clearly
defined in the methods section, and the same terms must then be used throughout the manuscript.

We now use obesity across the manuscript, to avoid the confusion of the reader. Also, we have defined these terms in the methods section.

(3) For one independent variable (subjective stress), a large number of dependent variables (12!) -which are closely intercorrelated - are analyzed. For more clarity, the authors should more precisely define the main outcome (metabolic syndrome? visceral fat accumulation? Subclinical atherosclerosis?). Other measures could (and should) still be reported as secondary outcomes. In the regression analyses, multicollinearity should be checked.

We corrected the results description, and we have clarified the main outcomes of the study.

(4) The cited literature is incomplete. There are already 14 longitudinal studies until 2009 included in the cited review article by Wardle and colleagues. 69% of these studies did not find an association between stress and obesity. The authors must more carefully summarize the existing knowledge in the background section, and their findings must be discussed in the context of the existing studies in the discussion section.

The cited literature has been corrected across the all manuscript.

(5) Stress measures were not available in 254 of 1500 control subjects, leaving 1246 for the analysis. Only 1243 subjects are reported in the results section and the tables. Please clarify.

We have noticed the mistake, now we mentioned that the stress measure were not available in 257 subjects.

**Did the drop-outs differ from the included subjects in sociodemographic in medical parameters?**

We have analyzed the excluded subjects in sociodemographic and medical parameters and these did not differed from the included, except for the age. We included a few part of the analyses.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>INCLUDED: 1243</th>
<th>EXCLUDED: 257</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.2 ± 9</td>
<td>49.2 ± 9.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Female gender</td>
<td>51 %</td>
<td>48.4 %</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>117.2 ± 17.5</td>
<td>118.2 ± 17.3</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C level</td>
<td>46.1 ± 13.5</td>
<td>45.6 ± 12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
<td>80%</td>
<td>84%</td>
<td>NS</td>
</tr>
</tbody>
</table>
How did the authors deal with other missing data?

We actually have analyzed all the subjects excluded for different reasons, and the results did not differed, therefore we made the final analysis with all the data that we count.

(6) The quality and the structure of the discussion have to be improved, and I am afraid that complete re-writing is necessary. Some helpful advice for the authors may be found using the following link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1115625/
I would recommend the following structure:
Summary of the main results
Discussing the findings in the context of the existing literature
The authors also have to discuss (1) why they observed differential associations of stress with health outcomes in men and women, and (2) why stress was only related to one outcome parameter each despite the close interrelationship of all outcome parameters (including the possibility that the finding(s) are due to chance alone given the multiple testing).

We have rewriting the entire discussion following the recommend structure.

Discussing the potential mechanisms linking stress and health outcomes.

In the new manuscript, we mentioned that the genetic predisposition also play a role in the pathological mechanisms that involved the stress as a risk factor for cardiovascular disease.

Strengths (although pretty obvious, these are not presented so far!) and limitations

Strengths and limitations are now included at the end of discussion section, page 12, last paragraph.

(7) Results: It would be interesting to have also the ORs for the whole study group. As the authors used a high cut-off for the stress score, only 126 subjects are in the high-stress group. Further subgroup analyses (men vs. women) may lead to further loss of power.

We have analyzed the population thought several subgroups using different cut-offs for the stress score, however, in the way we presented the results gives greater statistical power for the mean objective. We adjunct the crude regression analysis of the six answers to the stress question for obesity, to support the cut-off used in the paper.

<table>
<thead>
<tr>
<th>Self-perceived psychological stress</th>
<th>N= 1246</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never experienced stress</td>
<td>97</td>
<td>1.00 reference</td>
</tr>
<tr>
<td>Some periods of stress</td>
<td>264</td>
<td>1.3 (0.77-2.20)</td>
</tr>
<tr>
<td>Some periods of stress during the last 5 years</td>
<td>450</td>
<td>1.2 (0.74-2.02)</td>
</tr>
<tr>
<td>Several periods during the last 5 years</td>
<td>154</td>
<td>1.28 (0.72-2.25)</td>
</tr>
<tr>
<td>Permanent stress during the last year</td>
<td>153</td>
<td>1.55 (0.88-2.27)</td>
</tr>
<tr>
<td><strong>Permanent stress during the last 5 years</strong></td>
<td><strong>128</strong></td>
<td><strong>2.79 (1.57-4.94) p&lt;0.000</strong></td>
</tr>
</tbody>
</table>
Specific comments:

(8) Abstract: “...adiposity and subclinical atherosclerosis disease were determined by computed tomography” – that is incorrect: adiposity (obesity) was defined by BMI > 30, or central obesity as waist circumference > 90/80cm. Subclinical atherosclerosis was estimated using ultrasonography, and abdominal fat was assessed using CT scans (see above).

We have rewritten the methods described in the abstract to avoid mistakes.

(9) Abstract: “Adult Treatment Panel III (ATP III)”: omit the abbreviation in brackets (unnecessary)

We have omitted the mentioned abbreviation.

(10)Background: “A recent meta-analysis of longitudinal studies supports an association between psychosocial stress and obesity in adults (4).” – please be more precise here – what was the exact finding of the meta-analysis?

In this meta-analysis published on Obesity 2011, 19:771-778, demonstrated positive effect (25%) than a negative effect (6%) of stress on adiposity and appeared to play a greater part in the onset of obesity for men than women.

(11)Background: “Stress pathophysiological underlying mechanisms are poorly understood” – After decades of stress research, the underlying mechanisms are far from being “poorly understood” -although there is still room for improvement

We have corrected the background. Now it says: “Stress pathophysiological underlying mechanisms covers a wide field of research...” Follow by

(12)Background: “…some investigators have reported...hypercortisolaemia, neuroendocrine activation, and stimulation of the hypothalamic-pituitary-adrenal (HPA) axis...” – that is basically three times the same. Please change (or clarify).

We have changed the sentences. Now it says “…some investigators have reported inflammatory and immune process, alterations in activating hypothalamic pituitary adrenal axis, alterations of activities of autonomic nervous system among others...”

(13)Background: “These multiple risk factors have been identified as the principal cause of cardiovascular morbidity and mortality from atherosclerosis disease in adult population (7).” – It is unclear what is meant by “these multiple risk factors”. Atherosclerosis has a multifactorial etiology and not one “principal cause”.

We mean the principal diseases involved in the atherosclerosis, we have rewritten the sentences.
Background: “Epidemiological studies, like the INTERHEART Study (10), have used the subjective perception of psychological stress, measured by a semiquantitative single item question to know the prevalence and the impact of psychological stress.” That is incorrect! The INTERHEART study used 4 items to assess stress at work and at home, financial stress, and major life events in the past year. Additional questions assessed locus of control and presence of depression.

We have omitted from the sentences “measured by a semiquantitative single item”

Methods: A statement about approval by an ethics committee and about informed consent of the participants is missing.

This is specified in page 5. “The GEA study was approved by the Institutional Review Board of the National Institute of Cardiology “Ignacio Chavez”, and the Ethics Committee of the National Institute of Genomic Medicine (INMEGEN), and conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants”

Methods: Central obesity is defined in the methods section, but it does not appear later in the text body or the tables. Or does the term “obesity” used in the results and discussion sections and tables refer to central obesity (see above)?

We have omitted the terms central and abdominal form the paper.

Methods: Why were subjects with alcohol consumption > 20g / d excluded? Alcohol consumption might very well be a pathway partially explaining the relationship between chronic stress and metabolic factors.

The subjects with alcohol consumption >20gr per day were excluded from the principal population of GEA study for the implication of the etiology in the liver disease, because another objective of the study was the fatty liver disease.

Methods: “According to the literature, permanent but not periodic stress, as assessed with this measure, has previously been found to associate with cardiovascular risk, crude OR was calculated between obesity and the different answers of self-perceived psychological stress item and only score 6 (permanent stress during the last 5 years) showed significant association to an increased risk of obesity, therefore in the principal analyses and in subtype analyses.” What literature? As far as I can see, only one study using the same stress assessment instrument as in the present study is cited (Rosengren et al., 1991). In that study, scores 5 and 6 were combined, whereas in the authors here compare subjects with a score of 6 with all other subjects. The authors have to explain why they used this approach. Would be the results change if the same approach as in the Rosengren study was used? Was a sensitivity analysis performed?

We have previously support this analysis in question number 7.
(19) Methods: “…visceral adipose tissue areas as described by Kvist…” Please give a reference here.


(20) Methods: Please explain the abbreviations VAF, SAF and TAF.

We have explained the abbreviations.

(21) Methods: Please use the term “Mann-Whitney U test”, not U-Mann Whitney test.

We have changed the term not U-Mann Whitney test for “Mann-Whitney U test” in methods section.

(22) Methods / discussion: I do not see the sense in adjusting for BMI if obesity (elevated BMI by definition) is the dependent variable – it sounds illogical that stress is associated with obesity “independent of BMI”. Or do the authors mean “central obesity”.

We followed the recommendations for the adjustment in the analysis of model 2, and the results did not different when we excluded the BMI in the adjustment, so it was retired.

(23) Discussion: “In conclusion, we found that self-perceived permanent stress is an independent risk factor of lifestyle/social and biologic mediators for adiposity in men and carotid atherosclerosis in woman of Mexican population.” Please omit “of lifestyle/social and biologic mediators” (or clarify, as I do not understand that part of the sentence). Minor point: “In summary” (instead of “in conclusion”) would possibly be the better expression at this point of the manuscript. We made the suggested changes. In page 13, first paragraph: “In summary, we found that self-perceived permanent stress is an independent risk factor for obesity in men and carotid atherosclerosis in woman of Mexican population.”

These part of the discussion text have been modified as your suggestion.

(24) Discussion: “…shows that men who reported permanent stress in relation to condition at work or at home for period of over 5 years in the past, had a increased risk for abdominal obesity…” – please omit “abdominal” as the studies shows no increased risk for elevated intraabdominal fat in chronically stressed individuals. Or do the authors mean central obesity as assessed by waist circumference?

The word “abdominal” has been omitted, the sentences now reads: “men who reported permanent stress in relation to condition at work or at home for period of over 5 years in the past, had an increased risk for obesity”
(25) Discussion: “These associations were independent of lifestyle/social confounders, as age, current smoking, alcohol intake, physical activity and marital status, and biologic mediators included BMI, lipids, blood pressure and glucose.” – If the associations between stress and obesity/subclinical atherosclerosis are independent of all these factors, do the authors have hypotheses regarding other possible pathophysiological pathways?

As we mentioned before, we suggest that maybe the genetics factors in Mexican population could play an important role in the pathophysiological mechanism in atherosclerosis disease.

(26) Discussion: “Studies concerning associations between general stress, obesity and metabolic syndrome, are limited, but the results from these studies are in accordance with those of the present study.” – This is simply not true (see above).

We have corrected these sentences from the discussion.

(27) Discussion: “Chronic hypersecretion of stress mediators...”. Please be more precise here.

We have attached one reference that explains some of the chronic hypersecretion of stress mediators on the discussion.

(28) Discussion: “…carotid artery ultrasound only was measured in 914 subjects for a reason unrelated to this study.” – What was the reason?

We have corrected the sentence on page 12. Now it says: “Third, we had missing data of stress item in the first 254 subjects, and we were able to performed the carotid artery ultrasound only in 914 subjects.”
Reply to comments by Reviewer 3

Reviewer's report:
DISCRETIONARY REVISIONS:
1. To add sample information (Mean age, DS) in "abstract" and "subjects" sections.

The information is now given in abstract and subjects sections.

2. To add prevalence data of obesity in "Introduction" section.

MINOR ESSENTIAL REVISIONS:
3. I suggest to indicate as a limitation the lack of a standardized questionnaire to assess self-subjective stress.

In the present study, we used a single-item questionnaire in which stress was described in psychological terms, such as feeling tense, irritable, anxious, or as having sleeping difficulties. It may be argued that this measure is too crude. However, one advantage of this measurement is that it has been shown to predict overall stroke as well as myocardial infarction in prospective studies [2,11]. It has also been shown to correlate with other constructs representing psychological stress including financial stress, stressful life events and locus of control [6]. Taken together, these results indicate a validity of the single-item question in investigations of cardiovascular risk. BMC Medicine 2009, 7:53

4. I suggest to review the following articles, in order to extend the "discussion" section:

We appreciated the articles you suggested for our manuscript and we took some information as part of the new manuscript.