Author's response to reviews

Title: Ethinylestradiol 30ug-drospirenone and metformin: could this combination improve endothelial dysfunction in polycystic ovary syndrome?

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Author's response to reviews: see over
Dear Mr. Timothy Shipley, Executive Editor of BioMed Central

On behalf of my co-authors, I am re-submitting the revised manuscript (MS ID: 1638804262679309), entitled “Ethinylestradiol$_{30\mu g}$-drospirenone and metformin: could this combination improve endothelial dysfunction in polycystic ovary syndrome?” together with the cover letter, for possible publication in your journal – BMC Endocrine Disorders. The manuscript reports for the first time that the 6-month treatment with metformin 1700mg/day and the antiandrogenic oral contraceptive-drospirenone / ethinylestradiol$_{30\mu g}$ combined with diet has an overall beneficial effect on endothelial function and shows a neutral effect on hsCRP levels in obese and non-obese women with PCOS.

We appreciate both your promptitude and the accurate observations of your esteemed reviewers.
Regarding the latter’s comments, we are detailing the actions taken to remedy the errors detected and to clarify certain aspects that needed further explanation. The information has been organized per reviewer/comment.

Reviewer: Bee K Tan

We hereby express our appreciation for the positive feedback.

Reviewer: Neoklis Georgopoulos

MAJOR POINTS

1. Although flow-mediated dilatation (FMD) (%) was significantly increased ($p=0.033$) after 6 months of treatment, no significant increase in FMD was reported in either obese ($p=0.133$) or non-obese ($p=0.161$) group. The authors should comment on the discrepancy.

As far as the first observation is concerned, and following the advice of the reviewer, we have introduced a comment within the text of the manuscript stating that the FMD improvement which could be noted in the overall PCOS group could not be separately identified in obese and non-obese patients. This might be due to the small number of patients in the PCOS subgroup.

“Of note, FMD did not report a significant improvement when evaluating non-obese and obese PCOS subjects separately and this can be accounted for by the small number of subjects in the two groups.” (Quoted from the manuscript, page 15, paragraph 1)

2. The effect of DRP/EE30μg and metformin 850mg/day on PCOS women (no improvement of adiposity and deterioration of TG, IL-6) was compared to the effect of DRP/EE20μg + metformin 1500mg/day (improvement of insulin sensitivity, increase in HDL-cholesterol concentrations and no significant change in TG levels) in non-insulin resistant and non-obese young PCOS women. The effect of the combined administration of DRP/EE +
metformin on lipid metabolism cannot be evaluated, since doses of metformin and EE as well as basal metabolic profile were different between the groups of the studies. This observation has been particularly appreciated and followed. Consequently, we have modified the respective fragment as required, underlining, at the same time, that a valid comparison on the effects of this therapy on the lipid metabolism between these two studies as well as between them and our study is hampered by the fact that the doses of metformin and EE as well as the basal metabolic profile were different between the groups of the studies.

“Moreover, in a subsequent research by the same authors, the addition of small doses of metformin-850mg/day to the treatment with DRP/EE₃₀µg for 3 months failed to attenuate body adiposity of PCOS subjects and was accompanied by a further deterioration of TG and IL-6 levels from the norm [33]. In contrast, others observed that DRP/EE₂₀µg + metformin 1500mg/day improved insulin sensitivity, increased HDL-cholesterol concentrations and did not significantly change TG levels in a group of young but non-obese and non-insulin resistant women with PCOS [39]. However, a valid comparison between the study findings of Ibanez et al. and Fruzzetti et al., as well as a further one between their results and ours cannot be performed since the doses of metformin and EE used as well as the basal metabolic profile were different between the populations of these reports. Regarding lipid metabolism, although TG levels rose significantly over the study period in the whole study population as well as in the obese subgroup, most probably as a result of the medical therapy, they remained within the normal range. In other words, it can be hypothesized that metformin clearly does not outperform DRP/EE₃₀µg in increasing TG concentrations.” (Quoted from the manuscript, page 16)

3. In the present study, it was hypothesized that metformin and DRP/EE30µg may have either different or even opposing effects on chronic inflammation that may balance the risk out and
neutralize it overall or may both have neutral effects on hsCRP. The aforementioned hypothesis should be elaborated and supported by data sources from the literature.

As far as this suggestion is concerned, we have exemplified the possible effects of each individual substance by quoting specialty literature, where available. This has been the starting point of hypotheses on the possible effects resulted from combining these substances. It is worth mentioning, though, that, as far as we know, there are no reports evaluating the influence of DRP/EE$_{30\mu g}$ monotherapy on hsCRP levels in PCOS or in other populations. (Quoted from the manuscript, page 18, line 1-9)

4. Different effects of metformin, oral contraceptives and anti-androgens on CRP and hsCRP have been reported in the literature. Could the authors provide possible mechanisms implicated in each one of the agents? Additionally, the authors should comment on the findings of the present study.

Following this observation, we have added information (extracted from the available literature) on the possible mechanisms through which metformin, oral contraceptives and anti-androgens might influence hsCRP levels. As far as anti-androgens are concerned, we have strictly focused on flutamide and spironolactone, as there were no available studies on the relationship between drospirenone –CRP. Moreover, our results have been further discussed and the entire fragment on CRP inflammation has been reorganized, as follows:

“Most published data, yet not all [6, 40, 41], demonstrate increased levels of hsCRP in women with PCOS [5, 12, 42, 43], which may be associated with increased central fat excess rather than PCOS status per se [41, 44]. The change in hsCRP levels in our study was not significant by the end of the 6-month follow-up. However, the dosage of metformin and the duration of our study assured the detection of the effects of the medical intervention on hsCRP levels. Therefore, we can conclude
that even though moderate weight loss (6-8 %) definitely conferred significant metabolic benefits, it was not sufficient to improve the low-grade chronic inflammation in the total PCOS group and obese subgroup. The following results at the end of the study are noteworthy: both insulin resistance and body adiposity were still abnormal, particularly in the total PCOS group and the obese subgroup, on the one hand and the hsCRP concentrations correlated with trunk fat mass, BMI, TC and insulin resistance indices on the other hand. Therefore, it appears that a more aggressive therapeutic approach and a greater degree of weight loss may be required to achieve metabolic benefits, such as reductions in insulin resistance and body adiposity and consequent decrease of low-grade chronic inflammation. Sustaining our results, it was shown that a 4–5% weight loss improved lipid, glucose, and insulin profiles in women with and without PCOS, but was not effective in lowering CRP concentrations in PCOS women [45] whereas a 15% weight loss in a 2-yr dietary and exercise intervention study was associated with hsCRP reduction [46]. The results obtained in the non-obese subgroup of PCOS women and also those resulted after the adjustment for a decrease >5 % and 10% in body weight showed that the drug combination used in this study did not affect hsCRP levels. It could be hypothesized that metformin and DRP/EE30µg may have either different or even opposing effects on chronic inflammation that may balance the risk out and neutralize it overall or may both have neutral effects on hsCRP. Our hypothesis is based on previous results showing that metformin either decreased [11-13] or, used in lower doses (1000-1500mg/day) and for shorter period of time (3 months), caused no change in hsCRP [8, 47], as well as on the fact that the effect of DRP/EE30µg on chronic inflammation and especially on hsCRP has not been settled. As far as we know, there are no reports evaluating the influence of DRP/EE30µg monotherapy on hsCRP levels in PCOS or in other populations. What has been previously observed, though, is that, DRP/EE30µg further increased the abnormal levels of IL-6 found in young women with hyperinsulinemic hyperandrogenism [36].
Previous reports suggested that metformin may have direct actions on vascular cells [10, 48]. Additionally, metformin might decrease angiogenesis via nuclear factor-κB and Erk1/Erk5 pathways by increasing the antiangiogenic trombospondin-1, an adipokine, preferentially produced by visceral adipose tissue and highly expressed in obese insulin-resistant subjects [49]. However, it is uncertain whether metformin exerts direct effects on hsCRP levels or whether its beneficial changes on the concentration of this inflammation marker are only the result of improved glycemia, insulin resistance, abdominal fat excess and weight loss. Complex interactions and mechanisms might be implied. On the contrary, the serum CRP levels have been shown to increase after a 6-month treatment with COC in young overweight and obese women with PCOS, even if the COC contains an anti-androgen [11, 13]. Both estrogens and progestin content and dosage appear to be implicated in CRP regulation [50-52], even though the role of oestrogen might be more important than that of progestin [52]. As far as the mechanism of action is concerned, the literature has been sustaining the direct role of COCs in hsCRP determination by affecting the latter’s metabolic and genetic regulation [52]. Hence, a direct estrogen action on the liver was proposed since it is the oral estrogen, and not transdermal estradiol (the latter avoiding the first pass liver effect), which leads to increased serum CRP levels [50, 53]. Moreover, COCs have been demonstrated to increment CRP concentrations, without increasing IL-6 ones, suggesting that COCs stimulate hepatocytes to synthesize CRP in a direct way and not via IL-6 mediated inflammation [52]. Regarding anti-androgens, even though their mechanisms of action are not settled, they also appear to modulate and decrease inflammation in PCOS. Hence, low-dose flutamide added to metformin and a fourth-generation OC has been identified to attenuate the hypoadiponectinemia, lean mass deficit as well as central adiposity in young women with PCOS [33]. Up-regulated in states of insulin resistance, IL-6 differentially regulates androgen receptor transactivation via three distinct signaling transduction
pathways, the overall effect depending on the balance among these pathways and the androgen concentrations. For instance, at androgen concentration above normal female range and below normal male range, IL-6 and androgens could act synergistically on the androgen receptor [33, 54], hereby increasing androgen action. On the other hand, androgen excess in women favors an adipose body composition, including in the abdominal region. A vicious circle amplifying chronic inflammation is thus established. Moreover, adiponectin, which exerts insulin-sensitizing effects, is reversibly down-regulated by androgens and IL-6, but not by estrogens [55]. Therefore, the mechanism of action of flutamide might be at least partly explained by restoration of the androgen receptor transactivation balance and counteraction of the androgen – and IL-6- induced down-regulation of adiponectinemia [33]. Additionally, the mineralocorticoid and the androgen receptor antagonist spironolactone has been shown to inhibit the production of proinflammatory cytokines in patients with congestive heart failure, acting at the transcriptional level and independent of its antimineralocorticoid and antiandrogen activities [56]. We found no data on the effect of the combination metformin-DRP/EE30µg on hsCRP, only that Ibanez et al. showed that the treatment with flutamide-metformin plus DRP/EE30µg is associated with a consistent fall in CRP and TNF-α levels, especially in patients with the most abnormal values [57].

"(Quoted from the manuscript page 18-19-20)"

MINOR POINTS

1. Page 4: Women with polycystic ovary syndrome (PCOS) frequently cluster…

   We have modified “clusters” to “cluster”.

2. Page 15: …metformin 850 mg/day + DRP/EE30µg does not influence the insulin resistance

   We have deleted “to” from “to influence”.

"Quoted from the manuscript page 18-19-20"
3. Page 16: …the total PCOS group and obese and non-obese subgroups.

We have concluded that this observation was related to the following sentence: "Therefore, we can conclude that even though moderate weight loss (6-8%) definitely conferred significant metabolic benefits, it was not sufficient to improve the low-grade chronic inflammation in the total PCOS group and obese subgroup" and, therefore, taking into account that the 6-8% weight loss was valid exclusively for the total group (6.61%) and the obese one, respectively (8.78%), and that the non-obese group reported a weight loss of only 2.1%, we have considered not adding ‘non-obese subgroup’ to the sentence more appropriately.

Thank you very much for considering our manuscript for publication as well as for the feedback which has made us aware of our inaccuracies and has helped us improve the quality of our work.

We are looking forward to hearing from you.

Kind regards,

Ioana Rada Ilie