Author’s response to reviews

Title: The Effect of Lifestyle Intervention in Obesity on the Soluble Form of Activated Leukocyte Cell Adhesion Molecule.

Authors:

Alba Sulaj (Alba.Sulaj@med.uni-heidelberg.de)
Johanna Zemva (Johanna.Zemva@med.uni-heidelberg.de)
Ulrike Zech (Ulrike.Zech@med.uni-heidelberg.de)
Annika Woehning (Annika.Woehning@med.uni-heidelberg.de)
Maik Brune (Maik.Brune@med.uni-heidelberg.de)
Gottfried Rudofsky (gottfried.rudofsky@spital.so.ch)
Peter Nawroth (Peter.Nawroth@med.uni-heidelberg.de)
Thomas Fleming (Thomas.Fleming@med.uni-heidelberg.de)
Rüdiger von Bauer (ruediger.vonbauer@kkl.srh.de)

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Author’s response to reviews:

Response to Reviewer 1:

1. sALCAM represents the proteolytic cleavage of the full receptor from the sheddase ADAM17. However the physiological role of sALCAM needs still to be understood. It could be speculated that sALCAM might act as a decoy receptor by preventing ALCAM-ligand interaction as previously shown in our group for sRAGE-RAGE ligands interaction (Discussion section, page 10, line 192-198).

2. sALCAM levels in healthy controls have been reported in previous studies (Discussion section, page 10, line 186-187). We have measured sALCAM serum concentrations in healthy subjects and the values are significantly higher than those measured in obese subjects in this study (data under review) (Discussion section, page 10, 190-192).
3. In vitro experiments in previous work of our group showed significantly higher levels of ligand required to activate the full receptor (400nM) than the concentration of sALCAM measured in this study (as example: 224ng/ml sALCAM converts to 10nM, molecular mass of sALCAM approximately 22kDa, Ikeda et. al. 2004). Therefore the main functional role of sALCAM might be primarily in local tissue rather than serum (Discussion section, page 10, line 200-205).

4. A limitation of this study is the small sample of subjects recruited. We acknowledge that the model we propose explains only the variables that were considered and there might be other factors influencing the levels of sALCAM. However the likelihood ratio-test of the model was significant (LR=590, df=15), showing that the considered variables explain significantly part of the changes in sALCAM level. Unexplained changes due to non-included factors need to be further investigated (Method section, page 6, line 131-132; Result section, page 9, line 169; Discussion section, page 11, line 226-230).

5. sALCAM level was expressed in linear scale because the scales in between variables were comparable (sALCAM [97-367] vs. age [20-60] and HOMA [2-30]). Following the reviewer’s suggestion we performed the analysis after log-transformation and the changes in correlation coefficients were minimal (example: sALCAM corr. with age R=-0.5170, P=0.0034 on logscale and R=-0.5110, P=0.0046 on linear scale). We decided for presentational purposes to show the results in linear scale.

6. Collinearity analysis was performed for each variable and timepoint and no significant multicollinearity was found (VIF<3) (Methods section, page 7, line 132; Result section, page 9, line 170).

Response to Reviewer 2:

1. When age in between the groups with different ΔHOMA-IR was compared it was found that the subjects with the higher HOMA-IR improvement and higher sALCAM levels at baseline were younger, as it is expected since sALCAM level correlated negatively with age (37±2,3 vs. 50±3, P=0,005). It is previously shown that rather than chronological age itself, it is the age-related increase in adiposity that explains the reduction in insulin sensitivity. Therefore a pure chronological age effect is to be excluded (Results section, page 9, line 178-181; Discussion section, page 11, line 222-225).

2. Please refer to Point 2 in Response to Reviewer 1.

3. Please refer to Point 1 and 3 in Response to Reviewer 1.

4. Text for Figure 2 corrected (Results section, page 9, line 164).
Comments to Editor:

1. The affiliations of two of the coauthors have been updated (Dr. Thomas Fleming and Prof. Dr. Peter Nawroth) (Cover Page, line 7-9)

2. Regarding limitations of the study please refer to Discussion section, page 11, line 222-230.

3. Regarding future directions of the research please refer to Discussion section, page 11-12, line 231-234.

4. All changes in the manuscript are highlighted in yellow.