Author's response to reviews

Title: Leptin and smoking cessation: secondary analyses of a randomized controlled trial assessing physical activity as an aid for smoking cessation

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Author's response to reviews: see over
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Dear Mr. Proel Vargas,

We are submitting a revised version of the manuscript entitled “Leptin and smoking cessation: secondary analyses of a randomized controlled trial assessing physical activity as an aid for smoking cessation”. Kindly find below our answers to the reviewer’s comments and questions.

Reviewer’s comments and questions:

Major revisions
- there are no data on insulin sensitivity and insulin secretion. It seems difficult to discuss the change in leptin levels outside the context of insulin sensitivity, particularly because physical activity as well as smoking cessation impact on insulin sensitivity and secretion (Eliasson B et al Eur J Clin Invest 1997; Stadler M. et al. Eur J Endocrinol. 2014). The effects of smoking cessation on insulin sensitivity and secretion need to be addressed in the discussion.

This is an important comment. We have measured fasting insulin and fasting glucose levels in the study, and we added those variables to our analyses, as suggested by the reviewer (see Methods section, page 8: “To estimate insulin secretion and insulin sensitivity among participants, the Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin [mU/l] * fasting glucose [mmol/l]/22.5”, and results section, page 10: ” Fasting insulin and glucose levels did not impact this association (no significant p values, expected for insulin at 6 months, where p = 0.011)”; the actual values of HOMA-IR at baseline are given in the Table 1; the change of HOMA-IR between the baseline and the subsequent visits are given in the Tables 2 (by study group) and 3 (by smoking status); finally, the detailed results of a multivariate model including fasting insulin and glucose levels (i.e., the components of the HOMA-IR) are given in the Table 4). We described the importance of analyzing any changes in leptin together with any change in insulin in the present study, and added the suggested references in the last paragraph of the Introduction section, page 5: “We analyzed also if any variation in insulin secretion and sensitivity...
impacted the leptin levels—as they are both influenced by smoking cessation and physical activity”.

- Have the authors measured fasting insulin levels? If so, I would suggest calculating a fasting measurement of insulin sensitivity (e.g. HOMA-IR, QUICKI) and report these data.

Yes, we did. We calculated the HOMA-IR, as suggested, and put the baseline results in the Table 1. We also calculated any changes in this index over the study period and reported the results in the Tables 2 and 3. In the Table 4, we investigated any potential confounding effect of insulin and glucose levels to the association between change in leptin and the study intervention. For that purpose, we performed a multivariate linear regression and reported the results. We did not find any major confounding effect (see Results section, page 10: “In the multivariate linear regression, the change of the ratio leptin/body fat by study group was strongly significant, being lower in the intervention group (Table 4). Fasting insulin and glucose levels did not impact this association (no significant p values, expected for insulin at 6 months, where p = 0.011)” , and in the Discussion section, page 12:” Moreover, the observed increase of leptin was independent of any major influence of insulin secretion or sensitivity.” We thank the reviewer for this suggestion of analysis that strengthens our findings.

- The lack of insulin sensitivity and secretion data needs to be mentioned in the limitations section.

It does no longer seem to be needed.

- I would suggest simplification of the statistical methods and to clearly present what has been measured without trying to “inflate” the data by applying complex piecewise polynomial longitudinal models: Fasting leptin was measured at 4 time-points, there are no dynamic measurements (e.g. oral glucose tolerance test, post-meal leptin...), it was one adipokine. It would be sufficient to present clearly present the results of the measurements as 4 time points with mean and standard deviation to start with.

We simplified the Tables and Figures as suggested by the reviewer. Tables 2 and 3 show the differences (means and SD) of the main metabolic variables between the baseline and the 3 subsequent visits (at 3, 6 and 12 months). Table 2 shows it by study group and Table 3 by smoking status.

Please put in the models and the Figures 1-2 as supplemental material rather than in the main manuscript body.

We did it and we simplified the corresponding Methods and Results sections (see the Methods section, page 8: “We used a polynomial longitudinal model in order to compare the dynamics across the time of the ratio leptin/body fat in the two groups of randomization (the methods are described in the supplementary material).”); And in the Result section, pages 10 and 11: “Applying a longitudinal model, we found that the mean ratio leptin/body fat increased in the first 35 weeks, and decreased thereafter, following a quadratic curve (p < 0.001 for both the linear and quadratic term, Supplementary Figure 1). We also found a significant interaction between the study group and time (p = 0.04), attesting a different pattern of ratio leptin/body fat over the
follow up for the control and the intervention group. Namely, the intervention group showed an attenuated dynamics of the ratio leptin/body fat across the time, with a maximum gain of 0.04 ml/l instead of 0.07 ml/l at 35 weeks, and a final gain of 0.02 ml/l instead of 0.04 ml/l at the median follow-up duration of 55 weeks. Finally, with a piecewise longitudinal model applied to the 234 participants who stopped smoking at least once, we could give a separate estimate of the ratio leptin/body fat change during abstinence and relapse episodes during follow-up (Supplementary Figure 2). Physical activity still had an influence on leptin dynamics during abstinence periods, with a more important increase for the control than the intervention group (p = 0.03). The model also showed a significant effect of sex on ratio leptin/body fat initial levels and dynamics across time: women had a baseline ratio leptin/body fat level significantly larger than men (0.48 ml/l vs. 0.20 ml/l, p<0.001), and a more important dynamics over the follow-up. Relapse to smoking modified the quadratic pattern of the adjusted leptin, leading to an abrupt linear decrease of the mean adjusted leptin when the relapse episode started (p<0.001).”

- please describe why the number of participants differs from the main publication (271 vs 481).

This was explained in the Methods section (p. 8, 1st paragraph). For cost reasons, leptin was measured in the first 271 participants only.

- Please describe how you dealt with missing data

It has been added in the Methods section (p. 9, 1st paragraph). They were excluded from the analyses and the actual total numbers of participants are given in the Tables for the multivariate regression.

Minor revision
- Table 2: the percentage body fat seems low, are you actually reporting the change percentage body weight?

This Table 2 has changed. In the actual Tables 2 and 3, we indeed report the changes in body fat (i.e., the difference between the baseline and the subsequent visits).

In addition, an authors’ contribution section and the Clinical Trial Registration Number were added to the manuscript.

Yours sincerely,

Dr. Semira Gonseth,
On the behalf of all authors

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