Author’s response to reviews

Title: The minor C-allele of rs2014355 in ACADS is associated with reduced insulin release after an oral glucose load

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Author’s response to reviews:

Dear Editor and Reviewers,

Thank you for your valuable comments to our manuscript. Please find our amendments in response to your comments summarized below.

Editor:

In the present paper authors conclude that a common variant in ACADS gene is associated with reduced insulin secretion which partially could be mediated through an impaired b-oxidation of fatty acids. Though the hypothesis is well motivated it is not well elaborated.

Mainly authors should provide data whether this SNP influences lipid profile, under normal or hyperglycemic conditions as they speculate.

A table showing the effect of the SNP on the lipid profile under normal and hyperglycemic conditions has been included (table 2, page 18).

Secondly, they should look at insulin secretion during lipid infusion, data that authors have access to.

Unfortunately, we have never conducted such relevant experiments.

Thirdly, whether the effect on glucose-stimulated insulin secretion would change if adjusted for lipid levels.
The effect on glucose-stimulated insulin secretion is unchanged when adjusted for either cholesterol or triglyceride levels. This has been included in the manuscript (results section page 9, line 205-207).

Reviewer 1:

In this manuscript, Hornbak et. al examine the hypothesis that single nucleotide polymorphism (SNPs) at two genes encoding short (rs 2014355 at ACADS) and medium chain acylcoenzyme A dehydrogenase (rs 11161510 at ACADM) are associated with impaired beta cell function. The authors genotype subjects from cohorts whom they had available measures of insulin secretion and insulin sensitivity. The authors compare insulin secretion and insulin resistance measure among the three genotypes for each SNP. Their main finding is that subjects with homozygous for the minor allele (cc) at rs 2014355 have impaired insulin secretion compared to the other two genotypes. This study has two major limitations: (1) the methods utilized to quantitate insulin secretion and insulin sensitivity, and (ii) the interpretation of the results. Neither both beta cell function nor insulin sensitivity are directly measured with clamp study even at a subgroup of the subjects.

We agree that clamp data would have been more favorable in this study, but unfortunately we have no clamp data for this study population.

Instead, the assessment is made indirectly with surrogate measures derived from plasma glucose and insulin concentrations during the OGTT. The authors primarily rely on indices that were validated against the IVGTT and do not utilize indices that were validated against the gold standard techniques, like the matsuda and stumvoll indices for insulin sensitivity.

This is a very important point. We have therefore included the Matsuda index in our table 1 on page 16.

Further, the authors miscalculate the insulinogenic index. They divide the increment in plasma insulin concentration during the first 30 minutes of the OGTT by plasma glucose concentration at 30 minutes instead of the increment in plasma glucose concentration during the first 30 minutes (page 7, first paragraph).

We are aware that several different ways of calculating the Insulinogenic Index exist. We have now changed the Insulinogenic Index according to the comment above (page 7, line 161).

In addition, the authors have the information about insulin secretion during the entire OGTT (0-120 minutes, #I/#G0-120, but they chose not to present it.

We have included the area under the curve (AUC) for insulin/ AUC for glucose, which reflect the insulin secretion during the entire OGTT, in our table 1 on page 16.

Lastly, and most importantly, the authors missinterpretate their data. The beta cell responds to an increment in glucose by an increment in insulin, and this beta cell response should be related to the prevailing level of insulin resistance,
because the magnitude of beta cell response to glucose stimulus is larger in insulin resistant individuals compared to insulin sensitive subjects. Thus, to compare beta cell function amongst two groups of individuals, the disposition index should be used, not the absolute plasma insulin concentration nor an index of insulin secretion. Insulin sensitivity indices (HOMA-IR, and BIGTT-Si) indicate (Table 1) that subjects with CC genotype tend to be more insulin sensitive compared to other genotypes. The authors ignore this finding, perhaps because it did not reach statistical significance. But it is important for the interpretation of insulin secretion indices. Because subjects with the CC genotype tend to be more insulin sensitive, one would anticipate that they have smaller insulin secretion indices, and if one calculates the disposition index for each genotype (BIGTT-si X BIGTT-AIR, or insulinogenic index ÷ HOMA-IR) it is likely that it will be similar among the three genotypes. Thus, the smaller insulin secretion indices in CC subjects, that the authors interpretate as impaired beta cell function, is actually an adaptive response to the improved insulin sensitivity in this group.

Thank you for elaborating on an import part of our data presentation. When we calculated the Matsuda index, using the formula: (10,000 / # (fasting plasma glucose x fasting serum insulin) x (mean plasma glucose x mean serum insulin during OGTT)), we observed that subjects with the CC-allele do tend to be more insulin sensitive compared to other genotypes, exactly as HOMA-IR, and BIGTT-Si indicate. Also, the Disposition Index is similar among the three genotypes as you suggest. The area under the curve (AUC) for insulin/ AUC for glucose, which reflects the insulin secretion during the entire OGTT, is also pointing at a decreased insulin secretion among subjects with the CC-allele.

We have therefore changed the manuscript to take into account the newly included data, emphasizing that CC-allele carriers show improved insulin sensitivity, both in methods (page 7, first section), results (page 8, line 200-204), and discussion (page 10, first and second section).

Reviewer 2:
The authors have investigated whether two variants, suggested to impair fatty acid # oxidation in a recent genome wide association study using metabolite concentrations as proxies for enzymatic activity, associates with altered insulin release following an oral glucose load or with type 2 diabetes. They have been using a population-based cohort of 6,162 middle-aged individuals with available data from an oral glucose tolerance test (OGTT) as well as a case-control cohort including a total of 10,196 individuals. They report that one of the tested variants associate with reduced glucose-stimulated insulin release during an OGTT and suggest that this may in part be mediated through an impaired # oxidation of fatty acids, while the other tested variant showed no association to reduced insulin secretion or to type 2 diabetes. The manuscript is well written, the study is well powered and the conclusions are sane in relation to results.

In the aim you state that you will investigate indices of insulin release in relation to genotype in 6,162 individuals. According to your table you have analyzed 4,324 and 4,337 glucose-tolerant individuals, respectively, and in Methods, Study population, last paragraph you have stated that the Inter99 cohort has 4,567
glucose-tolerant individuals. You also state that you have a genotyping success rate of >97%, but only 94.7% and 95.0%, respectively, of the glucose-tolerant individuals have been analyzed. Which other phenotypes were you missing from these individuals? It should be stated in the abstract and aim how many of the individuals you have been analyzing for indices of insulin release in relation to genotype, tentatively: Abstract, Methods, first sentence: “… and investigated for associations with serum insulin levels following an oral glucose tolerance test (OGTT) in a population-based sample of 4,567 glucose-tolerant, middle-aged individuals”. And: Background, last paragraph (aim): “The aim of the present study was to investigate rs2014355 of ACADS and rs11161510 of ACADM in relation to indices of insulin release in a large population-based study of glucose-tolerant, middle-aged individuals (n=4,567) […].

Thank you for highlighting this very relevant point. The 4,567 individuals mentioned have at least one or both SNPs successfully genotyped. Furthermore, they have all phenotypes used for adjustments in the study, such as BMI, sex, and age. That 4,567 is a somewhat higher number, than what you see in the tables, is mainly because genotype data can be available for only rs2014355, only rs11161510, or both variants. The reason why we choose to disclose the total number of genotyped individuals is to provide an easy overview of data when several variants and several subsets of the study-population are used. We agree that this can be a confusing, and we have changed the numbers in the methods section (page 6, line 135-140) to reflect the actual number of individuals included in the analyses for each variant. As we had to include more individuals with different glucose-tolerance status from this cohort, we have changed the numbers in the abstract, introduction, and methods sections accordingly (page 3 and 5), and these numbers reflect the total number of individuals from the Inter99 included in rs2014355 and/or rs11161510 analyses without any missing phenotypes.

As above, the number of investigated individuals for type 2 diabetes association is not consistent between text and tables. According to the text you have analyzed 10,196 individuals for association between the genotypes and type 2 diabetes, but according to the tables the numbers are 8,313 and 8,344, respectively, which translates into 81.5% and 81.8%, respectively. This is not consistent with a genotyping success rate of >97%. Update the number of individuals with available phenotypes needed for each study, so that it is consistent with the tables.

As above, we agree that this can be a confusing, and we have changed the numbers in the methods section (page 6, line 139-140) to reflect the actual number of individuals included in the analyses for each variant.

Discretionary Revisions

Why was not study group 3 included in the analysis of indices of insulin release in relation to genotype, since these individuals also underwent an OGTT. Is this because of missing phenotypes such as insulin measurements?

This is a very relevant point. Insulin was not measured in this group, and they were therefore excluded due to missing phenotype. We have highlighted this in
the methods section (page 6, line 138).

I would suggest to combine the four tables into one for easier overview, and also to make a figure describing the results for rs2014355, that is, a figure over glucose and insulin measurements per genotype during the OGTT.

Thank you for a very helpful suggestion. We have combined the four tables into one, which gives a better overview of the data (table 1, page 16). However, we do not believe that a figure is the best way to present data after the inclusion of the new indices.

Minor issues not for publication Methods, Biochemical and anthropometric measures, first sentence: Misplaced parentheses: “Height (without shoes) and weight were measured in light indoor clothing, and BMI was calculated as weight (kg/height2 (m2))”. Change to: “[…] and BMI was calculated as weight (kg) / height2 (m2)”.

This has been changed (page 6, line 147).

Thank you for the opportunity to resubmit the paper.

Sincerely yours,
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