Author’s response to reviews

Title: Effects of Endogenous Sex Hormones on Lung Function and Symptom Control in Adolescents with Asthma

Authors:

Mark DeBoer (deboer@virginia.edu)
Brenda Phillips (bphillip@phs.psu.edu)
David Mauger (DMAuger@phs.psu.edu)
Joe Zein (jgz8@case.edu)
Serpil Erzurum (ERZURUS@ccf.org)
Anne Fitzpatrick (anne.fitzpatrick@emory.edu)
Benjamin Gaston (bmg46@case.edu)
Ross Myers (ross.myers@uhhospitals.org)
Kristie Ross (Kristie.Ross@uhhospitals.org)
James Chmiel (James.Chmiel@UHhospitals.org)
Min Jie Lee (min.jie.lee@emory.edu)
John Fahy (John.Fahy@ucsf.edu)
Michael Peters (michael.peters@ucsf.edu)
Ngoc Ly (Ngoc.Ly@ucsf.edu)
Sally Wenzel (wenzelse@upmc.edu)
Merritt Fajt (fajtml@upmc.edu)
Fernando Holguin (Fernando.holguin@ucdenver.edu)
Wendy Moore (wmoore@wakehealth.edu)
Stephen Peters (speters@wakehealth.edu)
Deborah Myers (dmyers@wakehealth.edu)
Eugene Bleecker (erbleecker@email.arizona.edu)
Mario Castro (castrom@wustl.edu)
Andrea Coverstone (acoverstone@wustl.edu)
Leonard Bacharier (bacharier_l@wustl.edu)
Nizar Jarjour (NJarjour@uwhealth.org)
Ronald Sorkness (ronald.sorkness@wisc.edu)
Sima Ramratnam (ramratnam@pediatrics.wisc.edu)
Anne-Marie Irani (airani@vcu.edu)
Elliot Israel (eisrael@partners.org)
Bruce Levy (blevy@partners.org)
Wanda Phipatanakul (Wanda.Phipatanakul@childrens.harvard.edu)
Jonathan Gaffin (Jonathan.gaffin@childrens.harvard.edu)
W. Teague (WGT2P@hscmail.mcc.virginia.edu)

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Anita Kozyrskyj (Reviewer 1): This is a clearly written manuscript of a rigorously-conducted study of sex differences in asthma severity/control with advancing puberty that addresses a significant gap in the literature. DeBoer et al found lung function in boys to be associated with DHEA levels but with estradiol levels in girls. Lung function tests (spirometry, maximum bronchodilator and methacholine challenge) were conducted by trained personnel; asthma severity and asthma control were assessed according to ATS guidelines and a validated questionnaire, respectively. Both physical and serum biomarkers of adrenarche, thelarche and puberty were evaluated using valid and reliable methods. Univariate comparisons in lung function were performed across puberty stage. Multivariate logistic regression was conducted to derive the most parsimonious model for sex hormones and DHEA in relation to lung function. Results were clearly displayed.
Major comments:

1. Asthma status in children was well characterized but I recommend the addition of the following parameters to Table 1: i) atopic sensitization status and ii) age of asthma diagnosis or years with asthma symptoms. This information would enable the reader to discern whether asthma phenotypes were comparable between boys and girls, namely whether asthma in boys was longstanding and atopic versus the non-atopic asthma of recent onset in girls.

RESPONSE: We have included these data in Table 1.

2. Since many readers may not be familiar with the expected changes to DHEA or DHEA-S with adrenarche, I recommend inserting a brief description in the introduction, with a citation by: Mouritsen et al. Longitudinal changes in serum concentrations of adrenal androgen metabolites and their ratios by LC-MS/MS in healthy boys and girls. Clinica Chimica Acta 2015. In general, the paper was lacking a description and citations of expected hormonal changes in adrenarche, thelarche, pubarche and menarche.

RESPONSE: This reference has been added, along with the description: “In boys, androgen production gradually increases both from testes producing testosterone and the adrenal glands producing weaker androgens—ultimately leading to pubarche. Girls experience increases in the production of estrogen from the ovaries (driving thelarche and ultimately menarche) and androgens such as androstenedione and DHEA-S from the adrenal glands (driving pubarche in girls)” (Introduction, page 4, paragraph 2, line 3).

3. Potentially, there were 2 confounding factors that were not controlled in study comparisons: i) child overweight and ii) child depression status. Adjusting biomarker models for these factors may not be helpful as they may explain away the association with DHEA or a sex steroid. Hence, I recommend that associations be tested in children with and without these factors. Since height and weight were measured, minimally I recommend this stratified analysis.
RESPONSE: We have analyzed the data by child overweight/obesity, with the original tables corresponding to supplementary tables as follows:

Table IIA: Supplementary Table II-A-1 (overweight/obese) and II-A-2 (normal weight)
Table IIB: Supplementary Table II-B-1 (overweight/obese) and II-B-2 (normal weight)
Table IIIA: Supplementary Table III-A-1 (overweight/obese) and III-A-2 (normal weight)
Table IIIB: Supplementary Table III-B-1 (overweight/obese) and III-B-2 (normal weight)

For the Tanner stage differences in lung function and ACQ2 between boys and girls, results were the same. Interestingly, for the associations of sex hormones with lung function, among boys these associations were only seen among overweight/obese adolescents—a finding not seen among girls. This is now stated in the Discussion: “We noted associations between DHEA-S and lung function that were present in overweight/obese but not normal weight. This differential effect by weight status, which was seen only in boys, is of unclear significance and requires further investigation” (Discussion, page 12, paragraph 2, line 9).

Unfortunately, we lacked data regarding depression status and have included this as a limitation of the study: “We lacked data on some participant characteristics, including depression status—which could affect stress response” (Discussion, page 14, paragraph 2, line 6).

4. Although the paper is on sex steroid differences, authors should be prepared to discuss other hormonal changes in puberty such as transient insulin resistance or elevated cortisol, which accompany sex steroid changes. Some of these have been found to be associated with asthma persistence. See Kozyrskyj et al. Am J Resp Crit Care Med 2014;190 and Bahreinian et al. Am J Respir Crit Care Med 2013;187.

RESPONSE: We have added the following paragraph to the Discussion: “It should also be noted that other hormonal changes during puberty could also affect lung function, including an increase in the degree of insulin resistance—which itself is associated with increased risk of asthma in adults and adolescent girls—and elevations in stress hormones, including cortisol—with such stress systems further associated with asthma risk. While temporally related (i.e.,
during puberty), the processes of insulin resistance and stress response go beyond sex hormone regulation alone. Unfortunately, the timing of our blood collection was not specifically in the early morning (thus not as helpful to assess cortisol regulation) or with fasting status (for assessing insulin resistance), and thus we were not able to assess how these processes may have interacted with changes in sex hormones. Nevertheless, because we were able to assess relationships between measured levels of sex steroids and outcomes important to asthma severity, our findings suggest a direct role for sex steroids themselves.” (Discussion, page 13, paragraph 3, line 1.)

Minor comments:

1) Page 4, line 61: Are authors able to comment on the temporality of the discord between peripheral and central changes in girls, namely whether peripheral androgen changes precede the central changes to estradiol and breast development?

RESPONSE: Because these data are cross-sectional in nature, we cannot comment on the timing of whether adrenarche or central puberty occurred first for the majority of these girls (except for those for whom there Tanner staging revealed evidence of one process and not the other). Over the course of adolescence, it is common for adrenarche to precede central puberty and for puberty to precede adrenarche. When there was a discrepancy between early and late puberty by breast or pubic hair development (n=7, On-line Supplement Table 1), 6 of these girls had pubic hair development before breast development. We have added acknowledgement of the cross-sectional nature to the limitations section: “We would point out that the veracity of the results is limited by the cross-sectional analytic approach, and our lack of knowledge for most female participants of whether central puberty or adrenarche began first.” (Discussion, page 14, paragraph 2, line 3).

2) Page 5, line 5-6: The qualifier "…..of these studies" confuses the purpose of the current study versus that of the studies cited in previous sentences.

RESPONSE: We have clarified this to refer to the current studies (Introduction, page 5, paragraph 1, line 6).
3) Methods section: Specify the timing of the blood draw to measure sex hormones in relation to lung function testing since the latter may have influenced the former.

RESPONSE: We have added this to the Methods section: “Blood was drawn during the visit but not with systematic timing related to the timing of the spirometry” (Methods, page 6, paragraph 2, line 6).

4) Page 8, line 16: It was unclear whether lack of a difference in the covariate distribution in Table 1 was statistically significant. Please add p values to the table.

RESPONSE: We have added this to Table 1.

5) Page 10, line 32: Stating the study N in the first sentence of the Discussion provides context to the discussion of study results that follow.

RESPONSE: This has been added (Discussion, page 11, paragraph 2, line 2).

6) Page 10, line 33: The following sentence was very vague: "In males, lung function was not different and symptom control improved, whereas in females lung function and symptom control were worse."

RESPONSE: We have clarified this as follows: “In males, lung function was not different and symptom control improved when comparing those in early vs. late puberty, whereas in females lung function and symptom control were worse” (Discussion, page 11, paragraph 2, line 4).
Joerg Mattes (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

This is a well-written paper and the following suggestion are to be considered:

1) Provide information on add-on treatments (e.g. montelukast, LABA)

RESPONSE: We have added this to the participant characteristics in Table 1.

2) The definition for severe asthma was modified with regard to two aspects, one is that add-on treatment were not considered to be necessary to make the diagnosis of severe asthma, and secondly if the child needed to have uncontrolled asthma despite being on maximum inhaled steroids therapy. Can the authors clarify how many asthmatics labeled severe were uncontrolled (e.g. based on ACQ) and discuss any potential implications of a modification of the ERS definition of severe asthma for the result interpretation in greater detail.

RESPONSE: Thank you. We are sorry that our use of the ERS/ATS definition of severe asthma was unclear and have clarified our description of how SARP-III implemented the ERS/ATS definition as follows: “Severe asthma was defined according to the ERS/ATS consensus definition. Children whose asthma required treatment with high-dose inhaled corticosteroids (≥440 mcg of fluticasone equivalents per day for children 6-11 years of age; ≥880 mcg fluticasone equivalents per day ≥12 years of age) plus a second controller and/or systemic corticosteroids to maintain asthma control or which was uncontrolled despite these medications were assigned to the severe sub-group. Medications at this level were required for at least 6 of the previous 12 months and the 3 months prior to enrollment for participants to qualify for the severe sub-group. Those who did not meet criteria for severe asthma were assigned to the non-severe sub-group.” (Methods, page 6, paragraph 1, line 1).
Regarding ACQ elevations, in the Results section, we now report numbers of severe and non-severe participants above the cut-off of 1.0 that is cited by Juniper et al.: “There were 110 severe and 77 non-severe participants in the analytic cohort. Of these, 74 severe- and 30 non-severe participants had an ACQ <1.0 as an estimate of poor control (67% and 39%, respectively)” (Results, page 8, paragraph 2, line 3).

3) Presumably the authors did not measure FeNO which would have allowed to correlate hormone levels with activity of Th2-high airways inflammation?

RESPONSE: We lacked FeNO and have added the following to the limitations section: “We also lacked measures such as FeNO, which would have permitted assessment of the relationship between hormone levels with activity of Th2-high airways inflammation” (Discussion, page 14, paragraph 2, line 8).

4) Can the authors comment to what extent age versus puberty was relevant for predicting ACQ6. In that regard as GLI reference values are age-adjusted, would this potentially have lessened the puberty stage effects on lung function?

RESPONSE: We used linear regression to assess whether ACQ6 scores were associated with age, and did not find such a correlation in either boys or girls (Online Supplement Table IV). We have addressed this and age-based GLI reference values as follows: “Given that GLI-based normal ranges of lung function are age-specific these maturational and hormone-associated measures appear to be present beyond the expected changes in lung function based on age alone. Furthermore, we did not note associations between ACQ6 and age in either boys or girls.” (Discussion, page 11, paragraph 3, line 8.)