Author’s response to reviews

Title: Androsterone Glucuronide to dehydroepiandrosterone sulphate ratio is discriminatory for obese Caucasian women with Polycystic Ovary Syndrome

Authors:

Li Wei Cho (liweicho@hotmail.com)
Thozhukat Sathyapalan (Thozhukat.Sathyapalan@hyps.ac.uk)
Eric Kilpatrick (ekilpatrick@sidra.org)
Brian Keevil (Brian.Keevil@UHSM.NHS.UK)
Adrian Miller (adrian.miller@UHSM.NHS.UK)
Anne Marie Coady (anne.coady@hey.nhs.uk)
Lina Ahmed (lha2002@qatar-med.cornell.edu)
stephen atkin (sla2002@qatar-med.cornell.edu)

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The Editor
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BMC Endocrine disorders

Dear Editor

Re: Androsterone Glucuronide to dehydroepiandrosterone sulphate ratio is discriminatory for obese Caucasian women with Polycystic Ovary Syndrome.

Many thanks for the editorial and reviewer comments. We have now incorporated the comments and recommendations that have markedly improved the manuscript. We have also collated a
point to point responses to the reviewers comments. We hope this manuscript is now acceptable for publishing in BMC Endocrine disorders.

Yours Sincerely

Stephen L Atkin

Reviewer 1: I was pleased to review this cross sectional, observational study of androgen levels in obese and non-obese subjects with PCOS. The eloquently designed study is clearly described in a well written manuscript.

Thank you for your positive comments.

General points:

Overall, the introduction would benefit from a more detailed discussion about the metabolism of ADTG and rationale for measuring it in this study. The average reader may not be familiar with the details of DHEA breakdown and a clearer explanation would augment the manuscript.

Thank you for that comment that we have addressed and in the Introduction it reads “Androsterone glucuronide (ADTG) reflects adrenal androgen secretion from hepatic 5α-reductase activity, and to a lesser extent peripheral 5α-reductase activity, that converts dehydroepiandrosterone sulphate (DHEAS) to ADTG[7, 8]. Thus, DHEAS and to a lesser amount DHEA (20%) are converted to ADTG by hepatic and peripheral 5α-reductase and therefore the concentration of ADTG will reflect both DHEAS levels and 5α-reductase activity. ADTG has been reported to be a more reliable marker for the effects of androgen at the target tissue level and studies have shown that ADTG is significantly elevated in hirsute compared to non-hirsute women with PCOS[9-11]. Previously ADTG has been measured by immunoassay that may be inaccurate due to cross-reactivity with other androgen metabolites such as DHEAS [12, 13], circumvented by tandem mass spectrometry.”
Similarly, the discussion would benefit from a clearer comparison with other studies of ADTG in PCOS as well as in other pathological states and healthy women.

Thank you for your comment that we have addressed and the discussion now reads “There is little data on the role ADTG in PCOS and androgen metabolism, however this study showing the increased ADTG:DHEAS ratio in obese PCOS using state of the art measurement free from assay interference addresses the discrepancy noted in one study that it was not correlated with PCOS {Thompson, 1990, Androsterone glucuronide is a marker of adrenal hyperandrogenism in hirsute women}, with another suggesting that ADTG levels are increased {Kiddy, 1990, Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases}”

While the results are of interest and may advance the scientific knowledge of the PCOS syndrome and allow us to better classify patients, I am unclear of the clinical utility of ADTG:DHEAS ratio. The authors conclude that "this may be a novel biomarker to identify obese PCOS patient". They should be more specific about the potential utility (particularly in the clinical setting) of this test. Is it just a reflection of insulin resistance for which we already have clinical and biochemical parameters

Thank you for that comment that we have addressed in the Discussion that now reads “Thus, in the case of diagnostic uncertainty, the presence of both obesity and raised ADTG:DHEAS ratio could help confirm the diagnosis of PCOS, and the ratio could be an indirect measure of 5α-reductase activity that may identify those individuals whose insulin resistance is having a marked effect on their androgen metabolism.”

Specific points:

Was this a prospective or retrospective study? This should be clearly stated in the methods section. If it was prospective, why were subjects and controls not matched for age?

Thank you for that comment that we have addressed in the Methods that reads “This was a prospective study that enrolled all patients that fulfilled the inclusion and exclusion criteria and as a consequence the 2 groups were not age matched”
In Table 1, was all the data normally distributed? If so, it may be best expressed as mean +/- SEM. If not normally distributed, it could be expressed as median and range.

Thank you for that comment that we have addressed in the Statistical Analysis that now reads “The data was normally distribution between individuals and none violated the assumptions of normality when tested using the Kolmogorov-Smirnov test and therefore are expressed as mean ± SD.”

We used the SD and not the SEM to give the reader a better indication of the spread between subjects.

Reviewer 2: 1. Was there a correlation between ADG:DHEAS and clinical phenotype of PCOS in the study subjects?

Thank you for that comment. As noted in the Methods all PCOS women had all the three diagnostic criteria for PCOS: irregular menses, biochemical hyperandrogenism and polycystic ovaries on ultrasound. It was therefore not possible to look at other phenotypes

2. Was urinary samples collected for the study subjects? If so, is there a correlation between ADG:DHEAS ratio and urinary Androsterone: etiocholanolone ratio (marker of 5alpha reductase)

Thank you for that comment and excellent suggestion, but urinary samples were not collected in the study. This has been noted in the limitations that reads ““Additional measurements of DHEA (the second largest source of ADTG) in blood and urine for the androstenedione:etiocholanolone ratio in urine as a marker of 5 alpha reductase would be useful to evaluate.”
3. Literature suggests that DHEA forms the second largest source of ADG accounting for upto 20%. What were the DHEA values in the study population?

Thank you for your comment but the DHEA values were not measured in this study this has been addressed in the Strengths and Limitations that reads “Additional measurements of DHEA (the second largest source of ADTG) in blood and urine for the androstenedione:etiocholanolone ratio in urine as a marker of 5 alpha reductase would be useful to evaluate.”

4. The contribution of Thozhukat Sathyapalan in the study is not included in the relevant section. Can the authors kindly update this?

Thank you this has now been addressed in the Authors Contributions and reads “LWC was involved in protocol development, patient recruitment, data analysis and first draft of the manuscript. ESK, BGK and AGM were involved in sample analysis. ESK, TS, BGK, AMC, LA and SLA reviewed and edited the manuscript. All authors approved the final version of the manuscript.”