Author’s response to reviews

Title: Endocannabinoid receptor blockade reduces alanine aminotransferase in polycystic ovary syndrome independent of weight loss.

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Author’s response to reviews:
Point by point answers to reviewers

Reviewer 1: In this interesting paper, the authors have to investigate the impact of rimonabant (CB1 antagonist) on alanine aminotransferase. They have concluded that Rimonabant through CB1 receptor blockade decreased serum ALT that was independent of weight loss and hepatic inflammatory markers in obese women with PCOS without NAFLD.

Materials and Methods:
1. No control study is the limitation of this study.
Thank you for that comment. We agree that not having a weight matched control population for each of the rimonabant, orlistat, metformin and pioglitazone arms was a limitation and we have acknowledged that in the Discussion that reads “The inclusion of a weight matched normal population would have ideally also been included for each intervention. However, for the
reduction in ALT with rimonabant not to be significant, all three other treatments would need to have actively increased ALT.”

2. How do you exclude non-PCOS women in the PCOS group? Do you check the level of cortisol, TSH and etc. for a differential diagnosis?
Thank you for that comment. As detailed in the methods “….. the diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenaemia (Ferriman–Gallwey score > 8 and FAI > 4, respectively), oligomenorrhoea or amenorrhoea, and polycystic ovaries on transvaginal ultrasound. ………. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing’s disease and androgen-secreting tumours were excluded by appropriate tests.”

3. What is the range of BMI in PCOS women?
Range of BMI at baseline was 34.2 to 41.0 and matched in each of the 4 groups and this is addressed in the discussion that reads “It can be seen that the four groups were weight matched with a BMI range of 34.2 to 41.0.”

Results:
1. Do you perform OGTT for PCOS and control? If yes, please provide the data of OGTT in both groups.
Thank you for that comment. Clinically as all of these patients were obese (and as confirmed in the clinical practice guidelines Legro et al JCEM 2013;98:4565) received a OGTT as routine practice. From the clinical notes all patients had a fasting plasma glucose less and 7mmol/L and 2hr value after the OGTT less than 7.8 excluding both impaired glucose tolerance and type 2 diabetes. As this was routine clinical practice then this data was not collected as part of this study, nor repeated at the end of each intervention

2. Please provide the data of quantitative insulin sensitivity check index (QUICKI) (Katz et al, J Clin Endocrinol Metab 2000; 85:2402-10) for the evaluation of insulin resistance in PCOS.
Thank you for your comment and thoughtful suggestion. As the review knows, both QUICKI and HOMA-IR function equally well as they give the same information, so doing both and may
confuse and that is the reason that the QUICKI was not added to the manuscript (Rossner SM et al. HOMA-IR and QUICKI: decide on a general standard instead of making further comparisons. Acta Paediatr 2010;99:1735)

3. It is important to know the status of insulin resistance of PCOS and its relationship with CB1. Thank you for that comment. As noted in the Results “insulin resistance improved in the group treated with rimonabant (calculated by HOMA-IR method) p<0.05” however; as seen in Table 1 Orlistat also reduced insulin resistance and therefore it is not clear if there insulin resistance was reduced by rimonabant through CB1 or indirectly through weight loss. However, in an animal model it has been shown that rimonabant countered age-induced insulin resistance (Lipina C et al. Aging cell 2016;15:325). This is addressed in the Discussion that reads “However, there was a reduction in weight and a reduction insulin resistance for both rimonabant and orlistat therefore it is not clear if their insulin resistance was reduced by rimonabant through CB1 or indirectly through weight loss, though in an animal model rimonabant countered age-induced insulin resistance[19].”

4. Is there any correlation between testosterone levels and reduction of body weight?
Thank you. As detailed elsewhere there was no correlation between testosterone and reduction of body weight (Sathypalan et al Clin Endocrinol 2009;70:124)

5. Table 2 should be deleted of its less important in this paper.
We agree that the results are normal but this is the liver inflammatory cytokine profile and we think that it is important to show that data, confirming that the changes were not due to cytokines activation.

Discussion:
1. The author should explained the finding of “the reduction of the inflammatory marker hsCRP was only seen for pioglitazone and not for rimonabant”.
Thank you. We have reworded that sentence to read “the reduction of the inflammatory marker hsCRP was only seen for pioglitazone and therefore likely modulated by peroxisome proliferator-activated receptor gamma, rather through potential CB1 blockade”
2. Similarly, the phenomenon of "... all treatments reduced the FAI there was no correlation to changes in ALT.".

This has been addressed in the discussion to read “all treatments reduced the FAI there was no correlation to changes in ALT, suggesting a hepatic dependent mechanism, and suggesting that the changes seen for rimonabant were independent of its affect on androgen reduction.”

Ikram Shah Bin Ismail (Reviewer 2): Page 5, Line 10: The second study did not involve Rimonobant at all, so this statement is not correct.

Thank you this has been altered to metformin, pioglitazone and orlistat

In both studies, metformin did not result in any change in Insulin Resistance whereas Rimonobant, Pioglitazone and Orlistat all resulted in improvement in Insulin Sensitivity. A comment should be made about this to explain the lack of effect on Insulin resistance with Metformin.

Thank you for that and the Discussion now reads “In both studies, metformin did not result in any change in insulin resistance whereas rimonabant, pioglitazone and orlistat all resulted in an improvement in insulin sensitivity; however, as we have noted before the insulin sensitising action of metformin may be lost or reduced as BMI increases[10, 12]”