Author's response to reviews

Title: Biological and other health related correlates of the long-term life dissatisfaction burden

Authors:

    Teemu Rissanen (teemu.rissanen@fimnet.fi)
    Soili M Lehto (soili.lehto@kuh.fi)
    Jukka Hintikka (jukka.hintikka@phsotey.fi)
    Kirsi Honkalampi (kirsi.honkalampi@uef.fi)
    Tarja Saharinen (tarja.saharinen@kuh.fi)
    Heimo Viinamäki (heimo.viinamaki@kuh.fi)
    Heli Koivumaa-Honkanen (heli.koivumaa-honkanen@kuh.fi)

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COVER LETTER / Responses to the reviewer

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Research article

**Long-term life dissatisfaction burden is associated with inflammatory alterations**
Teemu Rissanen, Soili M Lehto, Jukka Hintikka, Kirsi Honkalampi, Tarja Saharinen, Heimo Viinamäki and Heli Koivumaa-Honkanen

BMC Psychiatry (Section: Mood disorders)

We thank the reviewers for their constructive comments concerning our manuscript on long-term life satisfaction. We have addressed these comments *point by point* and made *alterations* according to them with *coloured text* in our revised manuscript. We hope that these alterations have improved the manuscript and that it is now suitable for publication in BMC Psychiatry.

Sincerely,

Dr. Teemu Rissanen, corresponding author
OUR RESPONSES

1. TO THE EDITOR

Editor’s comment: Please also address how the manuscript differs from previous manuscripts of your group on this topic and whether the current data set contains identical or similar data:


   BMC Psychiatry. 2011 Aug 23;11:140

RESPONSE: Only the original population-based study population is the same. However, the definitive differences lie in the topic, study sample, measures being used and data collection criteria after the fourth phase.

In the study of Lehto SM et al. (2010), the sample comprised 70 participants who were diagnosed with MDD, and a healthy control group (n = 70) with an age and gender distribution similar to that of the MDD group. It was conducted in a case-control setting with different inclusion criteria and it only investigated the association between MDD and adiponectin or resistin.

In the study of Rissanen T et al. (2011), only the association of the life dissatisfaction burden and mental health, including MDD, was investigated without paying attention to biological factors. Furthermore, in this study the long-term life dissatisfaction burden was based only on the sum of LS scores in the first three follow-up assessments (1998, 1999 and 2001) without that of 2005, which also resulted in different tertiles of
the life dissatisfaction burden. The highest tertile group (n = 116) and the lowest tertile (n = 107) were compared.

The present study is a step further from these two separate previous studies. It incorporates their findings with new uninvestigated material into the aims of the present study. It deals with various health-related factors, including biomarkers, in relation to long-term life dissatisfaction. Half of the selected subjects belonged to the symptomatic group (n = 209; 48.9%) and half to the asymptomatic group (n = 218; 51.1%) based on the previous reporting of adverse mental symptoms. The final sample included 305 cases. The life dissatisfaction burden score was based on assessments during a 7-year follow-up (1998, 1999, 2001 and 2005).

2. RESPONSES TO THE REVIEWER, Ines Kaufmann

Overview of the reviewer: Life dissatisfaction is characterized by a heterogeneous clinical picture with a decreased mental health status and changed inflammatory conditions. The authors RISSANEN et al. are reporting on long-term dissatisfaction in association with decreased adiponectin levels, poor sleep and poor social support. The study conducted by RISSANEN et al. is interesting because they showed for the first time that adiponectin is not only a marker of diabetes mellitus and coronary artery disease, but also of importance in inflammation.

Major compulsory revisions:

COMMENT 1. The title is misleading and does not demonstrate a thread for the topic. The title should have an adequate accuracy. Decreased adiponectin levels could be suggestive for inflammatory alterations; however, the causality has not been shown in this study. Therefore, the title must be adapted.
RESPONSE 1: We have changed the title in order to indicate that the study deals with correlates of the life dissatisfaction burden without causal considerations and that it also includes other than inflammatory factors.

COMMENT 2. Reading the background, the reviewer is missing a hypothesis for the investigation made by RISSANEN et al. Why did the authors consider adiponectin and resistin as biological factors associated with long-term dissatisfaction? Low adiponectin plasma levels has been reported in major depression (J Psychiatr Res. 2012 Aug;46(8):1081-5) as well as panic disorders (J Affect Disord. 2012 Aug;139(3):302-5). These facts could contribute to a well-founded hypothesis.

RESPONSE 2: We thank the reviewer for this comment and the references. The aims of the study needed some clarification. However, this information on the foundation of the hypothesis is a further benefit for the study and has been included in the text (see page 3, chapter 3, lines 7–9; page 3, chapter 4).

COMMENT 3. An anxious or depressed person often lives alone and suffers from poor quality of sleep or low social support. Did the authors assess GADI (Generalized Anxiety Disorder Inventory) anxiety measure, the BDI-II Cognitive–Affective factor measure or the 29-item Hamilton Depression Rating Scale?

RESPONSE 3: The aim of this study was to address the lack of knowledge of health-related factors, including biomarkers, underlying life satisfaction. Recently, we conducted a study concerning life dissatisfaction, major depressive disorder and various indicators of poor mental health, including several psychometric scales: life satisfaction (LS), depression (HDRS, BDI), hopelessness (HS), mental distress (GHQ), dissociative experiences (DES), and alexithymia (TAS) (article 1 in the list below). According to the results of that study, previous long-term life dissatisfaction associated significantly with poor mental health, which was the case with all of the previous psychometric scales (HDRS, BDI, TAS, HS, GHQ, DES, LS) of the study (1). Thus, in the present study the psychometric scales, e.g. HDRS, were not reused with respect to
LS. Furthermore, life satisfaction is not an illness-specific condition. Life dissatisfaction is high in psychiatric patients regardless of their diagnosis based on previous reports, the first being Koivumaa-Honkanen et al. (1996) (2). On the other hand, self-reported life satisfaction correlates with the health status as well as various long-term health hazards (1, 3-7), but its role and underlying mechanisms with respect to somatic health and biological factors require further research, in which the present study is the first step forward.


**COMMENT 4.** Hypoadiponectinemia has been demonstrated to be independently associated with metabolic syndrome, including type 2 diabetes, hypertension,
atherosclerosis, and non-alcoholic steatohepatitis. The authors should state every of these conditions in their patient populations.

**RESPONSE 4:** This is a population-based study sample, which limits the possibility to gather detailed information on the different disease groups. We do not have data on diagnoses of non-alcoholic steatohepatitis or atherosclerosis in general. However, we have self-reported data and/or clinical measurements for metabolic disorder, hypertension, cancer (which has now been included in the study design) and coronary heart diseases. These conditions are likely to at least partially cover biological alterations produced by the conditions the reviewer requested due to their overlapping pathogenesis. Furthermore, rheumatoid arthritis with its inflammatory sequels has also been taken into account in the present study.

**COMMENT 5.** Adiponectin levels are also changed in several cancer patient groups, e.g. it is lowered in endometrial, postmenopausal breast, gastric, prostate, and colon cancer. Did the authors exclude patients with malignant disorders?

**RESPONSE 5:** Thank you for the two comments above. In the study sample (n = 305) there were six (n = 6) individuals with a cancer diagnosis. According to the additional analyses, no significant association was found between the cancer diagnosis (yes vs. no) and the inflammatory markers, i.e. the level (mean; 95% confidence intervals) of adiponectin [µg/ml] (15.2; 8.8–39.2 vs. 15.9; 14.2–17.7; Z = -1.03; p = ns), resistin [ng/ml] (14.9; 0.8–29.4 vs. 10.8; 9.9–11.8; Z = -0.36; p = ns), hs-CRP [mg/l] (2.5; 0.3–5.4 vs. 3.0; 2.5–3.5; Z = -0.36; p = ns), IL-6 [pg/ml] (2.5; 1.8–6.9 vs. 4.0; 2.6–5.3; Z = -0.39; p = ns) and TNF-α [pg/ml] (2.5; 0.5–5.5 vs. 11.5; 6.2–16.7; Z = -0.21; p = ns).

Please see manuscript page 4, chapter 1, lines 2–3; page 6, chapter 1, line 4; page 9, chapter 1.

**COMMENT 6:** Adiponectin is increased in hemodialysis patients. Did the authors assess this condition in their patient groups?
RESPONSE 6: Please see responses 4-5. We have no data on hemodialysis, but the presence and prevalence of the hemodialysis patients would presumably be very marginal in this randomly selected population-based sample. Thus, the influence of hemodialysis on the results of present study would presumably be at most marginal. These limits (responses 4-6) have been discussed in the discussion section.

COMMENT 7. Resistin is a member of the cysteine rich family of resistin-like molecules that are associated with the activation of inflammatory conditions. However, more research is needed to clarify the exact role of resistin in inflammatory human diseases. Therefore, it is very speculative to determine this parameter in a psychiatric condition in which the underlying inflammatory process is not clearly defined. Furthermore, serum resistin concentrations are elevated in patients with non-alcoholic fatty liver disease, and increased resistin levels correlate with histological severity of liver disease. Did the authors consider this and other conditions changing resistin?

RESPONSE 7: Please see the responses 4-6 above. As inflammatory markers should be studied with respect to psychiatric conditions, this is also true with subjective well-being. We agree that these issues deserve vigorous and profound research in order to determine the mechanisms in these biological processes. However, in the present study we started with cross-sectional analyses with a population-based data set and analyzed biological factors together with other health-related factors with respect to long-term life dissatisfaction. Since life dissatisfaction has been shown to also affect somatic mortality and disability, these types of investigation are needed. Due to the lack of previous studies, we hope that this preliminary step will result in increasing interest in this area.

COMMENT 8: The thread in the section ‘Discussion’ is not identifiable. The authors should discuss their data not only in the light of long-term dissatisfaction, this would be a one-sided view. The clinical relevance of the conclusions drawn is not clear. The
conclusions of the authors are not strong enough to emphasize the clinical relevance of the findings.

**RESPONSE 8:** We have now clarified the manuscript with respect to its rationale and its different sections, including the discussion. We hope this has improved it and made it more logical from the very start to the discussion.

**Minor compulsory revisions:**

**COMMENT 9.** Did the authors explore different oligomeric forms of adiponectin? If no, what was the reason?

**RESPONSE 9:** Unfortunately, oligomeric forms of adiponectin were not available in the analytical kit that was used.

**COMMENT 10.** The authors measured TNF-#, one of the insulin resistance inducible factors, in their patient groups. TNF-# is not very stable, it has a short half-life time in blood after withdrawal. Therefore it is import to describe the blood sample processing to exclude a prolongation or mistakes in sample processing. Were the samples measured as a batch at the end of the study? If yes, please describe the storage conditions. How long was the sample processing delay after blood withdrawal?

**RESPONSE 10:** According to this suggestion, we added some information related to sample handling and analytical procedures to the text, but tried to keep the manuscript as concise as possible. However, all venous blood samples followed the same protocol and were frozen as soon as possible after the blood draw, and stored at -80 °C until run. The freezer temperature of -80 °C is commonly considered suitable for storing
samples for the measurement of substances that have short half lives and easily disintegrate. The samples were measured as a batch at the end of the study.

Adiponectin and resistin were analyzed with a human serum adipokine (Panel A) LincoPlex kit (Millipore, MA, USA) using a Bio-Plex Suspension Array System (Bio-Rad Laboratories Pty Ltd; Hercules, CA, USA). The assay conditions were controlled, standardized, and pre-optimized to ensure optimal repeatability and reproducibility of the assays, and the kit instructions and instrument manuals were carefully followed. The assayed kits were from the same lot, which allows better control of inter-assay variability. The correlation between multiplex analyses and traditional enzyme-linked immunosorbant assays (ELISA), the golden standard of current peptide immunoassays, have been shown to be high in several papers (8-10).

Specifically, adiponectin and resistin analyzed using xMAP technology and Lincoplex kits have been shown to correlate highly with the ELISA method (11). Before the analyses, samples were centrifuged for 15 min at 2165 g. For the analyses, the samples were diluted in the appropriate sample matrix to 1:400 according to the kit instructions. The beads were incubated overnight with the samples. A minimum of 50 events (beads) were collected for each analyte protein, and the concentrations calculated from the standard curves based on the median fluorescence intensities. BioPlex Manager Software 4.1 was utilized in calculating the concentrations. The intra-assay and interassay variations for the adipokine analyses were 1.4–7.9% and 21%, respectively.


3. RESPONSES TO THE REVIEWER, Nicolas Rohleder

**OVERVIEW:** The aim of the study presented here was to test whether “life dissatisfaction”, accumulated over 7 years in a longitudinal study, predicts current levels of a small selection of biomarkers. Results show lower serum adiponectin, together with lower social support and lower sleep quality in high life dissatisfaction. Testing prospective relationships of life dissatisfaction with biomarkers of inflammation seems to be an important approach, with potentially impactful findings. However, the present study appears to have too many problems to really make an impact. See below.

**COMMENT 1:** One of the larger shortcomings is that the authors are not making good use of their data. Although they have a sufficient number of participants (>300) and number of observations (4), the authors apparently try to simplify their data as much as possible, by (a) just summing up life dissatisfaction scores over the 4 assessment times, and (b) using those summed up scores for a median split. This is clearly insufficient. I would have expected at least to (a) test whether potential patterns in individual development of life dissatisfaction over time (i.e. decrease, increase, general low, general high, low vs. high variability, etc) are related differentially with biomarkers. This will require more sophisticated statistics.

This analytical weakness might also be (or might not be…) the reason for not finding relationships of life dissatisfaction with inflammatory biomarkers.
measured. The problem here is that from what the authors present here, it is impossible to draw any conclusions. Not finding a relationship might indicate that maybe life dissatisfaction is not a strong enough stressor to stimulate pathophysiological processes that could be picked up by increased inflammatory biomarkers, but it is entirely possible that we are just not finding these results because the authors did not analyze their data right. The summary of this point is thus simply a re-iteration of the point above: The authors are strongly encouraged to take another look at their data, and apply more sophisticated analysis techniques. Such techniques can and should also include a control for potential confounders / covariates.

**RESPONSE 1:** We thank the reviewer for this important comment. We have now revised the manuscript with respect to both the reviewer overview and this comment. To the best of our knowledge, this is the first study to search for biological correlates of long-term life satisfaction. However, we also investigated other health-related factors than biomarkers. According to previous studies, current sociodemographic factors, marital status, social status, and economic status, etc., have been more strongly associated with life satisfaction than health-related factors. However, inflammatory factors were not included in these previous analyses, as they were in the present study. Moreover, life satisfaction has been extremely stable among the general population (12). According to all this, and taking into account our sample size (n = 305), we felt that the median split provided a larger group size to seek significant health-related correlates of long-term dissatisfaction.

Now, according to reviewer’s suggestion, extra analyses have been performed in order to also include individual change in the life dissatisfaction level during the follow-up period. For this, the previously used cut-off point of 11/12 for the life satisfaction score was applied (1, 3, 4, 12-14) to categorize the subjects into the satisfied (LS: 4–11) and the dissatisfied (LS: 12–20) at baseline in 1998 and at the end of the study in
2005. After this, three different subgroups were formed according to the change in individual life satisfaction:

Group A “Stable Satisfied”: LS score 1998 ≤ 11 and LS score 2005 ≤ 11; \( N_A = 197 \)

Group B “Increasing Dissatisfaction”: LS score 1998 ≤ 11 and LS score 2005 ≥ 12; \( N_B = 14 \)

Group C “Stable Dissatisfied”: LS score 1998 ≥ 12 and LS score 2005 ≥ 12; \( N_C = 51 \)

Comparisons were made between group A vs. group B and group A vs. group C. The stability of life satisfaction was observed: 197/305 remained constantly satisfied and 51/305 constantly dissatisfied in the follow-up. Only 14 satisfied cases /305 became dissatisfied and 43 dissatisfied cases /305 became satisfied by the end of the follow-up. The result from the comparison between group A vs. group C was similar to the original comparison of the long-term dissatisfied with the long-term satisfied (Table 1). Likewise, comparing all the same biomarkers between group A vs. group B \( (n = 14) \), no significant differences were detected, but the group sizes were small.


### Table 1. Sociodemographic, health behavior, and biological factors in 2005.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stable_Satisfied</th>
<th>Stable_Dissatisfied</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Col% (n)</td>
<td>Col% (n)</td>
<td></td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>46.7 (92)</td>
<td>35.3 (18)</td>
<td>ns</td>
</tr>
<tr>
<td>Living alone, n (%)</td>
<td>11.2 (22)</td>
<td>29.4 (15)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Smoking (yes), n (%)</td>
<td>9.1 (18)</td>
<td>33.3 (17)</td>
<td>&lt;<strong>0.001</strong></td>
</tr>
<tr>
<td>Alcohol consumption ≥2/week, n (%)</td>
<td>16.2 (32)</td>
<td>13.7 (7)</td>
<td>ns</td>
</tr>
<tr>
<td>Social support (poor), n (%)</td>
<td>4.1 (8)</td>
<td>45.1 (23)</td>
<td>&lt;<strong>0.001</strong></td>
</tr>
<tr>
<td>Sleep (poor), n (%)</td>
<td>46.7 (92)</td>
<td>85.7 (12)</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Metabolic syndrome (yes) B, n (%)</td>
<td>33.3 (64)</td>
<td>44.0 (22)</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension (yes), n (%)</td>
<td>29.4 (58)</td>
<td>35.3 (18)</td>
<td>ns</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>8.6 (17)</td>
<td>2.0 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>Rheumatoid arthritis, n (%)</td>
<td>5.6 (11)</td>
<td>11.8 (6)</td>
<td>ns</td>
</tr>
<tr>
<td>Cancer (yes), n (%)</td>
<td>2.0 (4)</td>
<td>2.0 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>Use of oral corticosteroids, n (%)</td>
<td>2.0 (4)</td>
<td>5.9 (3)</td>
<td>ns</td>
</tr>
<tr>
<td>Use of NSAIDs, n (%)</td>
<td>3.6 (7)</td>
<td>5.9 (3)</td>
<td>ns</td>
</tr>
<tr>
<td>Use of statins, n (%)</td>
<td>29.9 (59)</td>
<td>19.6 (10)</td>
<td>ns</td>
</tr>
<tr>
<td>Variables</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>57.4 (55.9–58.8)</td>
<td>53.6 (51.5–55.8)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>BMI, kg/m</td>
<td>27.2 (26.5–27.9)</td>
<td>28.5 (26.5–30.6)</td>
<td>ns*</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>17.25 (14.90–19.60)</td>
<td>13.81 (10.63–17.00)</td>
<td>ns*</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>10.83 (9.80–11.86)</td>
<td>12.51 (9.26–15.78)</td>
<td>ns*</td>
</tr>
<tr>
<td>hs-CRP, mg/l</td>
<td>2.71 (2.11–3.33)</td>
<td>4.06 (2.74–5.38)</td>
<td><strong>0.026</strong></td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>12.49 (5.27–19.71)</td>
<td>16.69 (2.76–30.61)</td>
<td>ns*</td>
</tr>
<tr>
<td>IL6, pg/ml</td>
<td>4.22 (2.40–6.04)</td>
<td>5.92 (2.26–9.57)</td>
<td>ns*</td>
</tr>
</tbody>
</table>

*Fisher’s exact test; *Mann-Whitney U-test

Abbreviations: NSAID = non-steroidal anti-inflammatory drug, BMI = body mass index, hs-CRP = high-sensitivity C-reactive protein, TNF-α = Tumor Necrosis Factor α, IL6 = interleukin 6.