The relationship between reproductive hormones and asthma-related outcomes in boys residing in the rural Western Cape

Mandy Sigametsi Phuti

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Supervisors

Professor Mohamed Jeebhay, MBChB PhD
Professor Mohamed Aqiel Dalvie, PhD
Dr Toyib Olaniyan, PhD

Centre for Environmental & Occupational Health Research (CEOHR)
School of Public Health
Health Sciences Faculty, University of Cape Town

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PREFACE

Plagiarism Declaration

I, Mandy Sigametsi Phuti, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Background: Asthma is more prevalent and severe among boys but this pattern reverses after puberty. It has been suggested that reproductive hormones may play a role in explaining these sex differences after puberty, but the evidence is still limited especially for children living in low- and middle-income countries.

Objective: This study investigated the association between reproductive hormones and asthma-related outcomes among boys residing in a rural setting.

Methods: A cross-sectional study of 470 boys (6-18 years), residing in the rural Western Cape province of South Africa was conducted. General questionnaires were administered to the boys and their caregivers including an abbreviated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Testosterone (TST and FT), luteinizing hormone (LH), serum follicle-stimulating hormone (FSH), oestradiol and serum hormone-binding globulin (SHBG), were assessed using electrochemiluminescence immunoassays. A total of 314 (66.8%) boys had valid asthma outcome information and hormone level measurements that were analysed further. Multivariate logistic regression models were used to assess the relationship between asthma and measured endocrines levels.

Results: The mean age of the boys was 11.2 (Standard deviation -1.7 years). Current wheeze (CW), asthma symptom score ≥ 2 (ASS) and parental reported asthma (PA) prevalence were 6.1%, 6.7% and 8.0%, respectively. In the adjusted multivariate logistic regression models, total serum testosterone (TST) levels were negatively associated with CW (OR=0.66, 95% CI: 0.45-0.98), ASS (OR=0.64, 95% CI: 0.43-0.95) and PA (OR=0.86, 95% CI: 0.59-1.25). Free testosterone (FT) levels were similarly negatively associated with all three asthma-related outcomes. Similar associations were also found for LH and FSH, but there were no clear associations for estradiol and SHBG with asthma-related outcomes. The concentration-response curves confirmed that higher levels of testosterone (TST and FT) were associated with reduced risk of asthma-related outcomes.

Conclusions: This study provides evidence that increasing testosterone levels are associated with reduced asthma risk among rural boys in South Africa.

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PART A: RESEARCH PROTOCOL

1. Introduction

Asthma is a chronic lung illness largely defined by the constriction of the airways and/or hyperreactivity, inflammation, and mucus production presenting with intermittent respiratory symptoms (1). It is common in children and its incidence continues to increase in South African urban and rural communities (2). Children that develop airway inflammation and obstruction will wheeze and develop decreased lung capacity and also consistently experience disabling symptoms as adults because of poor lung development (3, 4). Asthma is one of the leading causes of school absences in children, and according to Toskala et al (5), it is one of the most common reasons for admission to the hospital in children under the age of 15. Although asthma symptoms can develop at any age, including adulthood, they usually appear for the first time during childhood (3).

Koper et al (2017) report that boys are about twice as likely as girls to develop asthma during childhood (1). Multiple studies show that, when compared to girls, boys have a higher prevalence of asthma-related outcomes (e.g., current asthma, wheeze and decreased lung function) during childhood. However, the prevalence tends to shift from being higher among males to higher in females around puberty (6). Asthma and its underlying characteristics, such as bronchial hyperresponsiveness, atopy, and increased IgE, are thought to be influenced by a variety of genetic, host-related factors and environmental exposures (5, 7, 8).

2. Literature Review

2.1 Asthma risk factors

Asthma is becoming more prevalent in both rural and urban inhabitants, especially in children owing to a variety of genetic and environmental factors. (2, 3). Genetic factors or susceptibility have quite a significant impact on the development, severity, and management of asthma. It is important to note that asthma is a complex genetic disorder and often occurs in families (3, 5). Several genetic variants, particularly in children, which contribute to the risk of asthma development have been recognized, for example, a study on twins found that there is a genetic aspect to the susceptibility to asthma (5, 9). The function of the genes is not well understood, however, polymorphisms in various genes (e.g. A disintegrin, metalloproteinase 33 (*ADAM33*) and filaggrin gene) have been shown to influence the onset, severity and responsiveness of asthma to treatment (5).

Other host risk factors include age, sex, obesity, current or previous medical history and smoking (7). The association between increased risk of asthma and obesity has also been identified by a number of studies both in children and adults (10). Other risk factors in children include low birth weight and growth retardation (3). History of preterm delivery, low birth weight and greater child weight gain are also contributing factors for developing persistent airflow limitation and thus risk factors for asthma (11). Moreover, eczema is a significant risk factor for asthma, with longitudinal studies showing that more than one-third of eczema children develop asthma (2).

There is also a strong link between asthma and atopy, but this connection is complex. A limited number of studies have shown that atopic people are particularly vulnerable to asthma and that a large portion of asthma prevalence may be attributed to various allergies in the domestic

environment (5). However, not everyone who is atopic develops asthma, and not everyone who has asthma has detectably heightened allergic responses.

Environmental factors are far more likely to contribute to the increased number of asthmatic people worldwide than genetic factors (3). It is important to note that asthma is an episodic condition triggered by, many different environmental stimuli, which include irritants (smoke), temperature variations, exercise, viruses and allergens such as animal fur, dust mites, pollens, allergens in the workplace and moulds (7). Furthermore, exposure to tobacco smoking, living in extremely polluted locations, and pesticide exposure may aggravate wheezing in young children. (2, 11). Prenatal exposure to tobacco smoke and smoking, in general, are significant risk factors for wheezing, both in children and adults (12). Previous research suggests a causal association, between asthma and tobacco smoke, since a decrease in asthma incidence and severity was related to a decrease in exposure to tobacco smoke (3, 11). Additionally, industrial pollution and pesticide exposure are also associated with a higher prevalence of asthma. (13, 14). Pesticide exposure is suspected to exacerbate asthma by causing airway irritation and inflammation, endocrine disruption, or immunosuppression. (14, 15)

2.2 Mechanism of asthma

Asthma is a heterogeneous condition with various underlying mechanisms. It also has a number of different phenotypes including allergic asthma, non-allergic asthma, cough-variant asthma, adult-onset asthma, aspirin-induced asthma, paediatric asthma, asthma with persistent airflow restriction, eosinophilic (type 2 inflammation) asthma, exercise-induced asthma and obesity-related asthma (4, 11).

While asthma is typically identified by acute asthma attacks, involving wheezing and often reversible airflow obstruction, it also has an important immune component. According to Toskala and Kennedy (5) dysregulated immunity is essential in the development of asthma

through high levels of serum immunoglobulin E (IgE), eosinophils penetration into the lungs, excess release of allergic mediators from mast cells, and airway inflammation accompanied by distorted T helper 1 (Th1) and Th2 responses (5).

Fahy et al (16) state that most asthmatics have type 2 inflammation, which is common in allergic diseases and as an immunological response to parasites. Type 2 inflammation is named from the type 2 T helper cell lymphocyte and it is characterised by a particular cytokine profile with elevated interleukin [IL]-4, IL-5, and IL-14 and inflammatory cells such as mast cells, eosinophils, type 2 T helper lymphocytes, basophils, and plasma cells that produce immunoglobulin E [IgE] (4, 16). Type 2 inflammation is facilitated by type 2 T-helper cells and the respiratory epithelium, which leads to bronchial inflammation, which in turn increases mucus production, and leads to bronchoconstriction and narrowing of airways due to airway remodelling and collagen deposition (4). Reproductive hormones are believed to interfere with the mechanisms of asthma leading to the differences in asthma prevalence, incidence, phenotypic manifestation, and severity of asthma between males and females. This is discussed in detail below.

2.3 Asthma and Gender/Sex differences

Several studies have found a gender difference in asthma prevalence or severity (1, 10, 17-21). However, most of these studies have been done in developed nations and have compared adult females and men, despite the fact that it has become clearer that boys are also a high-risk population in certain settings. In a study by Holguin (22) among adults and children, asthma was found to be more prevalent and severe in boys during childhood compared to girls, but women were more frequently affected than men in adulthood. Other studies have found that boys are twice as likely as girls to develop asthma during childhood and twice as likely as girls to be admitted to the hospital for an asthma exacerbation. (21, 23).

The reason for this difference in incidence is largely unknown, however, a potential explanation put forward relates to the development of the large airways in the child's respiratory system. The airway growth in boys lags behind lung parenchyma growth, a section of the lungs involved in gas exchange with alveolus being the most visible feature in this region, resulting in smaller airways in boys relative to girls (20). Furthermore, boys have higher allergen sensitivities and atopy than girls because they have higher levels of total immunoglobulin E (IgE), therefore the male gender is generally thought to be a potential risk factor for any sensitization (17). Zein et al (18) also found that young boys are more likely than young girls to have allergies, asthma symptoms, respiratory sensitization, and use asthma medication before and at school entry age.

A sex shift in the prevalence of asthma after puberty, with asthma being more prevalent and severe in women than in men has been observed in several studies (1). Adult females have reported more severe symptoms that tend to vary at different stages of life, such as menstruation, pregnancy and menopause, and are associated with the levels of their reproductive hormones (1). A review by Koper et al (1), reported that compared to women, men with non-allergic asthma have a statistically increased prevalence of eosinophilic airway inflammation symptoms. Another gender difference related to asthma is susceptibility to cigarette smoke, with females being more susceptible than males, resulting in increased asthma prevalence and severity in women than men (1). A study by Han, Forno (24), clearly highlights that reproductive hormones in adults play a role in these sex disparities in asthma, and also that the effects of reproductive hormones are modified by obesity in both adults and children (1). In general, changes in asthma prevalence correspond with changes in reproductive hormones, indicating that reproductive hormones modulate pathways linked with asthma pathogenesis (21).

2.4 Asthma and endogenous reproductive hormones

2.4.1 Reproductive hormones and asthma pathways

Reproductive hormones are considered to play a significant role in explaining the sex difference in asthma prevalence and severity (25). The mechanisms in which reproductive hormones affect the development of asthma are complex. Changes in the levels of reproductive hormones throughout life may explain some of the sex differences in asthma, however, the role of both female and male reproductive hormones in asthma pathogenesis is not completely understood and there are limited studies on this issue. Androgens, like testosterone, suppress innate and adaptive immunological responses, whereas progesterone and oestrogen may increase T-helper cell type 2 allergic airway inflammation in asthma (21).

The link between asthma and female reproductive hormones is unclear. Exacerbations of asthma in women may be caused by oestrogen changes, as it reduces TNF production, interferon expression, and NK cell activity by acting as an anti-inflammatory (26). The relationship between asthma and testosterone has the potential to shed light on the pathophysiology of respiratory diseases and reproductive hormones. Several studies have found a mechanistic connection between asthma and testosterone, through an inverse association with asthma-related outcomes or a positive association with lung function (22). Testosterone has been shown in biological mouse models to decrease lung innate type 2 cell levels, airway smooth muscle contraction, and decrease allergen-induced IL-13 and IL-5 production in innate type 2 cells (27). Additionally, testosterone and its metabolites improve the functional equilibrium of autoimmunity and protective immunity by retaining regulatory T-cells. In relation to this, Canguven et al (25) and Koper et al (1) report that testosterone has immunosuppressive properties and is potentially also effective against asthma-causing immunoinflammatory mechanisms. Therefore, testosterone is believed to have a protective effect against asthma compared to female-related reproductive hormones.

This is also supported by a study by Malkin et al (28), which suggests that testosterone can repress proinflammatory cytokine expression (IL-1 and IL-6) and enhances anti-inflammatory cytokine IL-10 expression. Evidence of improved asthma symptoms under testosterone intake in women has been observed in a previous study (29). However, Canguven et al (25) suggest that low testosterone levels can significantly modify the response of smooth muscle in the airways which could result in an increased risk of asthma among susceptible people. Moreover, there is evidence of improvements in asthma outcomes in women with testosterone intake. The effects of reproductive hormones on asthma symptoms and their development are therefore complicated, and they appear to be mostly linked to hormonal level fluctuations (1).

Furthermore, there is a relationship between asthma, female reproductive hormones, and leptin. Leptin is a protein produced by the obesity gene that is produced and released by adipocytes. According to Sood et al (30), there is an association between asthma and high serum leptin concentrations in adult women than men. However, in a children's study by Guler et al (31) higher serum levels of leptin were found in asthmatic male children than females. These elevated serum leptin levels in boys with asthma may help to explain why boys have a higher prevalence of childhood asthma (31).

2.4.2 Review of previous epidemiological studies investigating asthma and reproductive hormones

Search strategy method

A review of studies investigating the association between reproductive hormones and asthma was done. To do this, the search was conducted using the Pubmed search engine and the following search items were used: ((Asthma) OR (allergy)) AND ((children) OR (Adolescents) OR (Puberty) OR (Sex) OR (gender)) AND ((Reproductive hormones) OR (sex hormones) OR

(pituitary hormones) OR (Gonadal steroids)). The inclusion criteria were the following: Journal articles, published over the last 10 years and written in English.

The search was performed in five steps:

1. Keywords search.
2. Preliminary search utilizing article titles to include or exclude articles
3. A search was then done using their abstracts to include or exclude articles.
4. Full articles were then read, and additional exclusions were made.
5. Synthesis.

Tables 1 and 2 summarizes all studies identified in the literature that investigated the relationship between asthma and reproductive hormones in children and adults respectively from 2011 to 2021. Six studies investigating the relationship between asthma and reproductive hormones in children and adults were identified. Of these only two studies done in the United States (34) and Northern Taiwan (36) looked at children or adolescents only. Two studies were done in the United Kingdom (32) and the United States of America (35) investigated both children and adults and two studies from the United States (24) and the United Kingdom (33) investigated only adults. All 6 studies were cross-sectional of which two were sub-analysis of cohort studies.

The reproductive hormones of interest in these studies included total testosterone, serum-free testosterone, oestrogen or Estradiol Dehydroepiandrosterone sulfate (DHEAS) and progesterone. Additionally, sex hormone-binding globulin (SHBG) was also investigated. All six studies looked at serum testosterone as the reproductive hormone of interest. In all six studies, testosterone was associated with asthma and had a protective effect on asthma. Four studies measured sex hormone-binding globulin (SHBG) (24, 32,33, 34) and only one study by

Arathimos et al (32) analysed it and found a weak, non-significant association with asthma. Although there is limited evidence on the impact of SHBG on asthma outcomes among boys, the reason for the weak association observed in this study could be due to the lower sample size and therefore low statistical power. The study by Arathimos et al also found a protective effect of SHBG on asthma outcomes, and this could be due to the measurement of genetically elevated SHBG in the study. Furthermore, three studies looked at estradiol as the reproductive hormone of interest. Two of these found a significant positive association between asthma and lung function (24, 34). While only one study looked at Dehydroepiandrosterone sulfate (DHEA-S) as the reproductive hormone of interest and had a positive association with asthma outcome and one study investigated progesterone and oestrogen and both were negatively associated with an asthma related outcome (34)

Of the four studies conducted in children, two studies from the United States and the United Kingdom found significant associations between asthma and reproductive hormones (32, 34). The association between asthma outcomes and testosterone was negative suggesting a protective effect on asthma. While one study did not find any association between current asthma prevalence and serum testosterone among children younger than 12 years, which could be due to the low levels of testosterone among these children which were below the threshold to cause asthma (35). Of the four children's studies, one assessed the relationship between asthma and DHEA-S and found a positive association (34). One study found a negative association between female oestrogen and lung function (34).

In adults, all three studies used testosterone as the reproductive hormone of interest and found negative associations with asthma, which could be due to higher testosterone levels found in adults (24, 33, 35). Of the three adult studies, two looked at oestradiol as a reproductive hormone of interest. One study found a significant association in women (24) while the others never found a significant association between asthma and serum estradiol (33).

Four studies looked at obesity and its effect on the relationship between asthma and reproductive hormones (24, 33-35). Three of these studies found an interaction between obesity, asthma and reproductive hormone (24, 34, 35), while one did not find an interaction (33). These contrasting findings could be due to differences in sample composition with the latter study using data from relatively healthy participants that were less likely to be obese and had fewer self-reported health conditions. Five studies investigated the effect of smoking, and all found an interaction between smoking and reproductive hormones on asthma (24, 32, 33, 35, 36), with reproductive hormones having an association with an increased odds of asthma in participants who smoke. While only one study looked at the effects of genetics, evidence suggested that genetically elevated SHBG has a protective effect on asthma (32). Another one study looked at the effects of Tanner staging and also found an association between reproductive hormones and asthma in different Tanner stages with the surge of androgens during puberty conferring a protective effect in both sexes and estrogen having negative effects in females (34).

The main strength of these studies was that they had a large sample size ranging between 187 and 256 419 participants, and reproductive hormone levels were accurately measured to minimize bias. Furthermore, most of these studies focused on testosterone in both females and males. The measurement of asthma outcomes is a major constraint in epidemiological studies analysing the association between reproductive hormones and asthma. Despite the availability and widespread use of standardised methods such as the ISAAC questionnaire for measuring respiratory symptoms, self-reported measures are usually subjective as well as sensitive to misclassification and recall bias. Objective asthma outcome assessment includes asthma diagnoses given by a doctor as well as a combination of FeNO (exhaled nitric oxide test), ISAAC questionnaire and spirometry. And only three studies assessed asthma outcomes

objectively using methods such as lung function testing using spirometry and found an association with asthma and/or lung function. (33, 35, 36).

2.5 Areas for further research relating to hormones and asthma in children.

Studies looking into the relationship between reproductive hormones and asthma-related outcomes in children and the general population are limited. More research is needed to assess testosterone's possible protective effect since this would offer insight into the underlying reason for the sex discrepancy reported in asthma. These studies were mostly cross-sectional and also lacked control for confounding factors such as smoking, environmental exposures to endocrine disruptors, and medical history such as lung infections and obesity. Longitudinal research on reproductive hormones and asthma outcomes in younger children is lacking. Moreover, most of the previous research was undertaken in high-income countries (HIC), studies in low- and-middle income countries (LMICs) are limited, and no studies have investigated this relationship in sub-Saharan Africa.

Most of the previous studies investigated sex differences or bias and asthma in adults, and there is a need for studies investigating reproductive hormones and asthma in children. There is also limited evidence on the relationship between reproductive hormones and asthma among children, especially from rural communities particularly those in low-middle income countries where various underlying susceptibility and multiple risk factors, such as environmental elements play a significant role in asthma aetiology. This study will focus on the association of reproductive hormones and asthma-related outcomes of boys in rural areas (Piketberg, Grabouw and Hex River Valley) of the Western Cape in South Africa.

Table 1: Epidemiological studies of reproductive hormones and asthma-related outcomes in children.

Publication	Study population and design	Asthma outcome measurement/s	Descriptive statistics of hormones and biomarkers	Association between reproductive hormones and asthma-related outcomes	Strengths	Limitations	Overall conclusion
Bulkhi AA, et al. 2020 Elevated Testosterone Is Associated with Decreased Likelihood of Current Asthma Regardless of Sex (35)	**-Cross-sectional** study done in the United States using the National Health and Nutrition Examination Survey (2011-2012). -7584 participants (6-80 years)	-Questionnaires were used to collect data on current asthma. -The study also used spirometry and body measurements as well as fractional exhaled nitric oxide (FeNO) data.	-Eosinophil counts -Serum testosterone was determined. **Mean:** -Asthma: 147.8 (ng/dL), -No asthma: 200.6 (ng/dL)	-For both men and women testosterone was protective against asthma (current asthma). - Serum testosterone level (log-transformed) was associated with lower odds of experiencing asthma in males (aOR= 0.89, 95%CI 0.81-0.97) and in females (OR= 0.90, 95%CI 0.79-1.04). - There were decreased odds of having current asthma by 10% in females and 11% in males for every 1-unit rise in testosterone. -There was no association between serum testosterone and current asthma among children younger than 12 years.	-Large sample size -Exposure accurately measured to minimize bias	-Did not take account of the confounding factors e.g., environmental exposures endocrine disruptors -Missing data -Recall or misclassification bias.	Higher testosterone levels were protective of current asthma controlling for sex and other correlates.
Arathimos R, et al. 2019 Genetic and observational evidence supports a causal role of sex hormones on the development of asthma (32)	-UK Biobank and the study of asthma at the Trans-National Asthma Genetics Consortium genome-wide association -Cross-sectional study (n=512) - Two-sample MR data were meta-analysed.	-Questionnaires from the Avon Study of Parents and Children (ALSPAC). -Touchscreen questionnaire in the UK Biobank participants and participants were interviewed on whether they have ever been diagnosed with asthma.	-Total testosterone (TT) -Sex hormone-binding globulin (SHBG) -Bioavailable testosterone (BT)	-Observational findings suggested that serum testosterone had a mild protective effect on asthma in adolescent boys, but there was no consistent association pattern observed with SHBG. - After 11.8 years, BT and TT had an inverse association with asthma. -For SHBG and asthma had an OR of 0.96, 1.54, 1.32, 1.18 and 1.02 at 9.9, 11.8, 13.8, 15.5 and 17.8 years respectively. -TT and asthma had an OR of 1.12, 0.85, 0.80, 0.89 and 0.71 -BT there was an OR of 1.02, 0.79, 0.86, 0.87 and 0.87	-Large sample size -Exposure accurately measured to minimize bias - A combined genetic and observational epidemiology approach was used. -Using multiple independent data sets.	-Did not take account of the confounding factors e.g., environmental exposures endocrine disruptors -Lack precision	There is some evidence that genetically increased SHBG has a protective impact on asthma, which might explain the reported asthma sex disparity.

					-It is based on a strong biological rationale		
DeBoer MD, et, al. 2018 Effects of endogenous sex hormones on lung function and symptom control in adolescents with asthma (34)	-Cross-sectional study in the USA -Recruited (n=187) children (6-18 years).	Asthma control questionnaire (ACQ6) and Lung function	Testosterone (ng/dL) Dehydroepiandrosterone sulphate (DHEA-S), (μg/dL) SHBG 10 (nmol/L). Progesterone (ng/mL) and Oestrogen	Lung function was positively associated with DHEA-S in males (6–18 years). The pre-BD FVC % (β= 8.33, p = 0.01), pre and post-bronchodilator FEV1% (β = 8.05; p = 0.01), FEV1% (β = 8.82, p = 0.008), respectively had strongly positive coefficients. The ACQ6 indicated a 4-week symptom improvement with increased DHEA-S (β = − 0.59, p = 0.007), Androgen levels were associated with better lung function and ACQ6 in males, while estrogen was negatively associated with lung function in females.	-Large sample size -Exposure accurately measured to minimize bias	-Did not take account of the confounding factors e.g., smoking, environmental exposures endocrine disruptors. -Lower statistical power. -Recall or misclassification bias. -Selection bias.	The androgen surge that happens during puberty in both females and males is expected to have a protective effect on lung growth, but in females, estrogens may have negative effects that last into adulthood.
Zhou Y, et al. 2017 Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children (36)	Northern Taiwan **-Cross-sectional study.** -The Genetic and Biomarkers study for Childhood Asthma from 2009-2010. -231 asthmatic and 225 non-asthmatics children (10–15 years)	Physician-diagnosed asthmatics (past year).	Performed serum testosterone (nmol/L) **Median**-with asthma= 11.47 ± 7.72 (13.90), without asthma= 9.15 ± 8.99 (2.94). Estradiol (pmol/L) **median** with asthma= 178.67 ± 70.50 (166.72), without asthma= 151.47 ± 65.05 (140.55)	- In asthmatics, PFAS exposure was found to be negatively associated with testosterone levels and positively associated with estradiol levels. PFAS exposure was associated with asthma after controlling for hormone levels and this was consistently stronger among children (both males and females) with higher estradiol levels. Among girls, there were significant interactions between estradiol and PFASs, for PFOS (p = 0.026) and PFNA (p = 0.043). Testosterone significantly reduced the effect of PFOS on asthma across children (both males and females).	-Large sample size -Exposure accurately measured to minimize bias	-Did not take account of the confounding factors e.g., smoking, -Recall or misclassification bias. -Selection bias.	Asthma is affected by exposure to PFAS and hormone interaction among children. Testosterone significantly reduced the effect of PFOS on asthma across children (both males and females).

Table 2: Epidemiological studies of reproductive hormones and asthma-related outcomes in Adults

Publication	Study population and design	Asthma outcome measurement/s	Reproductive hormones and other outcomes measured	Results	Strengths	Limitations	Conclusion
Han YY, et al. 2020 Sex Steroid Hormones and Asthma in a Nationwide Study of U.S. Adults (24)	US A **cross-sectional** study of 7,615 adults (3,953 men and 3,662 women) aged 18-79 years	-Current asthma "Has a doctor or other health professional ever told you that you have asthma?" and "Do you still have asthma?"	Serum total testosterone (TT) nmol/L: men -14.7, 14.2, women-0.85, 0.75 Estradiol: men-91.6, 89.0, women-216.3, 204.4 SHBG (sex hormone-binding globulin) *Empirical-free testosterone (EFT) formula: men-209.2, 198.3, women-7.1, 6.1	-Association between asthma and free testosterone levels in women (odds ratio [OR] for the fourth quartile [Q4] vs. Q1, 0.56; 95% [CI], 0.39-0.80). - Association between asthma and increased free testosterone (OR for Q4 vs. Q1, 0.59; 95% CI, 0.37-0.91) - Association between asthma and estradiol levels in obese women (OR for Q4 vs. Q1, 0.43; 95% CI, 0.23-0.78), in nonobese men (OR for Q4 vs. Q1, 0.44; 95% CI, 0.21-0.90).	-Large sample size. -Exposure accurately measured to minimize bias. -The study focused on the effect of testosterone in both females and males.	-Did not take account of the confounding factors e.g., smoking, environmental exposures endocrine disruptors. -Association could not be detected as a result of reduced statistical power -Recall or misclassification bias. -Selection bias.	Sex hormones play a role in known sex differences in asthma in adults. The findings indicate that this relationship between asthma and sex hormones is also modified by obesity.
Han Yan, et al. 2020 Serum-free testosterone and asthma, hospitalisations and lung function in British adults (33)	UK **Cross-sectional** study. Recruited and interviewed 256 419 participants (40-69 years) between 2006 and 2010.	Questionnaire: Asthma Curent wheeze Spirometry	Serum total testosterone Estradiol Sex hormone-binding globin The empirical free testosterone (EFT) formula was used to calculate free testosterone levels.	), free testosterone levels above Q1(lowest quartile) were associated with reduced odds of asthma compared to Q4 (highest quartile) in both men and women (aOR=0.67, 95%CI: 0.64–0.71) and men (aOR=0.87, 95% CI=0.82-0.91). Among asthmatic participants (both women and men), higher levels of free testosterone were also associated with decreased odds of current wheezing. In addition, higher levels of free testosterone were found to be protective of asthma hospitalization (≥1) in women with asthma. In contrast, increased levels of free testosterone were weakly associated with lung function (FVC) in	-Large sample size -Exposure accurately measured to minimize bias.	-Selection bias -Might be difficult to generalize findings to the local population. -Recall bias and misclassification	Increased free testosterone levels were associated with reduced odds of current wheezing and asthma in both sexes, increased FVC and FEV1 in males, and reduced odds of being hospitalised for asthma in women.

				women but there was a positive association with lung function (FEV1 and FVC) in men.			
Bulkhi AA, et al. 2020 Elevated Testosterone Is Associated with Decreased Likelihood of Current Asthma Regardless of Sex (35)	-**Cross-sectional** study done in the United States using the National Health and Nutrition Examination Survey (2011-2012). -7584 participants (6-80years)	Questionnaires were used for Current asthma data -Study also used spirometry and body measurements as well as fractional exhaled nitric oxide (FeNO) data	Eosinophil counts Serum testosterone was determined. **Mean:** Asthma 147.8(ng/dL, No asthma 200.6 (ng/dL	-In men, serum testosterone was associated with reduced odds of current asthma (aOR= 0.8, 95%CI 0.81-0.97), but weakly associated with decreased odds of current asthma in women (aOR= 0.90, 95%CI 0.79-1.04). - There is decreased odds of having current asthma decreased by 10% in females and 11% in males for every 1-unit rise in testosterone	-Large sample size -Exposure accurately measured to minimize bias	-Did not take account of the confounding factors e.g., environmental exposures endocrine disruptors -Missing data -Recall or misclassification bias.	Higher testosterone levels were protective of current asthma.

3. Significance and relevance

Asthma tends to be more prevalent and more severe among boys compared to girls during childhood, however, asthma prevalence shifts based on sex differences after puberty, with asthma being more prevalent and severe in women than men. The reason for these sex differences in asthma prevalence is still unknown, but there is laboratory evidence that reproductive hormones affect asthma prevalence and severity. There is limited epidemiological evidence related to this and it is mostly in adults. Moreover, all other studies were done in non-African settings and this study will investigate whether the evidence is the same in Africa. Children, especially from rural communities particularly those in low-middle income countries have various underlying susceptibility and multiple risk factors, as well as different environmental exposures which play a significant role in asthma aetiology. There is a large research gap in children, especially in Africa. A study investigating the relationship between reproductive hormones and asthma in South African children (6-18years) would therefore contribute to addressing this research gap and may also inform strategies to asthma prevention.. (32).

4. Aim and objectives

Aim

The study investigated the association between reproductive hormones and asthma-related outcomes in male children residing in the rural Western Cape province of South Africa.

Objectives

1. To describe the socio-demographic characteristics of the study participants and asthma risk factors
2. To characterize the reproductive hormones and SHBG of the study participants
3. To determine the prevalence of asthma-related outcomes (current wheezing, asthma symptoms score and parental reporting asthma) in these children.
4. To investigate the association between asthma-related outcomes and reproductive hormone levels, accounting for observed confounders.

5. Research question

Are reproductive hormone levels associated with asthma-related outcomes among rural male children in the Western Cape province of South Africa?

6. Study Methodology

6. 1 Study Design

This is a sub-analysis of a cohort study conducted among 1000 school-going children from a rural setting in the Western Cape province of South Africa with the baseline measurements conducted in 2017 and the follow-up measurements in 2019. This current study is a cross-sectional analysis focusing on the boys (n = 470) who participated in the baseline study as hormone levels were not measured among girls. The main study investigated the neurobehavioral and reproductive health effects of exposure to environmental pesticides in the Western Cape province (37). During the two years, both the caregivers and children were interviewed. Additionally, the child asthma-related outcomes were obtained from the questionnaire administered to the parents or caregivers of these boys are part of this sub-study. The thesis involves a secondary analysis of data. The student did not take part in the design of

the study and in conducting field work, but was primarily involved in the data cleaning, coding management and analysis as well as the write up.

6.2 Study Population and sampling

The study population for this analysis were boys (aged 6-18) residing in the Western Cape rural areas of South Africa. Of the 1000 school-going children recruited in 2017, before the baseline study, who attended schools in three agricultural-intensive rural areas and non-agricultural neighbouring areas, Piketberg, Hex River Valley, and Grabouw, (N=470) were boys (32, 37). The sampling frame used in the main study was schools in the three areas of study (Piketberg, Grabouw and Hex River Valley). From the 32 local schools in these three research areas, only combined, intermediate and primary schools were approached for recruitment. First, principals as well as governing authorities of respective schools were engaged, and out of the 32 existing schools, seven schools agreed to participate. Then guardians or parents of all school pupils in grades 2-9 in these participating schools were sent information sheets describing the research and the school's involvement, along with permission letters. Home visits were then conducted to obtain consent from all guardians or parents that responded positively to the study invitation. Participants were then selected to obtain an approximately equal number of children by area, age, and sex, those living on farms and those in the nearby town. When the number of approving guardians or parents exceeds the number of children targeted for a particular category, stratified random sampling was used to select the children.

6.3 Sample size and Power calculation

Power for a logistic regression with a continuous predictor variable was calculated using G-Power version 3 software. Based on previous studies looking at the association between asthma and testosterone (24,33,35), the current study assumed an odds ratio of 0.77 for a one standard

deviation increase in testosterone levels. The study further assumed a lognormal distribution for the main predictor and used a sample size of 470 and a 95% confidence interval to calculate the desired statistical power. Based on these calculations the study sample should have adequate power (89.7%) to demonstrate the associations of interest.

Inclusion Criteria: The research included 6–18-year-old schoolboys from rural agriculturally intensive areas in the Western Cape, South Africa.

Exclusion Criteria: The sample excluded all boys older than the age of 18 years. All boys without asthma outcome information, and hormone level measurements were also excluded from the analysis.

6.4 Study Instruments

6.4.1 Questionnaires

Standardized questionnaires were administered by interviewers to the parents or guardians to collect information about their children's socio-demographic factors, general health, allergy, and asthma symptoms (Appendix 1). Home visits were done by trained fieldworkers to interview parents or guardians completing an hour-long questionnaire. Demographic information was also similarly obtained from the children attending school during the baseline survey using the participant questionnaire (Appendix 2). The Open Data Kit (ODK) application was installed to capture all individual interviews on mobile devices using a structured questionnaire. Fieldworkers were trained on how to conduct interviews using the ODK software and mobile phones through special workshops.

The interviews were held in the participant's preferred language (Afrikaans and Xhosa). The questionnaire was translated into the participant's preferred language and then back into English. The *guardian questionnaire* asked about the child's demographics, birth weight,

general medical history, and pesticide exposure in the environment. The asthma symptoms and medication questionnaire were an abbreviated version of The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Appendix 1) that included the following asthma-related questions:

- Has the [child] ever had wheezing or whistling in the chest at any time in the past?
- If yes (to 5.17), has your child had wheezing or whistling in the chest in the past 12 months?
- If yes (to 5.17) how many attacks of wheezing has the child had in the past 12 months?
- In the past 12 months, has the wheezing ever been serious enough to limit your **child's** speech to only one or two words at a time between breaths?
- In the past 12 months, how often on average, has your child's sleep been disturbed due to wheezing?
- In the past 12 months, has your **child's** chest sounded wheezy during or after exercise?
- Has your **child** ever had asthma? or Self-reported asthma?
- In the past 12 months, has your **child** had a dry cough at night, apart from a cough associated with a cold or chest infection?

In the **participant questionnaire,** demographic and confounding questions included:

- Participant age
- Participant sex
- Are you currently living on a farm?
- Have you ever tried to smoke?

6.4.2 Serum reproductive hormone levels

During the examinations, a qualified nurse collected whole blood samples (5 ml) from 470 male participants in the early hours (before 9 am). All blood samples obtained at the study location were sent for analysis within 24 hours to Groote Schuur Hospital's National Health Laboratory Services (NHLS) laboratory in Cape Town. Serum reproductive hormones including testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2) as well as SHBG were measured in blood samples using electrochemiluminescence immunoassays (ECLIA) on a Cobas e601 module of the MODULAR ANALYTICS E170 Roche system. The same tests were used to compare hormones to age-related laboratory guidelines.

As part of the Hypothalamic Pituitary Gonadal Axis, testosterone, FSH, LH, and E2 are markers of reproductive function in males. Significant changes in these hormone levels may indicate the potential anti-androgenic, estrogenic, or other endocrine-disrupting effects in vivo (38). SHBG was measured to determine the free testosterone concentration and as an independent predictor as indicated in the literature. We regarded the following as reference values Testosterone (T), (Normal: 2.49–30.6 nmol/L), Luteinizing hormone (LH), (Normal: <.1 to 3.7 IU/l), Follicle-stimulating hormone (FSH), (Normal: <.1 to 8.6 IU/l), Estradiol, (< 20–40 pg/ml) and SHBG (6–45 nmol/l) (39, 40)

Pilot Study

Two pilot studies were conducted after fieldworkers received adequate training on both the instruments and the research itself. The questionnaires' content and flow were evaluated in the first pilot study, which involved ten individuals (five girls and five boys). The second pilot study tested both measurements and the workflow on the first 100 participants.

7. Statistical analysis and data management

7.1 Data management and quality assurance

The Open Data Kit (ODK) application was installed on mobile devices and used for all individual interviews making use of a standardised questionnaire. Standard operating procedures (SOPs) were established, and all field staff was trained for more than a week before the collection of data which continued at several stages during the project to ensure data collection accuracy. After the researcher received the data, it was kept confidential in a password-protected database.

Predictor variables

The predictor variables that were used include serum concentrations of:

- Total Serum Testosterone - nmol/L
- Free Testosterone - (testosterone/SHBG ratio)
- Follicle-stimulating hormone (FSH) - IU/l
- Luteinizing hormone (LH) - IU/l
- Estradiol (E2) - pg/ml
- Sex-hormone binding globulin (SHBG) - nmol/L.

These predictor variables were analysed as both continuous variables and as categorical variables with quartiles serving as cut-offs.

Dependent variables: Asthma-related outcomes

Parental-reported asthma was measured based on a positive response to the following question: '*Has your child ever had asthma?',* with the negative response defined as not having asthma. *Current wheezing* was defined based on the experience of wheezing or whistling in the

chest in the past 12 months and with the negative response defined as not having current wheezing. We also defined the third outcome measure, *Asthma symptom score ≥2* which was calculated using the total of the responses (0=no, 1=yes) to four questions looking at symptoms in the last 12 months. These included: one or more attacks of wheezing, the child's sleep being disturbed due to wheezing; the child's chest sounding wheezy during or after exercise and wheezing serious enough to limit your child's speech to only one or two words at a time between breaths, as has been described in previous studies (41). These four asthma-related symptoms were combined to create a binary variable (≥ 2 symptoms vs 0-1 symptom). Having 0-1 asthma-related symptom was considered as 'less likely' to have asthma while ≥ 2 symptoms were considered as 'more likely to have asthma.

Confounder variables

The following potential confounders were considered in the analyses: Age, Body Mass Index (BMI), history of allergy or family history of allergy (as a marker for atopy), passive smoking, child current smoking, previous history of lung disease, low birth weight, fetal alcohol syndrome and living on a farm (pesticide exposure).

7.2 Statistical Analysis

Data was analysed using R software (version 4.0.3 - 2020). Firstly, each variable was assessed for the extent and type of missingness and any other variable issues. Descriptive analysis was conducted to assess the population's demographic characteristics, medical history, reproductive hormones, and the distribution of asthma-related outcomes across these covariates. In some cases, box-and-whisker plots were used for distribution assessments. For hormones, values below the level of detection (LOD) were converted using the formula LOD/square root of 2. Typically, biological concentrations are often not normally distributed therefore we took the natural logarithm of each hormone before including them in the models.

The differences between participants with and without asthma-related outcomes were assessed by a univariate analysis using the chi-square test. Univariate logistic regression was used to investigate the bivariate associations between asthma-related outcome variables and various predictor variables. Furthermore, multivariate logistic regression models were then fitted to assess the association between asthma-related outcomes and reproductive hormones controlling for potential and known confounders such as obesity, atopy, passive smoking, child current smoking and previous history of lung disease. The selection of confounders was done *apriori* based on the literature to control for all forms of confounding and also based on the bivariate analysis. A sensitivity analysis of asthma-related outcomes and hormone concentrations dichotomised at the 75th percentile was done.

Correlation between the hormones was used to choose whether to do a single-predictor model if the hormones were highly correlated or a sensitivity analysis called the multi-predictor model if there was a low correlation. This is important because no single hormone exists in isolation and as they are present together in the body, thus is important to assess their association with the outcome in a multi-predictor model. Concentration-response curves for the association between the log-transformed sex hormone levels and asthma-related outcomes were assessed using the lowest measured hormone level in the data as the counterfactual (i.e., the theoretical minimum).

Table 3: Dummy table of the Demographic, characteristics, anthropometric measurements, exposures at birth and medical history of boys residing in rural Western Cape province stratified by age group

		Age Category (years)			
Demographic/Host Characteristics	**Number**	**6 to 10, (N_1 = 122)**	**11 to 14, (N_2 = 176)**	**15 to 18, (N_3 = 16)**	**Overall, N = 314**
Height (cm) - median (IQR)					
Weight (kg) - median (IQR)					
BMI - median (IQR)					
Gestational age at birth (weeks) - median (IQR)					
Gestational age < 37 weeks					
Low birth weight (<2.5 kg)					
Family history of allergy (n_1=74, n_2=99, n_3=8)					
Current smoker					
Maternal smoking during pregnancy					
Household ETS exposure					
Fetal alcohol syndrome					
Previous tuberculosis					
Previous repeated lung infections					
Living on a farm					

Results are shown as median (IQR) for continuous variables, and as N (%) for binary variables.

Dummy figure: Graphs for correlation between the hormones: Total Serum Testosterone (TST), Free Testosterone (FT), Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Estradiol (E2) and Sex-hormone binding globulin (SHBG).

Table 4: Dummy table for Asthma-related outcomes reported in the past year for boys residing in the rural Western Cape province stratified by age group.

Asthma-related outcomes	Age Category (years)				
	Number	6 to 10 (n_1 = 122)	11 to 14 (n_2 = 176)	15 to 18 (n_3= 16)	Overall, N = 314
Current wheeze					
Wheeze disturbing sleep *					
Wheeze during exercise *					
Wheeze limiting speech *					
Wheezing attack/s *					
Asthma Symptom Score (≥2)					
Parental-reported asthma					

Table 5: Dummy table for reproductive hormone levels of boys residing in the rural Western Cape province stratified by age group.

Reproductive hormones	Age Category (years)				
	Total	6 to 10 (n_1 = 122)	11 to 14 (n_2 = 176)	15 to 18 (n_3= 16)	Overall, N = 314
Testosterone – nmol/L -median (IQR)					
Testosterone categories:					
- Low (<2.3-<6.5 nmol/L)					
-Normal (<0.09-≤30.6 nmol/L)					
- High (≥30.6nmol/L)					
Free testosterone (testosterone/SHBG ratio) median (IQR)					
LH– IU/L -median (IQR)					
LH categories:					
-Low (<0.1 IU/L)					
-Normal (≥0.1-≤4 IU/L)					
-High (>4 IU/L)					
FSH -IU/L- median (IQR)					
FSH categories:					
- Low (≤0.1 IU/L)					
- Normal (≥0.1-≤8.6 IU/L)					
- High (>8.6 IU/L)					
Estradiol (E2) – pmol/L median (IQR)					
Estradiol (E2) categories					
- Low					
-Normal (<40)					
- High (>40)					
SHBG - nmol/l median (IQR)					
SHBG categories					
-Low (<18.3 nmol/l)					
-Normal (≥18.3-≤54.1 nmol/l)					
-High (>54.1 nmol/l)					

Table 6: Dummy table displaying the association between host characteristics and asthma-related outcomes among boys in unadjusted logistic regression models (N=470)

	Current Wheeze	Asthma Symptom Score ≥2	Parental-reported Asthma
Host Characteristic	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Age (year)			
Age categories (6 to <11- reference)			
- 11 to <15			
- 15 to <19			
Height (cm)			
Weight (kg)			
BMI			
Current smoking			
Maternal smoking during pregnancy			
Household ETS exposure			
Low birth weight (<2.5 kg)			
Gestational age <37 weeks			
Living on a farm			
Family history of allergy			

OR-Odds ratio
95 % CI- Confidence Interval

Table 7: Dummy table displaying the relationship between sex hormone levels and asthma-related outcomes among boys in unadjusted logistic and adjusted multivariate regression models (N=470)

Hormones	Models	Current wheeze	Asthma symptom score ≥2	Parental-reported asthma
		OR (95%CI)	OR (95%CI)	OR (95%CI)
Testosterone (nmol/L)	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			
Free Testosterone	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			
Luteinizing hormone (IU/L**)**	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			
Follicle-stimulating hormone (IU/L**)**	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			
Estradiol (pg/ml)	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			
Sex-hormone binding globulin (nmol/l**)**	Unadjusted model			
	Adj model *(a)*			
	Adj model *(b)*			

Model (a) – Model adjusted for age (year), household member smoking, living on a farm, low birth weight and mother smoking during pregnancy; Model (b) - adjusted for all the covariates in model (a) in addition to other potential confounders that include, BMI, current smoking, and family history of allergy; All categorical covariates with missing values were coded as 9 to persevere the power of the study

8. Ethics

This research was carried out in accordance with the Human Research Ethics Committee of the University of Cape Town.

Ethics approval and consent to participate.

The ethics protocols for the main study were approved by the University of Cape Town's Human Research Ethics Committee (HREC reference number: 234/2009) (Appendix 6). Consent to conduct the study was obtained from the Department of Education. Consent to participate in the study was sought from the school principals and their boards. The guardians/parents of the children were invited to participate by a letter from the school once they had given their consent. Participants and their parents or guardians were asked to provide signed permission and written consent/assent to take part in the study. Before any data was collected, the participant learner was asked for assent after receiving their parent's consent (Appendix 8, 10). For the protection and benefit of the research participants, key ethical standards focused on the Helsinki Declaration (42) were considered.

The study involved physical examinations and a needle prick however, precautions were implemented to protect all participants to have little physical discomfort as possible during the collection of blood samples. All data about study participants will be kept confidential in a secure device and location. The benefit of the study includes helping with the creation of measures and the possible implementation of health policies to protect farm employees and their families from the harmful effects of being exposed to agricultural pesticides that are hormonally active. The sub-study benefits include the creation of knowledge that might help with the advancement of therapeutic interventions and strategies for the prevention of asthma-related outcomes.

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PART B: MANUSCRIPT

Preparation for submission

Journal: Prepared to be submitted for publication in the peer-reviewed journal, The Journal of Allergy and Clinical Immunology: In Practice, and author instructions have been adhered **apart from** the cover page with co-author details which are not included in the dissertation paper.

Title: The relationship between reproductive hormones and asthma-related outcomes in boys residing in the rural Western Cape

Competing financial interest declaration: None

Student: Mandy Sigametsi Phuti

Abstract

Background: Asthma is more prevalent and severe among boys but this pattern reverses after puberty. It has been suggested that reproductive hormones may play a role in explaining these sex differences after puberty, but the evidence is still limited especially for children living in low- and middle-income countries.

Objective: This study investigated the association between reproductive hormones and asthma-related outcomes among boys residing in a rural setting.

Methods: A cross-sectional study of 470 boys (6-18 years), residing in the rural Western Cape province of South Africa was conducted. General questionnaires were administered to the boys and their caregivers including an abbreviated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Testosterone (TST and FT), luteinizing hormone (LH), serum follicle-stimulating hormone (FSH), oestradiol and serum hormone-binding globulin (SHBG), were assessed using electrochemiluminescence immunoassays. A total of 314 (66.8%) boys had valid asthma outcome information and hormone level measurements that were analysed further. Multivariate logistic regression models were used to assess the relationship between asthma and measured endocrines levels.

Results: The mean age of the boys was 11.2 (Standard deviation -1.7 years). Current wheeze (CW), asthma symptom score ≥ 2 (ASS) and parental reported asthma (PA) prevalence were 6.1%, 6.7% and 8.0%, respectively. In the adjusted multivariate logistic regression models, total serum testosterone (TST) levels were negatively associated with CW (OR=0.66, 95% CI: 0.45-0.98), ASS (OR=0.64, 95% CI: 0.43-0.95) and PA (OR=0.86, 95% CI: 0.59-1.25). Free testosterone (FT) levels were similarly negatively associated with all three asthma-related outcomes. Similar associations were also found for LH and FSH, but there were no clear associations for estradiol and SHBG with asthma-related outcomes. The concentration-

response curves confirmed that higher levels of testosterone (TST and FT) were associated with reduced risk of asthma-related outcomes.

Conclusions: This study provides evidence that increasing testosterone levels are associated with reduced asthma risk among rural boys in South Africa.

Keywords: Asthma, sex disparities, boys, reproductive hormones, total serum testosterone, free testosterone, estradiol, sex hormone binding globulin, androgens, rural settings

Highlights

- **What is already known about this topic?** Reproductive hormones may explain the sex differences in asthma.
- **What does this article add to our knowledge?** Increasing levels of both total and free testosterone are associated with a reduced risk of asthma in boys from rural communities in an African setting.
- **How does this study impact current management guidelines?** Differences in sex hormone levels can affect the phenotypic manifestations of asthma in children and modify their response to treatment.

Abbreviations:

TST	Total serum testosterone
FT	Free testosterone
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
E2	Estradiol
SHBG	Serum hormone-binding globulin
BMI	Body mass index
ISAAC	International Study of Asthma and Allergies in Childhood

1. Introduction

Childhood asthma is a global public health problem (1), and its prevalence is increasing in both urban and rural settings in South Africa (2, 3). Previous studies show that there are sex differences in asthma prevalence among children and that these differences change over time (4, 5). Boys are twice as likely to develop asthma than girls of a similar age before puberty (3), but this reverses after puberty with asthma prevalence higher in girls (4, 6). This increased asthma prevalence in girls after puberty has been partly attributed to reproductive hormones, which have an effect on asthma pathogenesis (7), although the mechanism is not well understood. Increased asthma symptom severity in women during menstruation, pregnancy, and menopause, could also be associated with changing reproductive hormone levels (3).

There are limited epidemiological studies on the association between reproductive hormones and asthma, with most studies conducted among adults in high-income settings. In general, these studies found that increased androgen levels such as testosterone were associated with reduced asthma symptoms in both males and females (4, 7-11). Furthermore, studies in mouse models show that androgens, decrease adaptive and innate immunological responses, while progesterone and estrogen may enhance Th2 allergic inflammation of the airways in asthma (12). Testosterone has been shown to also have immunosuppressive properties and is potentially effective against asthma-causing immunoinflammatory mechanisms among males (3, 13). In addition to testosterone, SHBG, a glycoprotein that is a major regulator and transporter of androgens and estrogen, has also been reported to have asthma-protective effects (14)

Overall, there is limited evidence on the relationship between reproductive hormones and asthma among children living in low-and middle-income countries, where various underlying

susceptibility and multiple environmental risk factors play a significant role in asthma manifestation. This current study sought to investigate the association between reproductive endocrines (the hormones, testosterone, and LH, FSH and oestradiol that and SHBG that are all part of the pituitary-gonadal axis) and asthma-related outcomes in young boys residing in a rural setting in South Africa.

2. Methods

2.1. Study Design and Population

This study is a sub-analysis of data collected from a cohort study conducted among school children (n=1000) residing in the rural Western Cape province of South Africa (Piketberg, Grabouw and Hex River Valley). The baseline measurements were conducted in children that were attending schools in 2017 and the follow-up was done in 2019. During these two years, a questionnaire on childhood asthma-related outcomes was also administered to caregivers at their homes. The main cohort study investigated reproductive and neurobehavioral health effects associated with environmental pesticide exposure in farm areas in the Western Cape province (15). The current sub-study is a cross-sectional analysis of data collected from boys aged 6-18 years (n = 470) that participated in the baseline study, and whose caregivers responded to the home-based survey.

2.2. Data collection

Standardized questionnaires were administered to parents or guardians through home visits conducted by trained interviewers to record information on the children's socio-demographic factors, general health status and the presence of allergy and asthma-related symptoms (Appendix 1). Demographic information was also obtained from the children at school using the *participant questionnaire* (Appendix 2). The Open Data Kit (ODK) application was used to capture interview information using mobile devices. The interviews were held in the

participant's preferred language (Afrikaans and Xhosa) using a questionnaire that was translated into these languages and then back-translated to ensure reproducibility prior to it being used. The *asthma symptom and medication questionnaire* was compiled from an abbreviated version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire.

Serum reproductive hormone levels: A qualified nurse collected early morning whole blood samples (5 ml) from 470 boys attending schools. All blood samples obtained at the study location were sent for analysis within 24 hours to Groote Schuur Hospital's National Health Laboratory Services (NHLS) laboratory in Cape Town. Serum reproductive hormones including testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2) as well as SHBG were measured in blood samples using electrochemiluminescence immunoassays (ECLIA) on a Cobas e601 module of the MODULAR ANALYTICS E170 Roche system. The same tests were used to compare hormones to age-related laboratory guidelines. SHBG was measured to determine the FT concentration. The following reference values were used: for TST –(Normal: 2.49–30.6 nmol/L), Luteinizing hormone (LH) –(Normal: <0.1-3.7 IU/l), Follicle-stimulating hormone (FSH) –(Normal: <0.1-8.6 IU/l), Oestradiol –(< 20-40 pg/ml) and SHBG –(6-45 nmol/l) (16, 17) .

2.3. Measures

This study had three main asthma-related outcome variables namely: (i) p*arental-reported asthma* defined as a positive response to the following question: *Has your child ever had asthma?* (ii) c*urrent wheezing*–defined as having had wheezing or whistling in the chest in the past 12 months, and (iii) *asthma symptom score (≥ 2)* –a binary indicator (≥ 2 symptoms vs 0-1 symptom) computed by the sum of answers to four questions of asthma-related symptoms reported in the past 12 months. These included: *how often on average, has your*

child's sleep been disturbed due to wheezing; *has your child's chest sounded wheezy during or after exercise; has the wheezing ever been serious enough to limit your child's speech to only one or two words at a time between breaths*; *and how many attacks of wheezing has the child had in the past 12 months,* as has been described in previous studies (18).

The independent variables of interest in this study were all continuous variables including serum concentrations of TST - nmol/L, FT - (testosterone/SHBG ratio), Luteinizing hormone (LH) - IU/L, FSH - IU/L, Estradiol (E2) - pg/ml and Sex-hormone binding globulin (SHBG) - nmol/L.

Potential confounders that were considered included: age, body mass index (BMI), history of allergy or family history of allergy (as a marker for atopy), passive smoking, child currently a smoker, previous history of lung disease, low birth weight (LBW), foetal alcohol syndrome (FAS), and living on a farm (pesticide exposure).

2.4. Statistical analysis

Data was analysed using R software (version 4.0.3 - 2020). The preliminary analysis included descriptive analysis and bivariate logistic regression analysis between asthma outcomes with host characteristics and other covariates. Hormones values below the level of detection (LOD) were converted using the formula LOD/square root of 2. Due to the skewness of the data, the natural logarithm concentration of each hormone was included in statistical models. Multivariate logistic regression modelling was used to assess the associations between asthma-related outcomes and the various endocrine variables. The first multivariate model (model (a)) was adjusted for confounders which were found to be statistically significant during bivariate analysis ($p < 0.05$). The second model (model (b)) was adjusted for all the covariates used in model (a) in addition to other known *apriori* confounders such as BMI, a child currently a smoker and a family history of allergy. Concentration-response curves for

the association between the log-transformed sex hormone levels and asthma-related outcomes were assessed using the lowest measured hormone level in the data as the counterfactual (i.e., the theoretical minimum). A sensitivity analysis of asthma-related outcomes and hormone concentrations dichotomised at the 75th percentile was done.

2.5. Ethics approval and consent to participate.

This study was carried out in accordance Helsinki Declaration (19). The University of Cape Town's Health Sciences Human Research Ethics Committee approved the main study (HREC reference number: 234/2009) and this sub-study (HREC reference number: 722/2021). The Department of Education also approved the study in schools. Consent to participate in the study was sought from the school principals, parents or guardians, and assent from respective children.

3. Results

From the 470 boys enrolled in the main study, a total of 314 boys aged between 6-18 years with complete asthma outcome and hormone level data were included in this current study. Their mean age was 11.2 years ± SD (1.71). There were no significant differences between participants retained and those excluded from the analysis with respect to demographic characteristics such as age and weight. More than half the participants had low birth weight and 73 % reported a family history of allergy, with the latter prevalence higher in the two younger age group categories. About a quarter of the boys in the older age group were current smokers. More than a third of mothers reported smoking during pregnancy (38%). Almost half of the participants lived on a farm.

Table 1. Demographic characteristics, anthropometric measurements, exposures at birth and medical history of boys residing in rural Western Cape province stratified by age group.

Demographic/Host Characteristics	Number	Age Category (years)			
		6 to 10, (N_1 = 122)	11 to 14, (N_2 = 176)	15 to 18, (N_3 = 16)	Overall, N = 314
Height (cm) - median (IQR)	309	131 (126, 137)	145 (138, 152)	165 (152, 168)	140 (132, 149)
Weight (kg) - median (IQR)	309	27 (24, 31)	36 (31, 43)	50 (44, 54)	33 (27, 40)
BMI - median (IQR)	309	15.3 (14.5, 16.2)	16.7 (15.3, 19.1)	18.9 (18.3, 20.6)	16 (14.9, 18.4)
Gestational age at birth (weeks) - median (IQR)	134	38 (36, 40)	38 (36, 40)	36 (35.5, 37.5)	38 (36, 40)
Gestational age < 37 weeks (n_1=44, n_2=83, n_3=7)	134	21 (48%)	36 (43%)	5 (71%)	62 (46.0%)
Low birth weight (<2.5 kg) (n_1=91, n_2=137, n_3=14)	242	65 (71.4%)	85 (62.0%)	11 (78.6%)	161 (66.5%)
Family history of allergy (n_1=74, n_2=99, n_3=8)	181	55 (74.4%)	74 (74.7%)	3 (37.5%)	132 (72.9%)
Current smoker	314	10 (8.2%)	29 (16.5%)	4 (25.0%)	43 (13.7%)
Maternal smoking during pregnancy (n_1=120, n_2=172)	308	47 (39.2%)	69 (40.1%)	1 (6.2%)	117 (38.0%)
Household ETS exposure (n_1=121, n_2=170)	307	75 (62.0%)	112 (65.9%)	5 (31.3%)	192 (62.5%)
Fetal alcohol syndrome	314	4 (3.3%)	0 (0.0%)	0 (0.0%)	4 (1.3%)
Previous tuberculosis	314	2 (1.6%)	3 (1.7%)	0 (0.0%)	5 (1.6%)
Previous repeated lung infections	314	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.3%)
Living on a farm	314	47 (38.5%)	81 (46.0%)	8 (50.0%)	136 (43.3%)

n_1, n_2, and n_3 represent the number in each age group based on the number of respondents to the specific question (variable) which may defer from the respective N1, N2, and N3; ETS: environmental tobacco smoke at home. Height, weight, and BMI had 5 missing values; Gestational age had 180 missing values (no road to health cards); Low birth weight had 72 missing values (no road to health cards); Family history of allergy had 133 missing values; Maternal smoking during pregnancy had 6 missing and Household ETS exposure had 7 missing values.

While the overall prevalence of current wheezing, wheezing attacks, and asthma symptom score ≥2 was 6.1%, 7.6% and 6.7% respectively these were higher in the two older age groups compared to the younger age group (Table 2) However, parental-reported asthma (8%) was highest in the youngest age group compared to the older age groups.

Table 2. Asthma-related outcomes reported in the past year for boys residing in the rural Western Cape province stratified by age group.

Asthma-related outcomes	Age Category (years)				
	Number	6 to 10 (n_1 = 122)	11 to 14 (n_2 = 176)	15 to 18 (n_3= 16)	Overall, N = 314
Current wheeze	314	4 (3.3%)	13 (7.4%)	2 (12.5%)	19 (6.1%)
Wheeze disturbing sleep *	314	3 (2.5%)	13 (7.4%)	1 (6.2%)	17 (5.4%)
Wheeze during exercise *	314	8 (6.6%)	15 (8.5%)	0 (0.0%)	23 (7.3%)
Wheeze limiting speech *	314	1 (0.8%)	5 (2.8%)	0 (0.0%)	6 (1.9%)
Wheezing attack/s *	314	5 (4.1%)	17 (9.7%)	2 (12.5%)	24 (7.6%)
Asthma Symptom Score (≥2)	314	5 (4.1%)	15 (8.5%)	1 (6.2%)	21 (6.7%)
Parental-reported asthma	314	11 (9.0%)	13 (7.4%)	1 (6.2%)	25 (8.0%)

*Asthma symptom score derived from the sum of positive responses to the marked variables

As expected, the mean FSH, LH, FT and TST hormone levels increased across age group categories (Table 3 and Figure S2-4). All testosterone and estradiol levels were below the detection limit in the youngest age group category. More than 90% of samples for SHBG levels, 10.2-11% for testosterone, and estradiol levels, and 3.2-3.8% for FSH and LH levels were higher than the normal range. All measured hormone levels were significantly positively correlated with each other and negatively correlated with SHBG (Table S2).

Table 3. Reproductive hormone levels of boys residing in the rural Western Cape province stratified by age group.

Reproductive hormones	Total	Age Category (years)			
		6 to 10 (n_1 = 122)	11 to 14 (n_2 = 176)	15 to 18 (n_3= 16)	Overall, N = 314
Total Serum Testosterone – nmol/L -median (IQR)	314	0.1 (0.1, 0.1)	0.7 (0.1, 5.9)	11.2 (6.2, 15)	0.1 (0.1, 3.3)
Testosterone categories:	314				
- Low (<2.3-<6.5 nmol/L)		0 (0.0%)	43 (24.4%)	4 (25.0%)	47 (15.0%)
-Normal (<0.09-≤30.6 nmol/L)		114 (93.4%)	109 (62.0%)	12 (75.0%)	235 (74.8%)
- High (≥30.6nmol/L)		8 (6.6%)	24 (13.6%)	0 (0%)	32 (10.2%)
Free testosterone (testosterone/SHBG ratio) median (IQR)	314	0.05 (0.04, 0.1)	0.7 (0.1, 7.3)	22.2 (16.1, 34.8)	0.08 (0.05,3.35)
LH– IU/L -median (IQR)	314	0.07 (0.07, 0.07)	1.4 (0.4, 2.20)	3.4 (2.2, 4.78)	0.4 (0.07, 1.8)
LH categories:	314				
-Low (<0.1 IU/L)		-	-	-	-
-Normal (≥0.1-≤4 IU/L)		122 (100%)	171 (97.2%)	11 (68.8%)	304 (96.8%)
-High (>4 IU/L)		0 (0%)	5 (2.8%)	5 (31.2%)	10 (3.2%)
FSH -IU/L- median (IQR)	314	1.1 (0.8, 1.7)	2.8 (1.9, 4.3)	4.75 (3.15, 5.95)	2.1 (1.1, 3.3)
FSH categories:	314				
- Low (≤0.1 IU/L)		-	-	-	-
- Normal (≥0.1-≤8.6 IU/L)		122 (100%)	165 (93.8%)	15 (93.8%)	302 (96.2%)
- High (>8.6 IU/L)		0 (0%)	11 (6.2%)	1 (6.2%)	12 (3.8%)
Estradiol (E2) – pmol/L median (IQR)	314	13 (13, 13)	13 (13, 13)	40 (26, 70)	13 (13, 13)
Estradiol (E2) categories	314				
- Low		-	-	-	-
-Normal (<40)		121 (99.0%)	152 (86.0%)	8 (50.0%)	281 (89.0%)
- High (>40)		1 (0.8%)	24 (14.6%)	8 (50.0%)	33 (11%)
SHBG - nmol/l median (IQR)	314	146 (115, 176)	107 (74, 156)	50 (34, 53)	123 (82, 167)
SHBG categories	314				
-Low (<18.3 nmol/l)		-	-	-	-
-Normal (≥18.3-≤54.1 nmol/l)		1 (0.8%)	14 (8.0%)	12 (75.0%)	27 (8.6%)
-High (>54.1 nmol/l)		121 (99.2%)	162 (92.0%)	4 (25.0%)	287 (91.4%)

The relationship between host factors and asthma-related outcomes (Table 4) showed that maternal smoking during pregnancy was positively associated with current wheeze (OR: 3.52, 95% CI: 1.33-10.4) and asthma symptom score ≥ 2 (OR: 3.29, 95% CI: 1.30 – 8.98), as was exposure to environmental tobacco smoke in the home, while living on a farm was associated with a reduced odds of parental reported asthma (OR: 0.39, 95% CI: 0.14 -0.94).

Table 4. Association between host characteristic and asthma-related outcomes among boys in unadjusted logistic regression models (n=314)

	Current Wheeze	**Asthma Symptom Score ≥2**	**Parental-reported Asthma**
Host Characteristic	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Age (year)	1.02 (0.77, 1.33)	0.92 (0.69, 1.20)	0.82 (0.62, 1.05)
Age categories (6 to 10- reference)	1	1	1
- 11 to 14	2.35 (0.81, 8.52)	2.18 (0.82, 6.85)	0.81 (0.35, 1.89)
- 15 to 18	4.21 (0.55, 23.7)	1.56 (0.08, 10.6)	0.67 (0.04, 3.85)
Height (cm)	1.01 (0.97, 1.05)	1.00 (0.96, 1.04)	**0.96 (0.92, 0.99)**
Weight (kg)	1.00 (0.95, 1.04)	0.98 (0.93, 1.03)	0.96 (0.91, 1.00)
BMI	0.99 (0.85, 1.13)	0.93 (0.78, 1.07)	0.95 (0.81, 1.07)
Current smoking	0.33 (0.02, 1.69)	0.30 (0.02, 1.49)	0.85 (0.19, 2.60)
Maternal smoking during pregnancy	**3.52 (1.33, 10.4)**	**3.29 (1.30, 8.98)**	1.70 (0.73, 3.97)
Household ETS exposure	**5.49 (1.53, 35.0)**	2.70 (0.97, 9.55)	0.89 (0.39, 2.11)
Low birth weight (<2.5 kg)	0.65 (0.22, 2.05)	0.74 (0.26, 2.28)	**4.66 (1.29, 29.9)**
Gestational age <37 weeks	0.27 (0.04, 1.12)	0.41 (0.09, 1.48)	0.28 (0.01, 1.95)
Living on a farm	1.87 (0.74, 4.96)	1.48 (0.61, 3.65)	**0.39 (0.14, 0.94)**
Family history of allergy	-	6.62 (1.29, 121)	-

OR: odds ratio, CI: Confidence Interval, "- "odds ratio not calculatable, each OR is a separate unadjusted regression model

In the unadjusted logistic regression models, there was a negative association between LH and parental-reported asthma (OR: 0.74, CI: 0.54 -0.97), but no other significant associations between asthma outcomes and other endocrine levels (Table 5). Increasing levels of Testosterone, LH and FSH concentrations were associated with reduced odds for all three asthma-related outcomes after adjusting for covariates in the multivariate logistic regression models. The associations between TST concentrations and current wheeze (OR = 0.66, 95% CI: 0.45 - 0.98) and asthma symptom score ≥2 (OR = 0.64, 95% CI: 0.43 - 0.95) were

statistically significant in the extended adjusted model (b). Furthermore, FT was also negatively associated with asthma symptom score ≥2 (OR = 0.68, CI: 0.48 - 0.97) in these models. Stratification of testosterone levels by age was not possible due to the low levels of samples (25 participants) with detection of testosterone above the detection limit. FSH was negatively associated with wheeze (OR = 0.39, 95% CI: 0.16 - 0.94) and asthma symptom score ≥2 (OR = 0.40, 95% CI: 0.17-0.92) in the fully adjusted model as well. There were no clear associations for estradiol. Although not statistically significant, an increase on SHBG was associated with increased odds of current wheezing and asthma symptom score ≥2 but reduced odds of parental reported asthma.

Table 5. Relationship between sex hormone levels and asthma-related outcomes among boys in unadjusted and adjusted multivariate regression models (n=314)

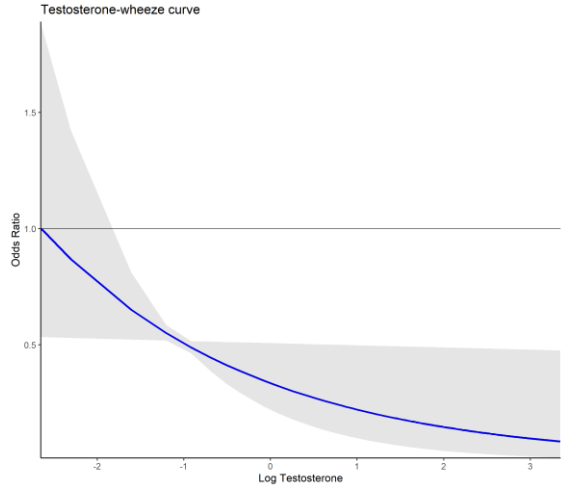
Hormones	Models	Current wheeze	Asthma symptom score ≥2	Parental-reported asthma
		OR (95%CI)	OR (95%CI)	OR (95%CI)
Total serum Testosterone (nmol/L)	Unadjusted model	0.91 (0.70 - 1.14)	0.81 (0.60 - 1.03)	0.83 (0.64 - 1.02)
	Adj model *(a)*	0.70 (0.48 - 1.02)	**0.64 (0.43 - 0.95)**	0.90 (0.63 - 1.28)
	Adj model *(b)*	**0.66 (0.45 - 0.98)**	**0.64 (0.43 - 0.95)**	0.86 (0.59 - 1.25)
Free Testosterone	Unadjusted model	0.92 (0.73 - 1.12)	0.83 (0.64 - 1.02)	0.85 (0.68 - 1.03)
	Adj model *(a)*	0.74 (0.53 - 1.03)	**0.68 (0.48 - 0.95)**	0.94 (0.69 - 1.27)
	Adj model *(b)*	0.70 (0.49 - 1.00)	**0.68 (0.48 - 0.97)**	0.89 (0.63 - 1.25)
Luteinizing hormone (IU/L**)**	Unadjusted model	0.93 (0.68 - 1.25)	0.85 (0.63 - 1.13)	**0.74 (0.54 - 0.97)**
	Adj model *(a)*	0.71 (0.44 - 1.13)	0.73 (0.46 - 1.15)	0.76 (0.50 - 1.16)
	Adj model *(b)*	0.68 (0.43 - 1.09)	0.73 (0.47 - 1.15)	0.70 (0.45 - 1.08)
Follicle-stimulating hormone (IU/L**)**	Unadjusted model	0.78 (0.43 - 1.42)	0.64 (0.35 - 1.13)	0.63 (0.36 - 1.07)
	Adj model *(a)*	0.51 (0.22 - 1.14)	0.48 (0.22 - 1.04)	0.82 (0.41 - 1.64)
	Adj model *(b)*	**0.39 (0.16 - 0.94)**	**0.40 (0.17 - 0.92)**	0.73 (0.35 - 1.53)
Estradiol (pg/ml)	Unadjusted model	0.82 (0.24 - 1.92)	0.53 (0.11 - 1.43)	0.74 (0.24 - 1.63)
	Adj model *(a)*	0.94 (0.30 - 2.97)	0.64 (0.17 - 2.46)	1.01 (0.33 - 3.04)
	Adj model *(b)*	0.93 (0.25 - 3.42)	0.73 (0.17 - 3.07)	1.04 (0.32 - 3.40)
Sex-hormone binding globulin (nmol/l**)**	Unadjusted model	1.23 (0.48 - 3.58)	1.84 (0.70 - 5.57)	1.49 (0.63 - 3.92)
	Adj model *(a)*	1.35 (0.41 - 4.45)	1.77 (0.53 - 5.90)	0.78 (0.26 - 2.39)
	Adj model *(b)*	1.40 (0.36 - 5.46)	1.66 (0.45 - 6.18)	0.88 (0.27 - 2.92)

Model (a) – Model adjusted for age (year), household member smoking, living on a farm, low birth weight and mother smoking during pregnancy; Model (b) - adjusted for all the covariates in model (a) in addition to other potential confounders that include, BMI, current smoking, and family history of allergy; All categorical covariates with missing values were coded as 9 to persevere the power of the study

The concentration-response curves for the association with testosterone (Figure 1) further demonstrated that higher levels of testosterone were associated with reduced risk of current wheeze, asthma symptom score ≥2 and parental reported asthma. A sensitivity analysis of asthma outcomes and hormone concentrations dichotomised at the 75th percentile produced similar but weaker associations (Table S3).

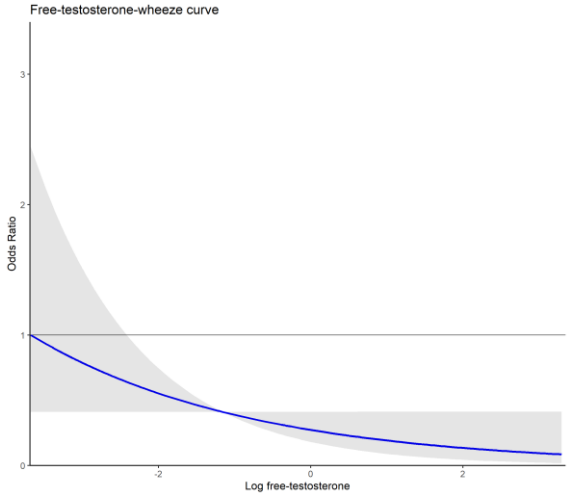
Total serum Testosterone

A1

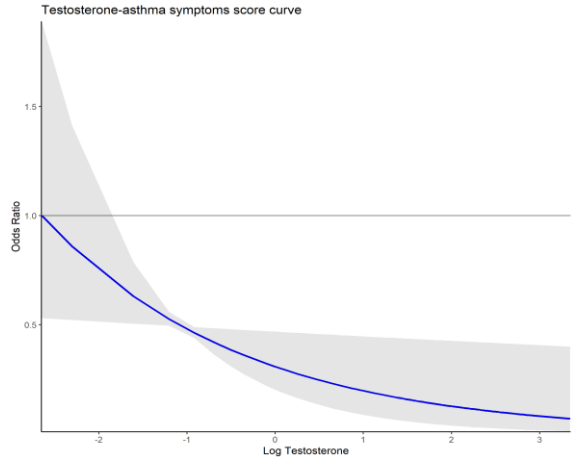


Free testosterone

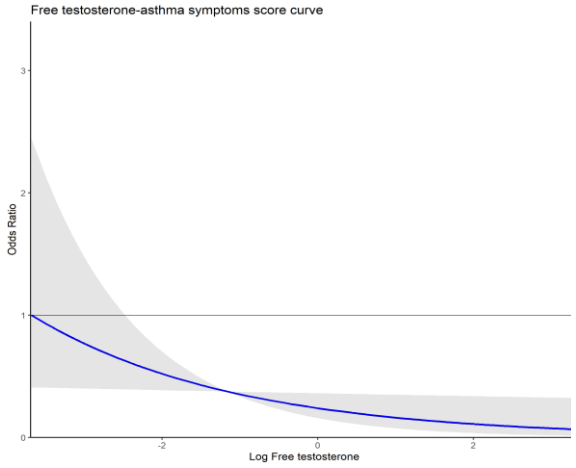
A2



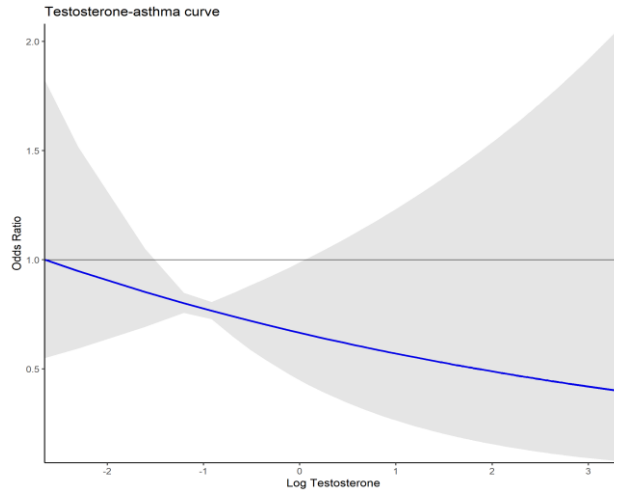
B1



B2



C1



C2

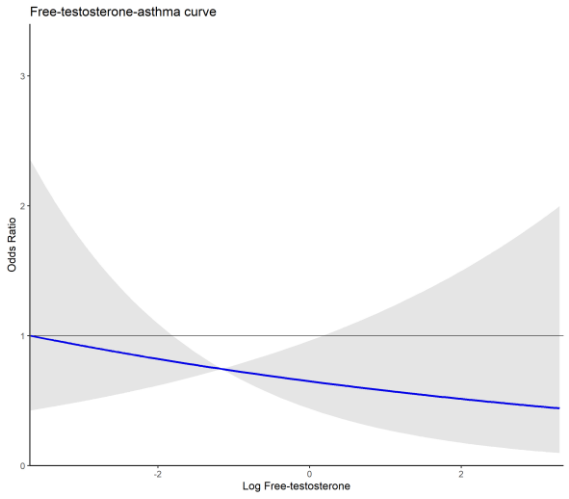


Figure 1. Concentration-response curves for the association of Total serum testosterone and free Testosterone on current wheeze (A1,2), asthma symptom score ≥2 (B1,2) and parental reported asthma (C1,2) in boys residing in the rural Western Cape province.

4. Discussion

In this study, increasing levels of total (TST) and free testosterone (FT) were associated with reduced odds of asthma-related outcomes, after accounting for important covariates relevant to the current setting. These findings provide further insight into the underlying susceptibility and risk factors for asthma in children.

The prevalence of parental-reported asthma (8.0%) was on the upper end of the range of earlier asthma prevalence studies reported in children in African, ranging from 2.5% in Burkina Faso and Ethiopia, to nearly 10% in Swaziland (20, 21). While most of these findings included data from both sexes, the prevalence of current wheeze among boys in the current study was lower (6.1%) than in earlier South African studies conducted in urban and peri-urban areas, which ranged from 12.8% in informal settlements in the Western Cape (15.4% amongst boys) (22), 18.2% in Tembisa and Kempton Park (23), 20.3% in Cape Town, and 24.5% in Durban (24). The discrepancies in asthma prevalence may be due to people living in different settings and therefore different household and environmental exposures and differences socio-economic characteristics.

Deeper insights into some key underlying risk factors are crucial to understanding the associations observed in the current study. For instance, living on farms was associated with increased odds of asthma-related outcomes (current wheezing and asthma symptom score), which may be due to increased pesticide exposure (25). On the other hand, the study also showed that there was a reduced odds of parental-reported asthma as has been shown in other studies that living on farms has a protective effect against asthma, which is due to the development of immune tolerance associated with early childhood endotoxin exposure on farms (26). This study also found that maternal smoking during pregnancy, exposure to environmental tobacco smoke in the household and low birthweight, increased the likelihood of asthma-related outcomes, which is also consistent with the reported literature (25, 27-29).

Higher rates of smoking and exposure to tobacco smoke could be related to asthma related symptoms such as wheezing. However, future research could valuably unpack the effect of tobacco smoke exposure on asthma related outcomes in children. No association was observed for BMI and asthma in the current study as has been reported in previous studies (30-32). This could be explained by the large proportion (70.1%) of participants that were underweight.

Most importantly, this current study found that TST, as well as FT concentrations, were associated with a reduced odds of having any of the three asthma-related outcomes that were investigated. The negative associations observed between TST and FT concentrations and asthma-related outcomes, as demonstrated by concentration-response curves shows that testosterone has a protective effect against asthma (3, 33). These findings are similar to those reported in previous studies conducted in other settings, mostly on adults, which found that increased FT levels are associated with reduced odds of current wheezing and asthma in both men and women (8, 9). In a US study among children aged 6 -18 years, DeBoer, Phillips (8) found that increasing androgen levels in males was associated with improved lung function and asthma symptom control score (ACQ6) (which is similar to the asthma symptom score computed in the current study). Similarly, a study of British children also reported that increased TST and FT levels in children older than 11.8 years were associated with reduced odds of asthma (14). Other studies have shown inconsistent results - a study of both sexes (age: 6 to 80 years) in the US Bulkhi, Shepard (4) found that current asthma prevalence was inversely associated with increased TST levels regardless of sex in adults but not in children younger than 12 years. These findings in different contexts reveal the need for continuous assessment of the impact of TST and FT levels on asthma-related outcomes among children in developing low-middle income countries.

The findings of the current study are also supported by laboratory-based studies in mice Fuseini, Yung (33), which showed that airway inflammation caused by Th2 cells in mice can be reduced by androgen receptor signalling. These laboratory experiments showed that elevated levels of testosterone decreased the release and expression of interleukin 17A (IL-17A) cytokine, which results in reduced neutrophilic and eosinophilic lung inflammation induced by dust mites.

In this study a weaker negative association was observed between testosterone and parental-reported asthma. This suggests that parental-reported asthma as a measure may be a less sensitive indicator of asthma than the presence of current symptoms as measured by the other two asthma-related parameters. There is a possibility for reporting bias to be present, which may be related to underreporting of parental-reported asthma either due to differential health-seeking behaviours of low-income parents or the lack of recognition of asthma symptoms by the parents to warrant a doctor's visit (22, 34).

Furthermore, weak negative associations of FSH and LH levels with asthma-related outcomes were observed in this current study. This could be explained by their positive correlation with testosterone since both LH and FSH are released before testosterone in the male gonadal-pituitary axis (35, 36). Additionally, a weak negative association was also found between estradiol and two of the asthma-related outcomes (current wheezing, asthma symptom score ≥ 2), while a weak positive association was found with parental-reported asthma. This inconsistent relationship with estradiol levels could be due to lack of power since estradiol levels were detectable in only 18% of samples (most boys studied may have been too young for these relationships to be explored further) or unmeasured confounding bias. In general, these findings are similar to other studies DeBoer, Phillips (8) that reported increase in estradiol levels were associated with deleterious effects of asthma in females, which extends into adulthood (more asthma symptoms were reported in children in the late tanner stages).

However, contrasting results were reported in US adults showing higher serum estradiol levels in non-obese males to be associated with lower odds of asthma (10). Laboratory-based studies have also demonstrated that Th17 cells from ovariectomized mice given progesterone and estradiol, produced more IL-17A and expressed more IL-23 receptors (37). This might explain why women have a greater prevalence of severe asthma than males. However, given these discrepancies, the evidence for the association between estradiol levels and asthma is inconclusive and further studies are needed (7).

In general, there is scant evidence on the effect circulating SHBG on asthma. In this current study, no consistent associations between SHBG and asthma symptoms were found. Previously, Arathimos et al. (2019) found a protective effect of SHBG on asthma related outcomes. (14). A sensitivity analysis stratified by sex showed that the protective effect of SHBG was higher in females. This was the first study to show that genetically increased SHBG has a protective effect against asthma. However, because of the complicated genetic correlation between testosterone and SHBG reported by Arathimos et al. (2019) (14), further research is needed to provide greater insights into the nature of these associations and to elaborate on the mechanistic pathways through which SHBG levels influence asthma-related outcomes.

In light of the significant findings demonstrating a protective effect of testosterone on asthma-related outcomes, there are important attributes of this study that merit further mention. The multiple strengths of the study included the measurement of multiple sex hormones and SHBG in relation to three validated asthma-related outcomes (current wheeze, asthma symptoms ≥2 and parental-reported asthma). This enabled the ability to demonstrate consistent associations across the parameters of interest. The most reliable parameter was asthma symptom score as it required more than just wheeze as a symptom. Furthermore, the regression models also controlled for several known confounders for which the findings were

consistent with evidence in the literature. However, this study had some limitations. Hormone concentrations were only available for boys, which limited exploration of associations common to both sexes. Future studies should include additional objective measures of lung function and airway inflammation to provide further specificity than outcomes based on questionnaire data and also adjust for tanner stages of children as most hormonal level changes are related to age (38). While data on certain missing covariates may have reduced the power of the study for certain hormones, this does not detract from the consistent associations observed for testosterone concentrations and asthma-related outcomes. Finally, the fact that some participants did not consent to participate in the study may have led to a potential selection bias in the study, but given the findings, it is unlikely that it played a major role.

In conclusion, this study found evidence that boys, from rural settings in the Western Cape province of South Africa, with increasing testosterone levels were less likely to report asthma-related outcomes after accounting for important underlying risk factors. While weaker evidence suggesting a negative association between asthma-related outcomes with either FSH or LH was present, no evidence was present for estradiol and SHBG. Since differences in sex hormone levels can affect the phenotypic manifestation of asthma in children and modified by treatment, further studies are recommended to obtain further evidence. Longitudinal studies investigating the relationship between measures of endocrine function (and disruption caused by environmental factors such as phthalates and pesticides) and asthma-related outcomes among both boys and girls are new avenues for future research. Furthermore, the incorporation of additional objective measures of asthma to further understand these associations, particularly among children residing in rural low-income settings.

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Supplementary material

Table S 1: Descriptive statistics of the study population by asthma-related outcomes.

Demographic Characteristic	Overall, N = 314	Current Wheezing (Yes), N = 19	Parent - reported asthma (Yes), N = 25	Asthma Symptom Score (≥2), N = 21
Age (year)	11 (10,12)	11 (11,11.8)	11 (10,11)	11 (10.8,11)
Height (cm) - median (IQR)	140 (132, 149)	142 (132, 146)	137 (128, 142)	142 (130, 145)
Weight (kg) - median (IQR)	33 (27, 40)	34 (28, 40)	29 (24, 37)	30 (27, 38)
BMI - median (IQR)	16 (14.9, 18.4)	17 (15.2, 18.9)	15.9 (14.8, 18.3)	16.0 (15, 18.3)
Gestational age (weeks) - median (IQR)	38 (36, 40)	39 (38, 39)	39. (38, 39)	39 (37.5, 39)
Birth weight categories - n (%)				
Normal	81 (100%)	6 (7.4%)	2 (2.5%)	6 (7.4%)
Low	161 (100%)	8 (5.0%)	17 (11%)	9 (5.6%)
Childbirth length (cm) - median (IQR)	49 (45, 52)	47 (45, 51)	45.5 (43.2, 50)	47 (45, 51)
Head circumference (cm) - median (IQR)	32.5 (31.3, 33)	32 (31.3, 32.8)	32 (32, 33)	32 (31.3, 32.8)
Family history of allergy (Yes) **n1 (%)**	132 (100%)	15 (11%)	14 (11%)	16 (12%)
Current smoking (Yes) **n1 (%)**	43 (100%)	1 (2.3%)	3 (7.0%)	1 (2.3%)
Mother smoking during pregnancy (Yes) **n1 (%)**	117 (100%)	12 (10%)	12 (10%)	13 (11%)
Smoking at home- secondary TS (Yes) **n1 (%)**	192 (100%)	17 (8.9%)	15 (7.8%)	17 (8.9%)
Foetal Alcohol Syndrome (Yes) **n1 (%)**	4 (100%)	0 (0%)	0 (0%)	0 (0%)
Tuberculosis (Yes) **n1 (%)**	5 (100%)	0 (0%)	0 (0%)	0 (0%)
Lung infections (Yes) **n1 (%)**	1 (100%)	1 (100%)	0 (0%)	1 (100%)
Farm residence- pesticide exposure (Yes) **n1 (%)**	89 (100%)	6 (6.7%)	3 (3.4%)	6 (6.7%)
Reproductive Hormones				
FSH - median (IQR)	2.1 (1.10, 3.3)	1.80 (1.0, 2.80)	1.70 (0.70, 2.90)	1.30 (1.0, 2.80)
FSH categories				
Normal	302 (100%)	19 (6.3%)	25 (8.3%)	21 (7.0%)
Abnormally High	12 (100%)	0 (0%)	0 (0%)	0 (0%)
LH - median (IQR)	0.4 (0.07, 1.8)	0.3 (0.07, 2.0)	0.07 (0.07, 0.50)	0.07 (0.07, 1.0)
LH categories				
Normal	304 (100%)	19 (6.2%)	25 (8.2%)	21 (6.9%)
Abnormally High	10 (100%)	0 (0%)	0 (0%)	0 (0%)
Testosterone – nmol/L - median (IQR)	0.1 (0.1, 3.3)	0.1 (0.1, 0.9)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)

Testosterone categories				
Normal	235 (100%)	17 (7.2%)	20 (8.5%)	19 (8.1%)
Abnormally High	32 (100%)	1 (3.1%)	3 (9.4%)	1 (3.1%)
Abnormally Low	47 (100%)	1 (2.1%)	2 (4.3%)	1 (2.1%)
Estradiol (E2)– pmol/L				
Normal	281 (100%)	17 (6.0%)	23 (8.2%)	20 (7.1%)
Abnormally High	33 (100%)	2 (6.1%)	2 (6.1%)	1 (3.0%)
SHBG median (IQR)	123 (82, 167)	149 (86, 162)	144 (86, 164)	154 (86, 168)
SHBG categories				
Normal	27 (100%)	2 (7.4%)	1 (3.7%)	1 (3.7%)
Abnormally High	287 (100%)	17 (5.9%)	24 (8.4%)	20 (7.0%)
Asthma-related outcomes				
Wheezing attack (Yes) **n1 (%)**	24 (100%)	19 (79%)	8 (33%)	21 (88%)
Wheezing disturbing sleep (Yes) **n1 (%)**	17 (100%)	15 (88%)	2 (12%)	17 (100%)
Wheezing during exercise (Yes) **n1 (%)**	23 (100%)	7 (30%)	8 (35%)	8 (35%)
Wheezing limiting speech (Yes) **n1 (%)**	6 (100%)	5 (83%)	4 (67%)	6 (100%)

Table S 2: Correlation matrix of sex hormone levels among boys residing in the rural Western Cape

Hormones	**Testosterone**	**Free testosterone**	**Estradiol (E2)**	**Follicle-stimulating hormone (FSH)**	**Luteinizing hormone (LH)**	**Sex-hormone binding globulin (SHBG)**
Testosterone	1	0.88*	0.65*	0.70*	0.86*	-0.52*
Free testosterone		1	0.61*	0.66*	0.79*	-0.81*
Estradiol (E2)			1	0.41*	0.54*	-0.52*
Follicle-stimulating hormone (FSH)				1	0.84*	-0.43*
Luteinizing hormone (LH)					1	-0.49*
Sex hormone-binding globulin (SHBG)						1

**Spearman correlation coefficient; * p< 0.05*

Table S 3: Relationship between sex hormone levels and asthma-related outcomes among boys in the rural Western Cape in unadjusted logistic regression models (N=314)

	Current wheeze	Asthma symptom score ≥2	Parental-reported Asthma
Reproductive Hormones	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Testosterone (nmol/L)	0.91 (0.70, 1.14)	0.81 (0.60, 1.03)	0.83 (0.64, 1.02)
Testosterone ($\geq 75^{th}$ percentile)	0.78 (0.22, 2.23)	0.48 (0.11, 1.46)	0.54 (0.15, 1.48)
Testosterone ($\leq 25^{th}$ percentile)	1.66 (0.64, 4.84	2.52 (0.96, 7.86)	2.02(0.85, 5.32)
Free testosterone	0.92 (0.73, 1.12)	0.83 (0.64, 1.02)	0.85 (0.68, 1.03)
Free testosterone (≥ 75th percentile)	0.78 (0.22, 2.23)	0.48 (0.11, 1.46)	0.54 (0.15, 1.48)
Estradiol (E2)	0.82 (0.24, 1.92)	0.53 (0.11, 1.43)	0.74 (0.24, 1.63)
Estradiol (≥ 75th percentile)	0.84 (0.19, 2.62)	0.46 (0.07, 1.63)	0.59 (0.14, 1.79)
Follicle-stimulating hormone (FSH)	0.78 (0.43, 1.42)	0.64 (0.35, 1.13)	0.63 (0.36, 1.07)
FSH (≥ 75th percentile)	0.73 (0.20, 2.08)	0.44 (0.10, 1.36)	0.51 (0.14, 1.38)
Luteinizing hormone (IU/L)	0.93 (0.68, 1.25)	0.85 (0.63, 1.13)	**0.74 (0.54, 0.97)**
LH (≥ 75th percentile)	1.13 (0.35, 3.06)	0.72 (0.20, 2.03)	0.25 (0.04, 0.88)
Sex-hormone binding globulin (nmol/l).	1.23 (0.48, 3.58)	1.84 (0.70, 5.57)	1.49 (0.63, 3.92)
SHBG (≥ 75th percentile)	1.07 (0.34, 2.89)	1.21 (0.42, 3.09)	0.73 (0.24, 1.87)

OR: odds ratio; each OR is a separate unadjusted regression model.; CI = Confidence Interval; Statistically significant at 5% level is denoted by bold text.

Table S 4:Relationship between sex hormone levels and asthma-related outcomes among boys residing in the rural Western Cape in adjusted logistic regression models (N=314)

		Current wheeze	Asthma symptom score ≥2	Parental-reported asthma
Hormones	**Models**	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**
Testosterone	Model- 1	0.70 (0.48 - 1.02)	**0.64 (0.43 - 0.95)**	0.90 (0.63 - 1.28)
	Model- 2	0.70 (0.48 - 1.02)	**0.65 (0.43 - 0.96)**	0.89 (0.62 - 1.27)
	Model- 3	0.71 (0.49 - 1.02)	**0.65 (0.44 - 0.95)**	0.90 (0.63 - 1.28)
	Model- 4	**0.66 (0.44 - 0.98)**	**0.61 (0.41 - 0.91)**	0.87 (0.60 - 1.25)
	Model- 5	**0.66 (0.45 - 0.98)**	**0.64 (0.43 - 0.95)**	0.86 (0.59 - 1.25)
Free Testosterone	Model- 1	0.74 (0.53 - 1.03)	**0.68 (0.48 - 0.95)**	0.94 (0.69 - 1.27)
	Model- 2	0.73 (0.52 - 1.03)	**0.68 (0.48 - 0.97)**	0.92 (0.67 - 1.27)
	Model- 3	0.75 (0.54 - 1.03)	**0.68 (0.49 - 0.96)**	0.94 (0.69 - 1.27)
	Model- 4	**0.71 (0.50 - 0.99)**	**0.65 (0.46 - 0.92)**	0.90 (0.66 - 1.24)
	Model- 5	0.70 (0.49 - 1.00)	**0.68 (0.48 - 0.97)**	0.89 (0.63 - 1.25)
Luteinizing hormone (LH)	Model- 1	0.71 (0.44 - 1.13)	0.73 (0.46 - 1.15)	0.76 (0.50 - 1.16)
	Model- 2	0.71 (0.44 - 1.14)	0.74 (0.47 - 1.17)	0.75 (0.49 - 1.16)
	Model- 3	0.70 (0.44 - 1.11)	0.72 (0.46 - 1.13)	0.76 (0.50 - 1.16)
	Model- 4	0.69 (0.43 - 1.10)	0.71 (0.45 - 1.12)	0.70 (0.46 - 1.08)
	Model- 5	0.68 (0.43 - 1.09)	0.73 (0.47 - 1.15)	0.70 (0.45 - 1.08)
Follicle-stimulating hormone (FSH)	Model- 1	0.51 (0.22 - 1.14)	0.48 (0.22 - 1.04)	0.82 (0.41 - 1.64)
	Model- 2	0.51 (0.22 - 1.15)	0.48 (0.22 - 1.06)	0.83 (0.41 - 1.67)
	Model- 3	0.44 (0.19 - 1.03)	**0.44 (0.20 - 0.97)**	0.82 (0.41 - 1.64)
	Model- 4	0.47 (0.21 - 1.07)	**0.45 (0.21 - 0.99)**	0.71 (0.34 - 1.48)
	Model- 5	**0.39 (0.16 - 0.94)**	**0.40 (0.17 - 0.92)**	0.73 (0.35 - 1.53)
Estradiol	Model- 1	0.94 (0.30 - 2.97)	0.64 (0.17 - 2.46)	1.01 (0.33 - 3.04)
	Model- 2	0.96 (0.29 - 3.18)	0.72 (0.18 - 2.82)	1.00 (0.32 - 3.10)
	Model- 3	0.99 (0.30 - 3.25)	0.67 (0.17 - 2.65)	1.00 (0.33 - 3.04)
	Model- 4	0.82 (0.24 - 2.74)	0.57 (0.14 - 2.29)	0.99 (0.31 - 3.15)
	Model- 5	0.93 (0.25 - 3.42)	0.73 (0.17 - 3.07)	1.04 (0.32 - 3.40)
Sex-hormone binding globulin (SHBG)	Model- 1	1.35 (0.41 - 4.45)	1.77 (0.53 - 5.90)	0.78 (0.26 - 2.39)
	Model- 2	1.39 (0.36 - 5.30)	1.60 (0.43 - 5.93)	0.81 (0.25 - 2.63)
	Model- 3	1.37 (0.41 - 4.61)	1.80 (0.54 - 6.05)	0.78 (0.25 - 2.39)
	Model- 4	1.46 (0.43 - 4.97)	2.01 (0.59 - 6.83)	0.89 (0.29 - 2.73)
	Model- 5	1.40 (0.36 - 5.46)	1.66 (0.45 - 6.18)	0.88 (0.27 - 2.92)

Model 1- (Base model) Age (year), Household member smokes, Living on a farm, Low Birthweight and Mother smoking during pregnancy.
Model 2- Base model + BMI
Model 3- Base model + Current smoking
Model 4- Base model + Family history of allergy
Model 5- (Full model) Age (year), Household member smokes, Living on a farm, Low Birthweight, Mother smoking during pregnancy, BMI, Current smoking and Family history of allergy

Supplementary Figures

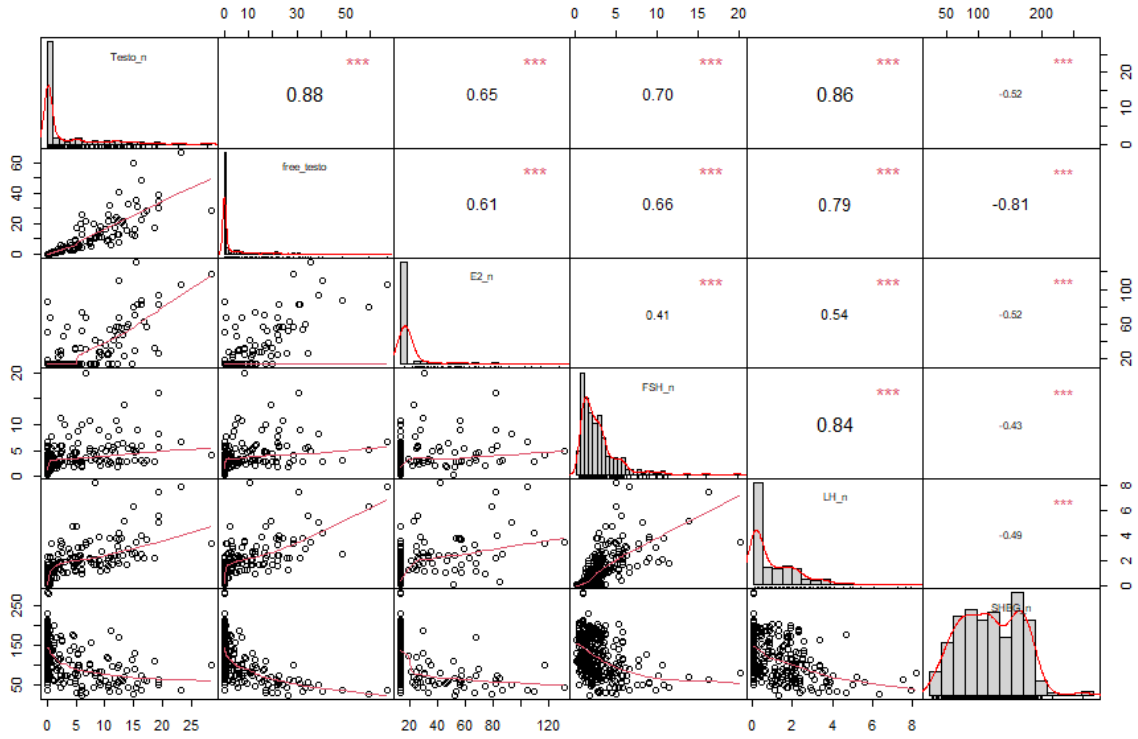


Figure S 1: Hormonal spearman's correlation analysis

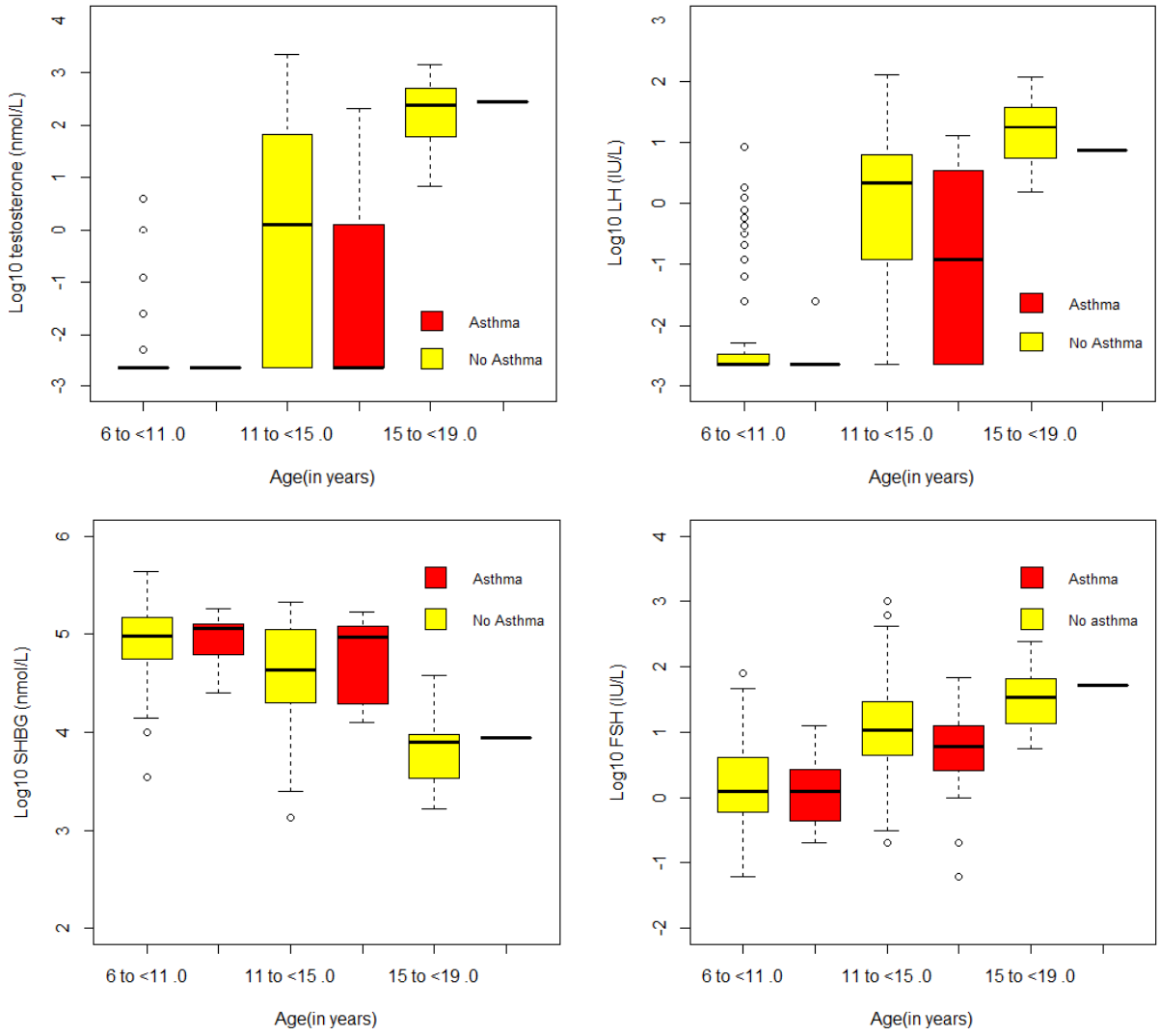


Figure S 2: Hormonal levels by self-reported asthma status, and age of boys residing in the rural Western cape. Hormonal levels were log-transformed for ease of exposition.

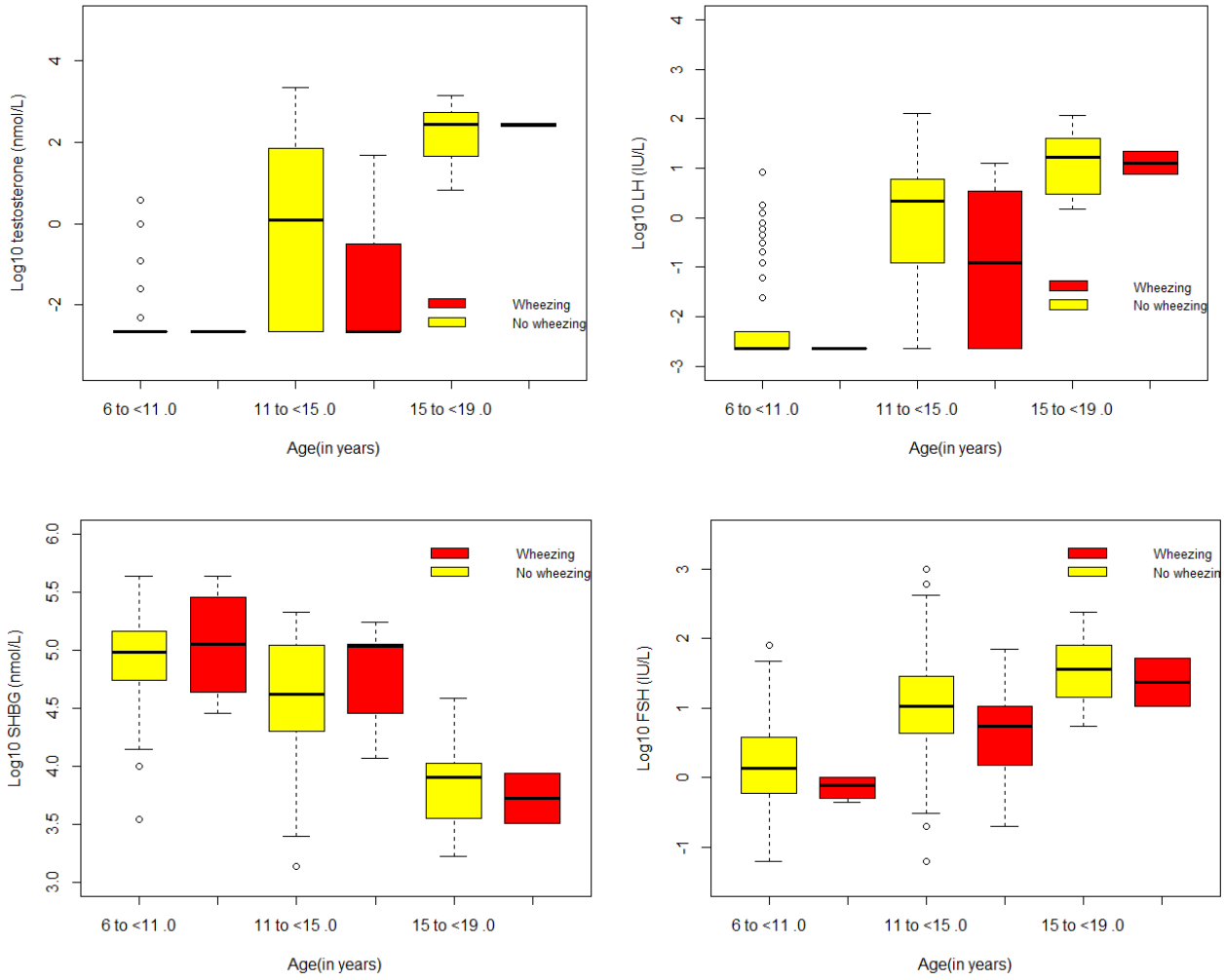


Figure S 3: Hormonal levels by current wheezing status, and age of boys residing in the rural Western cape. Hormonal levels were log-transformed for ease of exposition.

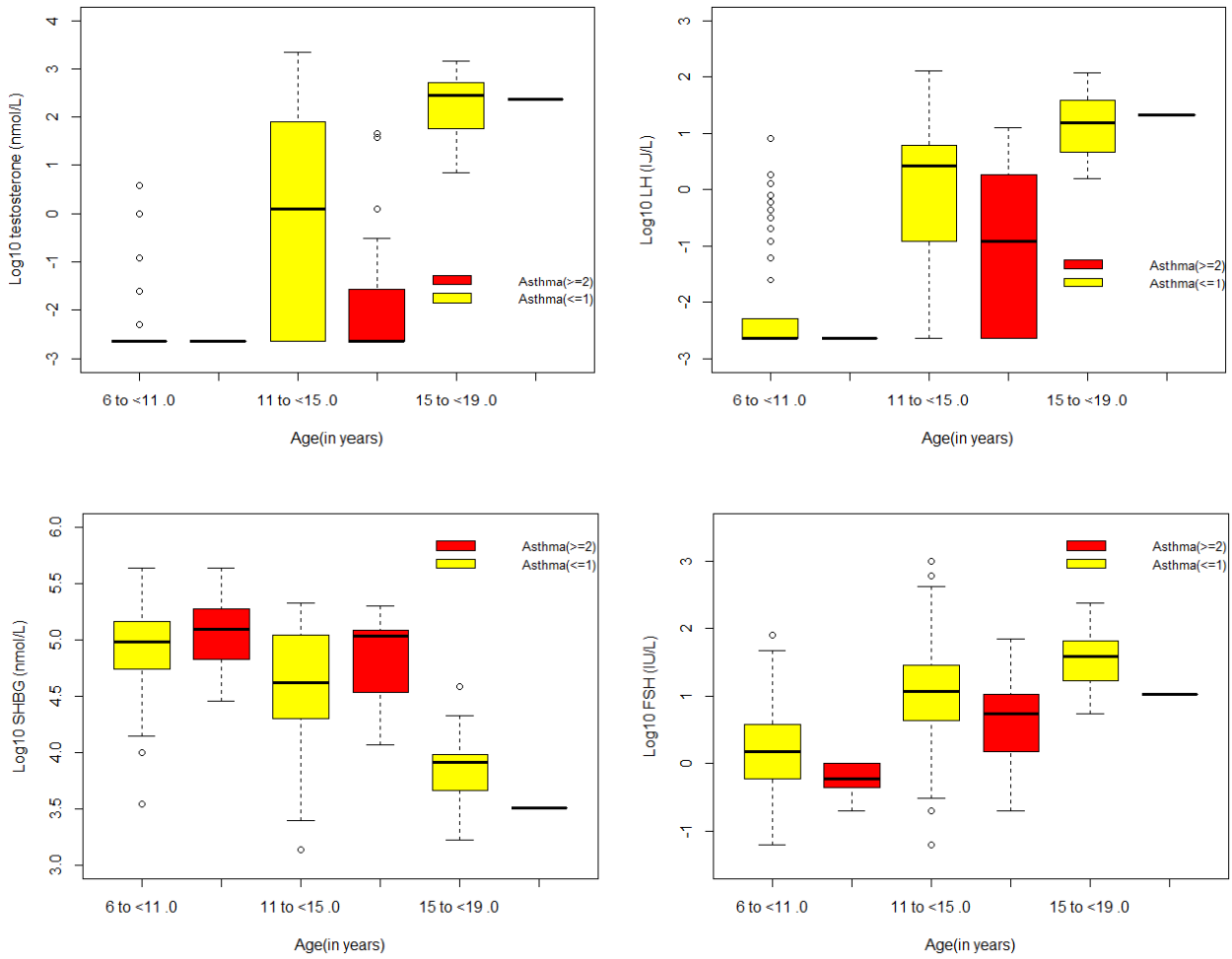
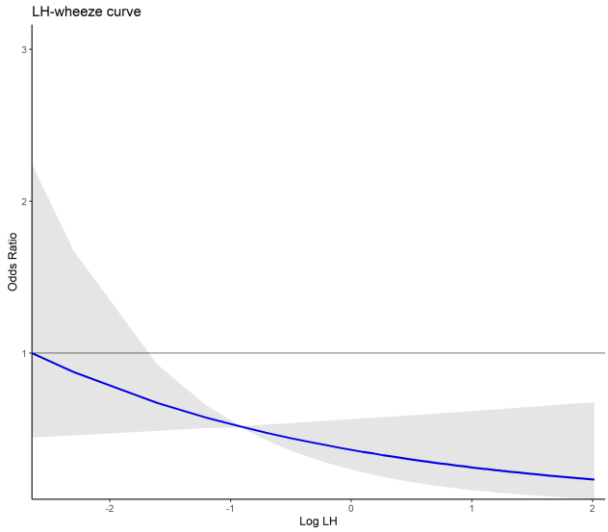
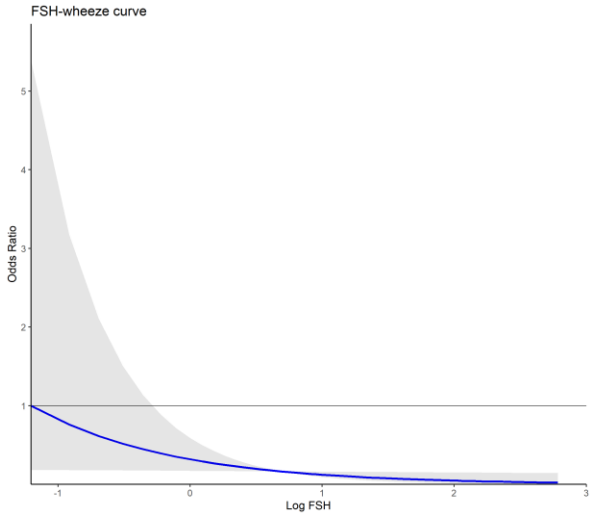


Figure S 4: Hormonal levels by asthma status (based on Asthma symptom score), and age of boys residing in the rural Western cape. Hormonal levels were log-transformed for ease of exposition.

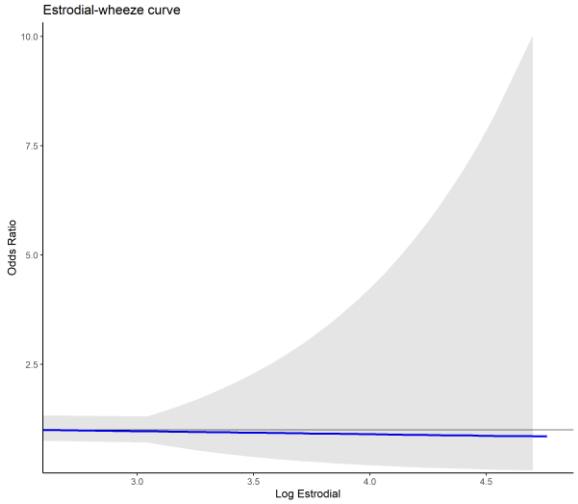
Luteinizing hormone (LH)



Follicle-stimulating hormone (FSH)



Estradiol



SHBG

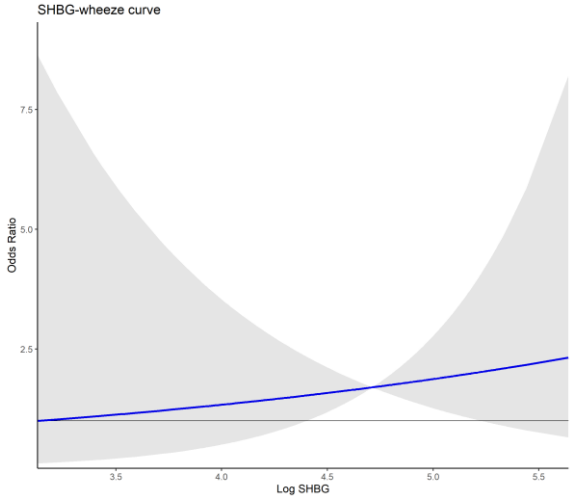
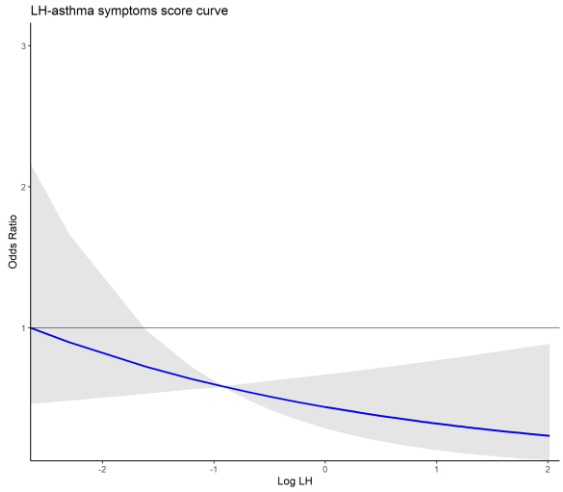
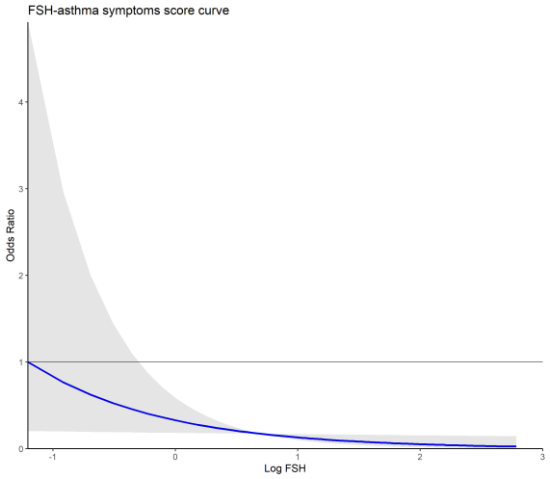


Figure S 5: Concentration-response curve for the effect of reproductive hormones on Current wheeze

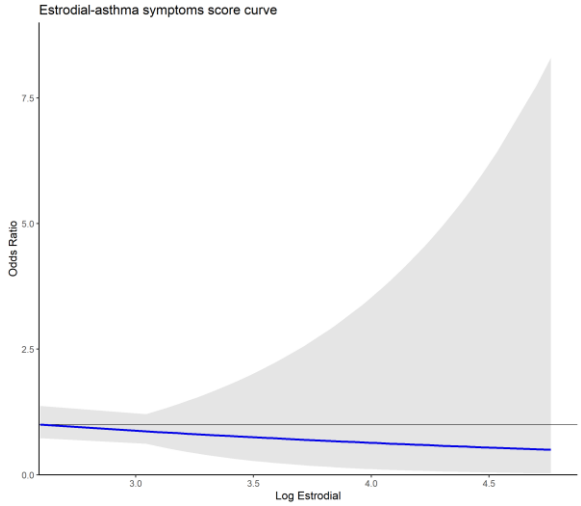
Luteinizing hormone (LH)



Follicle-stimulating hormone (FSH)



Estradiol



SHBG

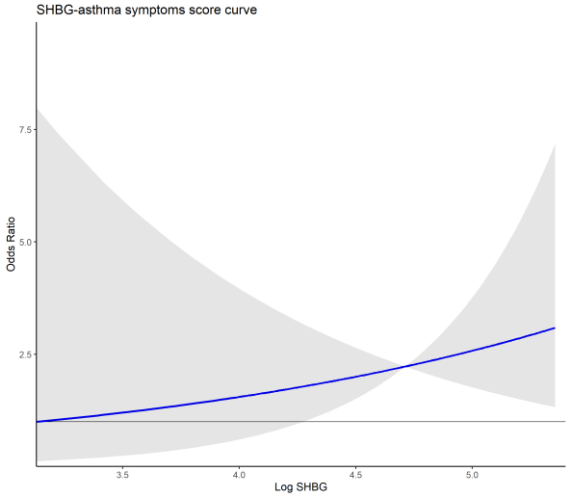
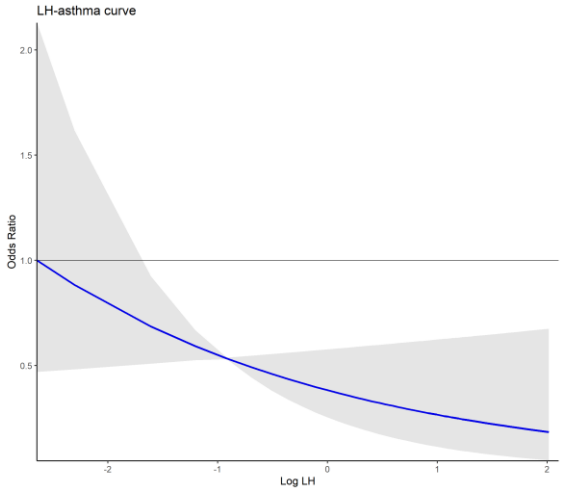
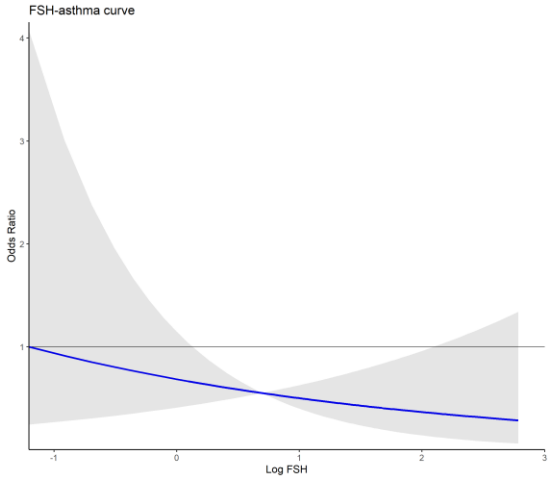


Figure S 6: Concentration-response curve for the effect of reproductive hormones on Asthma Symptom score >2

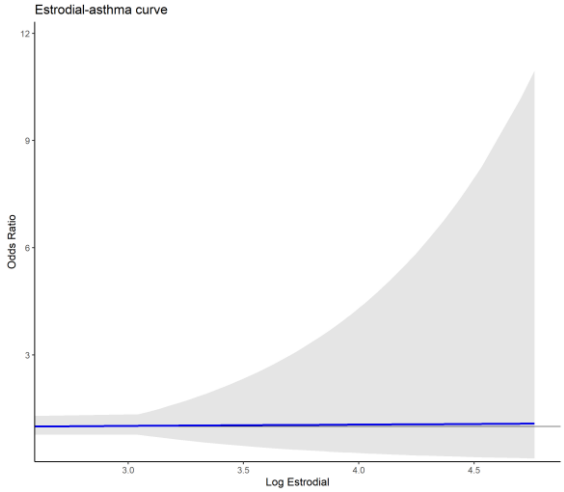
Luteinizing hormone (LH)



Follicle-stimulating hormone (FSH)



Estradiol



SHBG

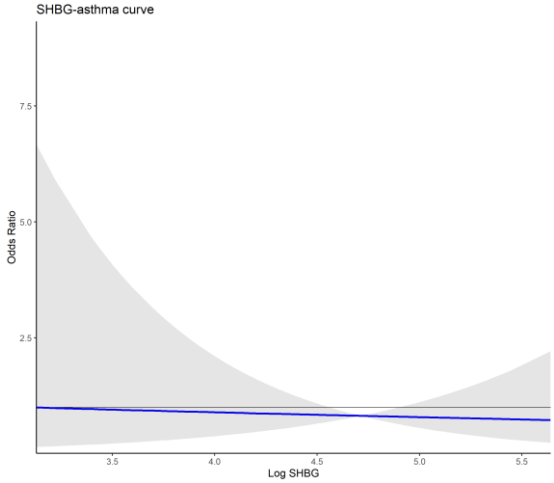


Figure S 7: Concentration-response curve for the effect of reproductive hormones on Parental- reported Asthma

PART C: APPENDINCES

Appendix 1: Guardian Questionnaire

(Sections highlighted in yellow and blue in the questionnaires apply to this study)

General Medical History of the child

Outcome: Asthma-related questions (highlighted)
Covariates and/or confounders: highlighted

5.1 What is the mother's date of birth?	_____ (dd/mm/yyyy)
5.2 What is the mother's age?	_____ (years)
5.3 Which health facility (MOU) did the mother attend during her pregnancy with this participant?	_____
5.4 What was the weight of the mother during pregnancy? *(Hint: refer to maternal health records if available)*	_____ (kg) ☐98 Don't know
5.5 What was the height of the mother during pregnancy? *(Hint: refer to maternal health records if available or measure the current height of mother)*	_____ (cm) ☐98 Don't know
5.6 What was the weight of the mother at the birth of the participant? *(Hint: refer to maternal health records if available)*	_____ (kg) ☐98 Don't know
5.7 What was the duration of pregnancy (gestational age) at the birth of the child? *(Hint: refer to Road to Health card if available)*	_____(weeks)
5.8 What was the **child's** birth weight (grams)? *(Hint: refer to Road to Health card if available)*	_____(g)
5.9 What was the **child's** birth length (cm)? *(Hint: refer to Road to Health card if available)*	_____(cm)
5.10 What was the **child's** birth head circumference (cm)? *(Hint: refer to Road to Health card if available)*	_____(cm)
5.11 What was the **child's** APGAR score? *(Hint: refer to Road to Health card if available)*	_____(1 min) _____(5 min)

5.12 During the 1st and 2nd trimester of the mother's pregnancy, did she carry a heavy load >5 kg?	☐1 Yes ☐2 No ☐98 Don't Know
5.13 If yes (to **5.11)**, how frequently were you involved with the task of carrying a load >5kg?	☐1 Seldom ☐2 Sometimes ☐3 Often ☐98 Don't Know
5.14 Currently, how would you rate your **child**'s health in general?	☐1 Poor ☐2 Fair ☐3 Good ☐4 Very good ☐5 Excellent

5.15 Has your **child** ever been diagnosed by a doctor to have/have had any of the following conditions?

Condition/Illness	**Response**	**If Yes, age of child when diagnosed**
5.15.1 Diabetes	☐1 Yes ☐2 No ☐98 Don't know	**5.15.1.1** _____ (years)
5.15.2 Obesity/Overweight	☐1 Yes ☐2 No ☐98 Don't know	**5.15.2.1** _____ (years)
5.15.3 Cancer	☐1 Yes ☐2 No ☐98 Don't know	**5.15.3.1**_____ (years)
5.15.3.2 If Yes (to **5.15.3.)**, what was the type of cancer?		_____
5.15.4 Fits/Epilepsy/Seizures	☐1 Yes ☐2 No ☐98 Don't know	**5.15.4.1** _____ (years)
5.15.5 Foetal Alcohol Syndrome (FAS)	☐1 Yes ☐2 No ☐98 Don't know	**5.15.5.1** _____ (years)
5.15.6 Heart problems	☐1 Yes ☐2 No ☐98 Don't know	**5.15.6.1** _____ (years)
5.15.7 Back problems	☐1 Yes ☐2 No ☐98 Don't know	**5.15.7.1** _____ (years)

5.15.8 Attention Deficit Hyperactivity Disorder (ADHD)	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.8.1** _____ (years)
5.15.9 Autism	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.9.1** _____ (years)
5.15.10 Tuberculosis	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.10.1** _____ (years)
5.15.11 High Blood Pressure	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.11.1** _____ (years)
5.15.12 Mumps	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.12.1** _____ (years)
5.15.13 Thyroid condition - Hyper/Hypothyroidism	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.13.1** _____ (years)
5.15.14 Any Lung infections	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know	**5.15.14.1** _____ (years)
5.15.15 Was your **child** born with an abnormality in their reproductive organ/ have they been diagnosed by a doctor with a reproductive health problem?		☐$_1$ Yes ☐$_2$ No (go to **5.15.19.**) ☐$_{98}$ Don't know (go to **5.15.19.**)
5.15.16 If yes (to **5.15.15.**), what was/is the name of the condition?		
5.15.17 If yes (to **5.15.15.**), did your **child** go for an operation for the abnormality?		☐$_1$ Yes ☐$_2$ No
5.15.18 If yes (to **5.15.15.**), did your **child** receive medication for the abnormality?		☐$_1$ Yes ☐$_2$ No
5.15.19 Has your **child** ever experienced an injury, resulting in a swelling of the reproductive organ area that has this been diagnosed by a doctor?		☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
If the child is male, go to question 5.17		
5.16 If the child is a female, complete the following questions with the mother / female guardian:		
5.16.1 Has your daughter started menstruating?		☐$_1$ Yes

	☐$_2$ No ☐$_{98}$ Don't know
5.16.2 If yes (to **5.16.1)**, at what age did she start menstruating?	_____ (years)
5.16.3 What is your daughter's average menstrual cycle length?	☐$_1$ 24 days or less ☐$_2$ 25–30 days ☐$_3$ 31–35 days ☐$_4$ 36–42 days ☐$_5$ 43 days or more ☐$_6$ Too irregular to say ☐$_{98}$ Don't know
5.16.4 Has your child ever been diagnosed by a doctor with any of the following reproductive conditions? *(Tick all that apply)*	☐$_1$ Polycysitc Ovarian Syndrome ☐$_2$ Premature Ovarian Failure ☐$_3$ Endometriosis ☐$_4$ Uterine Fibroids ☐$_{98}$ Don't know
5.16.5 During the past 12 months, did you (the mother) ever go for 6 weeks or more without a menstrual period? *(Hint: Do not count times when you were pregnant, breastfeeding, or using birth control pills)*	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.16.6 During the past 12 months, did you ever bleed or spot between menstrual periods? *(Hint: Do not count spotting at the beginning or end of your period)*	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.17 Has your **child** ever had wheezing or whistling in the chest at any time in the past?	☐$_1$ Yes ☐$_2$ No → go to **5.22** ☐$_{98}$ Don't know
5.18 If yes (to **5.17**), has your **child** had wheezing or whistling in the chest in the past 12 months?	☐$_1$ Yes ☐$_2$ No → go to **5.22** ☐$_{98}$ Don't know
5.19 If yes **(**to **5.17)** how many attacks of wheezing has the **child** had in the past 12 months?	☐$_0$ None ☐$_1$ 1 to 3 times ☐$_2$ 4 to 12 times ☐$_3$ >12 times ☐$_{98}$ Don't know
5.20 In the past 12 months, how often on average, has your **child's** sleep been disturbed due to wheezing?	☐$_0$ Never woken with wheezing ☐$_1$ Less than one night per week

	☐$_2$ One or more nights per week ☐$_{98}$ Don't know
5.21 In the past 12 months, has the wheezing ever been serious enough to limit your **child's** speech to only one or two words at a time between breaths?	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.22 Has your **child** ever had asthma?	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.23 In the past 12 months, has your **child's** chest sounded wheezy during or after exercise?	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.24 In the past 12 months, has your **child** had a dry cough at night, apart from a cough associated with a cold or chest infection?	☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Don't know
5.25 Does the **child** have/had any other illness diagnosed by a doctor that needs you to attend the clinic regularly for medication (≥ 3 months) apart from those listed above (in **5.15**)?	☐$_1$ Yes ☐$_2$ No → go to **5.27** ☐$_{98}$ Don't know → go to **5.27**
5.26 If yes (to **5.25**), specify condition? *(Hint: if they don't know, look in-clinic card and write down diagnosis or specific clinic they attend)*	_____ _____
5.27 Has your **child** taken any daily medication during the last three months?	☐$_1$ Yes ☐$_2$ No → go to **5.31** ☐$_{98}$ Don't know
5.28 Was the medication prescribed by a doctor?	☐$_1$ Yes ☐$_2$ No
5.29 What is the name of the medication? *(Hint: if they don't know, ask for the medication box and write down the information)*	
5.30 Specify the frequency of taking this medication? *(Hint: if they don't know, ask for the medication box and write down the information)*	
5.31 Does anyone in the biological family have any of the following allergic diseases? (*Tick all that apply)*	☐$_1$ Asthma ☐$_2$ Eczema ☐$_3$ Hayfever (Rhinitis) ☐$_4$ Food allergy ☐$_{98}$ Don't know

SMOKING AND ALCOHOL CONSUMPTION

Introduction: Interviewer Reads to Respondent

To understand pesticide exposure, we need to know what other exposures the child may have had. This is a section on the smoking and alcohol exposure that the child may have had before, during and after pregnancy.

(HINT: If the biological mother is NOT answering the questions, please phrase them accordingly)

Note: Now I am going to ask you some questions about drinking alcohol		
9.1	Does the mother currently drink alcohol?	☐$_0$ Never ☐$_1$ Less than 1 glass a day ☐$_2$ About 1 glass a day ☐$_3$ More than 1 glass a day
9.2	Did the mother drink alcohol during pregnancy?	☐$_0$ Never ☐$_1$ Less than 1 glass a day ☐$_2$ About 1 glass a day ☐$_3$ More than 1 glass a day
9.3	Has the mother ever drank alcohol in the past?	☐$_0$ Never **(**go to **9.8)** ☐$_1$ Less than 1 glass a day ☐$_2$ About 1 glass a day ☐$_3$ More than 1 glass a day
9.4	Who is the responder on the mother's smoking and alcohol consumption?	☐$_1$ Mother ☐$_2$ Guardian

Note: For Questions **9.5. – 9.11.**: Please, complete the **correct option** as indicated.		
9.5 <u>CURRENT DRINKING</u> **9.5.1** (*Hint: Question for the mother)* Have you ever felt that you should cut down on your drinking? **9.5.2** (*Hint: Question for the guardian)* Has she (the mother) ever felt that she should cut down on her drinking?	**<u>Mother:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**<u>Guardian:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.6 <u>CURRENT DRINKING</u> **9.6.1** (*Hint: Question for the mother)* Have people annoyed you by criticizing your drinking? **9.6.2** (*Hint: Question for the guardian)* Have people annoyed her (the mother) by criticizing her drinking?	**<u>Mother:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**<u>Guardian:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.7 <u>CURRENT DRINKING</u> **9.7.1** (*Hint: Question for the mother)* Have you ever felt bad or guilty about your drinking? **9.7.2** (*Hint: Question for the guardian)* Has she (the mother) ever felt bad or guilty about her drinking?	**<u>Mother:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**<u>Guardian:</u>** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know

9.8 CURRENT DRINKING **9.8.1** (*Hint: Question for the mother)* Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover? **9.8.2** (*Hint: Question for the guardian)* Has she (the mother) ever had a drink first thing in the morning to steady her nerves or to get rid of a hangover?	**Mother:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**Guardian:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.9 PAST DRINKING **9.9.1** (*Hint: Question for the mother)* When you did drink alcohol, did you ever feel that you should cut down on your drinking? **9.9.2** (*Hint: Question for the guardian)* When she (the mother) did drink alcohol, did she ever feel that she should cut down on her drinking?	**Mother:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**Guardian:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.10 PAST DRINKING **9.10.1** (*Hint: Question for the mother)* When you did drink alcohol, did people annoy you by criticizing your drinking? **9.10.2** (*Hint: Question for the guardian)* When she (the mother) did drink alcohol, did people annoy her by criticizing her drinking?	**Mother:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**Guardian:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.11 PAST DRINKING **9.11.1** (*Hint: Question for the mother)* When you did drink alcohol, did ever feel bad or guilty about your drinking? **9.11.2** (*Hint: Question for the guardian)*	**Mother:** ☐$_1$ Yes	**Guardian:** ☐$_1$ Yes

When she (the mother) did drink alcohol, did she ever feel bad or guilty about her drinking?	☐$_2$ No ☐$_{98}$ Do not know	☐$_2$ No ☐$_{98}$ Do not know
9.12 PAST DRINKING **9.12.1** (*Hint: Question for the mother)* When you did drink alcohol, did you ever have a drink first thing in the morning to steady your nerves or to get rid of a hangover? **9.12.2** (*Hint: Question for the guardian)* When she (the mother) did drink alcohol, did she ever have a drink first thing in the morning to steady her nerves or to get rid of a hangover?	**Mother:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know	**Guardian:** ☐$_1$ Yes ☐$_2$ No ☐$_{98}$ Do not know
9.13 Did the mother smoke during pregnancy?	☐$_0$ Never ☐$_1$ Less than 1 cigarette a day ☐$_2$ 1-5 cigarettes a day ☐$_3$ 6-20 cigarettes a day ☐$_4$ More than a packet a day	
9.14 Does anyone in the household currently smoke or ever smoked at home?	☐$_0$ None ☐$_1$ One ☐$_2$ Two ☐$_3$ More than two	
9.15 Has the mother ever taken any recreational drugs during pregnancy eg: Tik, Marijuana? *(Hint: Interviewer to record the name(s) of the drug(s) used)*	☐$_1$ Never ☐$_2$ Less than 1 times a week ☐$_3$ 1-6 times a week	

	☐4 Once a day ☐5 More than once a day

Signature: ..... Date: .....

Mother/Father/Other (please specify:)_____

Thank you

Appendix 2: Participant questionnaire

Your answers will be treated strictly confidential and only analysed anonymous

(Hint: Fill information for question a to i out according to the information in the participant envelope, and remember to fill the individual station from in parallel)

a. Study number: _____

b. Physical Address: _____

c. Participant Grade: _____

d. Participant Age: _____

e. Date seen: ___/_____/_____
Day Month Year

f. What is the name of your school:

1. Glen Elgin
2. De Rust Combined
3. Kathleen Murray Primary
4. Van Cutsem Combined
5. FJ Conradie Primary
6. Groenvlei Primary
7. Steynville Primary
8. Steynville Secondary
9. Hex Vallei Secondary
10. Hex River High School
11. Groenberg Secondary

g. Participant first/home language:

1. Afrikaans
2. isiXhosa
3. English
4. Other (**h.** Specify: _____)

i. Interviewed by:

1. Wisdom Basera
2. Phillancia Januarie
3. Chad Dirks
4. Zanele Gwanya
5. Althea Claasen

96. Other (**j.** Specify: _____)

SECTION A: Pesticide Exposure The following section is about possible contact with pesticides.	
1.1. Are you currently living on a farm?	0. No 1. Yes

1.1.1. Please specify the name of the farm?	
1.2. Is any one of your family members who lives with you working on a farm?	0. Nobody works on a farm 1. Mother 2. Father 3. Siblings 4. Grandparents 5. Other
1.2.1. Please specify the name of the farm or farms if there is more than one?	
Since the last time, we saw you for the urine sample and questionnaire	
1.3. Did you see any of these spraying activities happening on the field/vineyard/orchard close to you? **Hint**: show spraying activities cheat sheet 1	0. Never 1. Aeroplane spraying 2. Tractor spraying 3. Knapsack spraying
1.3.1. Specify how many times you saw aeroplane spraying? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.3.2. Specify how many times you saw tractor spraying? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.3.3. Specify how many times you saw knapsack spraying? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
Since the last time, we saw you for the urine sample and questionnaire	
1.4. How often could you smell the pesticides that were being sprayed on the nearest field/vineyard/orchard? Even if you did not see that they were sprayed? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.5. How often did you go into field/vineyard/orchard after pesticides were sprayed? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.6. How often did you play/swim/bath in the nearest dam/river/or any other water body? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
In the past seven (7) days, how often did you do the following	

1.7. How many days did you eat crops *(including fruits)* from the field/vineyard/orchard that were sprayed?	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.7.1. Did you wash the crops/fruits you ate?	0. No 1. Yes
1.7.2. Specify which crop/fruit it was? **Hint**: show cheat sheet 2.2 point out days in the week	1. Table grapes 2. Apples or pears 3. Citrus fruits such as oranges 96. Other (1.7.2.1. Specify: _____)
1.7.3. Eat Table grapes **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.7.4. Apples or pears **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.7.5. Citrus fruits such as oranges or lemons **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.7.6. Any other fruit **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
Since the last time, we saw you for the urine sample and questionnaire	
1.8. How often were chemicals or pesticides used in your home (indoors or outdoors) to control fleas, cockroaches, ants, termites, or other insects and weeds? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week

Section B Farming activities

The next few questions will be about how often you helped with activities or work on the farms since the last time we saw you for the urine sample and questionnaire

1.5. Picking fruits in the field/vineyard/orchard? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week

1.5.1. When helping with picking fruit, were you given any protective clothes like clothes or boots? **Hint**: show PPE cheat sheet	0. No 1. Yes
1.6. Pesticide or chemicals spraying mixing or loading (this could be transporting the pesticides in any way)?	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.7. Helped with cleaning farm equipment? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.8. Assist in pesticide storage? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
1.9. Help with burning any pesticide or chemical containers? **Hint**: show cheat sheet 2.2 point out days in the week	0. Never 1. One day a week 2. Two days a week 3. Three or more times a week
Section 3: Injury and exposure to drugs The following questions are about possible injury and other activities you may be involved in since the last time we saw you for the urine sample and questionnaire.	
1.10. Have you ever had an accident while playing/working where you fell and hit your head very badly?	0. No 1. Yes 99. Don't know
1.11. If yes (to 1.25), did you have any serious bleeding to your head that you had to go to the hospital?	0. No 1. Yes 99. Don't know
1.12. How long were you in the hospital for?	1. 1-6 days 2. 1-4 weeks 3. >1 month
1.13. Did you experience vomiting or losing consciousness or maybe confused, not sure where you were at the time of the accident? *(define to participant passing out/fainting)*	0. No 1. Yes 99. Don't know
1.14. Please tell us how often this happens to you since the last time we saw you for the urine sample and questionnaire **Hint**: show never, rarely, sometimes, always cheat sheet	

1.14.1. Difficulty in falling asleep at night?	0. Never 1. Rarely 2. Sometimes 3. Always
1.14.2. Agitated during your sleep at night?	0. Never 1. Rarely 2. Sometimes 3. Always
1.14.3. Waking up during your sleep at night?	0. Never 1. Rarely 2. Sometimes 3. Always
1.14.4. Waking up too early in the morning?	0. Never 1. Rarely 2. Sometimes 3. Always
1.15. Have you ever tried to smoke? *(If participant has said they only tried once, then tick never; otherwise, we want to recall regular smokers)* **Hint**: show cigarette amount cheat sheet	0. Never 1. 1-2 cigarettes a week 2. 3-4 cigarettes a week 3. More
1.16. Have you ever tried to drink alcohol? *(If participant has said they only tried once, then tick never; otherwise, we want to recall regular drinkers)* **Hint**: show alcohol amount cheat sheet	0. Never 1. 1-2 glasses a week 2. 3-4 glasses a week 3. More
1.17. Have you ever tried taking recreational drugs *(e.g. tik, marijuana)*? *(If participant has said they only tried once, then tick never; otherwise, we want to recall regular drug issues)* **Hint**: show amount cheat sheet	0. Never 1. 1-2 times a week 2. 3-4 times a week 3. More

APPENDIX 3: Physical examination for boys – Tanner staging

Study Number: _____

Date: _____

Physician/Nurse: _____

Measures/evaluations of height, weight, testes disposition, varicocele and hydrocele have been performed with the man in a standing position.

Evaluation of pubic hair should be according to the stages of Tanner, for which illustrations have been provided.

For evaluation of testes size, the orchidometer provided has to be used.

Characteristic	**Response**		
1. Height (cm)			
2. Weight (kg)			
3. Birth weight (kg)			
Genital region			
4. Scars due to surgery	☐$_1$ Yes → *describe in "other remarks" below* ☐$_2$ No		
5. Pubic Hair and penis: Tanner stage	☐$_1$ 1 ☐$_2$ 2 ☐$_3$ 3 ☐$_4$ 4 ☐$_5$ 5		
6. Penis	☐$_1$ Normal ☐$_2$ Abnormal → *describe in "other remarks" below*		
Testiscular morphology	Size (ml)	Consistency *N = Normal (1)* *S = Soft (2)* *H = Hard (3)*	Abnormality *1 = Yes* *2 = No*
7. Left testes		☐	☐
8. Right testes		☐	☐
9. Other remarks			

APPENDIX 4: Ethics Acceptance for current sub-study

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45, E-52- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

23 November 2021

HREC REF: 722/2021

Prof M Jeebhay
Centre for Environmental and Occupational Health Research
Falmouth Building-FHS
Email: Mohamed.Jeebhay@uct.ac.za
Student: mandyphuti@gmail.com

Dear Prof Jeebhay

PROJECT TITLE: THE RELATIONSHIP BETWEEN REPRODUCTIVE HORMONES AND ASTHMA RELATED OUTCOMES IN BOYS RESIDING IN THE RURAL WESTERN CAPE. (MASTER'S DEGREE – MANDY S PHUTI)-SUB-STUDY LINKED TO 234/2009

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020: 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 November 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Mandy Phuti will also be involved in this study.

Please quote the HREC REF 722/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

APPENDIX 5: Ethics Acceptance of follow -up study 2009

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za

01 June 2009

REC REF: 234/2009

Dr MA Dalvie
Public Health & Family Medicine

Dear Dr Dalvie

PROJECT TITLE: FOLLOW-UP STUDY OF REPRODUCTIVE HEALTH EFFECTS DUE TO ENVIRONMENTAL PESTICIDE EXPOSURE AMONG BOYS IN THE WESTERN CAPE, SOUTH AFRICA.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 05th June 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

sariefdien

APPENDIX 6: Ethics Acceptance- Annual approval for the study

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/06/22
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	22/7/21

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	11 June 2021		
HREC REF Number	234/2009	Current Ethics Approval was granted until	30/06/2021
Protocol title	An epidemiological cohort study of school-going children investigating reproductive and neurobehavioral effects due to environmental pesticide and cell phone use in the Western Cape		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ✓ ☐ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study	597/2017, 637/2018, 645/2018, 181/2019,		
Principal Investigator	Professor Aqiel Dalvie		
Department / Office Internal Mail Address	School of Public Health and Family Medicine, room 4.31, 4th floor, Falmouth Building, Faculty of Health Sciences		

HUMAN RESEARCH ETHICS COMMITTEE
22 JUL 2021
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ No ✓

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes	
1.3 Annual Approval for **full committee** review	- R 3450 (inclusive of vat)
For invoicing purposes, please provide:	
Sponsor's name	
Contact person	
Address	
Telephone number	
Email Address	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	Closed to enrolment (tick ✓)
☐✓	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	1002
Number of participants enrolled, since last HREC Progress report (continuing review)	1002
Additional number of participants still required	0

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	0

6. Cumulative summary of participants

Total number of participants who provided consent	1002
Number of participants determined to be ineligible (i.e. after screening)	0
Number of participants currently active on the study	1002
Number of participants completed study (without events leading to withdrawal)	-
Number of participants withdrawn at participants' request (i.e. changed their mind)	0
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	0
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
The baseline survey of the children was completed in 2017, the follow-up study in 2019 and all the follow-up of urinary samples, hair samples and participant exposure questionnaires were completed at the end of 2019. During 2020 and early 2021, most of the remaining guardian questionnaires interviews were conducted telephonically due to the COVID19 pandemic restrictions with overall 95% now completed. Urine specimens collected was couriered to the Lund University laboratory for pesticide analysis. Data analysis and write-up has progressed during the reporting producing several research outputs. Further fieldwork will only be considered if the COVID19 restrictions are lowered and it is safe to do so. Strict COVID19 procedures will followed.

8. Protocol violations and exceptions (tick ✓ all that apply)

✓☐	No prior violations or exceptions have occurred since the original approval

☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐✓	No prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☐ ✓ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☐ ✓ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☐ ✓ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ No ✓
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☐ ✓	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☐ No ✓
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

14. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	11 June 2021

APPENDIX 7: Approval letter from Department of Education

Audrey.wyngaard@westerncape.gov.za
tel: +27 021 467 9272
Fax: 0865902282
Private Bag x9114, Cape Town, 8000
wced.wcape.gov.za

REFERENCE: 20150629-846
ENQUIRIES: Dr A T Wyngaard

Prof Aqiel Dalvie
School of Public Health and Family Medicine
Health Sciences Faculty
Anzio Road
Observatory
7729

Dear Prof Aqiel Dalvie

RESEARCH PROPOSAL: REPRODUCTIVE HEALTH EFFECTS DUE TO PESTICIDE EXPOSURE AMONGST CHILDREN IN THE RURAL WESTERN CAPE IN SOUTH AFRICA

Your application to conduct the above-mentioned research in schools in the Western Cape has been approved subject to the following conditions:

1. Principals, educators and learners are under no obligation to assist you in your investigation.
2. Principals, educators, learners and schools should not be identifiable in any way from the results of the investigation.
3. You make all the arrangements concerning your investigation.
4. Educators' programmes are not to be interrupted.
5. The Study is to be conducted from **20 July 2015 till 30 September 2017**
6. No research can be conducted during the fourth term as schools are preparing and finalizing syllabi for examinations (October to December).
7. Should you wish to extend the period of your survey, please contact Dr A.T Wyngaard at the contact numbers above quoting the reference number?
8. A photocopy of this letter is submitted to the principal where the intended research is to be conducted.
9. Your research will be limited to the list of schools as forwarded to the Western Cape Education Department.
10. A brief summary of the content, findings and recommendations is provided to the Director: Research Services.
11. The Department receives a copy of the completed report/dissertation/thesis addressed to:

The Director: Research Services
Western Cape Education Department
Private Bag X9114
CAPE TOWN
8000

We wish you success in your research.

Kind regards.
Signed: Dr Audrey T Wyngaard
Directorate: Research
DATE: 01 July 2015

APPENDIX 8: Permission request letter for the school principal and school b

Date: _____/_____/_____

Dear Principal

Re: An epidemiological cohort study of school-going children investigating reproductive and neurobehavioral effects due to environmental pesticide and cell phone use in the Western Cape, South Africa

We would like to ask for your permission to include Grade 4 – 9 learners at your school in the important study above conducted by the University of Cape Town, Centre for Environmental Health and Occupation Research.

This study will investigate the reproductive and neuro-behavioural health effects that pesticides and radiation from cell phone usage may have on children. This will be of benefit to people who make use of cell phones and those exposed to pesticides in the environment that can be absorbed through the skin, breathed in and ingested through contaminated drinking water. The learners will undergo free medical testing and will benefit educationally from participation in the study.

This is a 3-year study, starting in 2017 and ending in 2019. Our sample population is 510 boys and 510 girls from 3 different farmland areas so we will require 340 learners from each area and about ±55 learners from each grade. In the 1st year, the learner will be required to complete a questionnaire at their home on demographic details, health and pesticide exposure and they will be required to perform the following tests at school: produce a urine and blood sample, undergo a physical examination of the genital area; perform a neurobehavioural test on a computer and complete a short questionnaire on pesticide exposure and cellphone use. These tests will be repeated in 2019. The tests will cause minimal disruption as they will last for only 2 hours at most. Additionally, a urine sample will be collected from each learner and a short questionnaire on pesticide exposure administered at school every 3 months during 2017-2019

Participation by your school involves identification of Grade 4-9 classes at the school, making available a copy of the class lists and their birth certificates if possible, distributing letters to all Grade 4-9 parents (copy enclosed) asking them for permission to include their child in the study and arranging an appropriate venue at the school on the days of testing during 2017-2019.

We would like to ensure that you, the learner and their guardian/parent offer your consent to participation before we conduct the study.

The results of the study will help to inform regulations to reduce harmful environmental exposures in residential areas in the Western Cape.

The survey has the approval of the Department of Education and Research Ethics Committee of the University of Cape Town.

Yours sincerely

Associate Professor MA Dalvie (Principle Investigator)

Cell phone number: 0827863781

APPENDIX 9: Caregiver consent form

Consent to participate in a study investigating reproductive and neurobehavioral effects due to environmental pesticide and cell phone use exposure in the Western Cape

1. **Title of the research project**

   An epidemiological cohort study of school-going children investigating reproductive and neurobehavioral effects due to environmental pesticide and cell phone use exposure in the Western Cape, South Africa

2. **Names of researchers**

   Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)

   Wisdom Basera (HBMLS, MPH)

   Shala Mhlanga (BSc (Hons), MSc)

3. **Purpose of the research project**

   This study will investigate the reproductive and neuro-behavioural health effects that pesticides and radiation from cell phone usage may have on children. This study will be of benefit to communities who make use of cell phone use and those exposed to pesticides in the environment that can be absorbed through the skin breathed in and ingested through contaminated drinking water. Your child will undergo free medical testing and will benefit educationally from participation in the study.

4. **Description of the research project**

   This is a 3-year study, starting in 2017 and ending in 2019. In the 1st year, you will be required to complete a questionnaire at your home on your child's demographic details, health and pesticide exposure and your child will be required to perform the following tests at school: produce a urine and blood sample, undergo a physical examination of the genital area; perform a neurobehavioural test on a computer and complete a short questionnaire on pesticide exposure and cellphone use. These tests will be repeated in 2019. The tests will cause minimal disruption as they will last for only 2 hours at most. Additionally, a urine and hair sample will be collected from your child and a short questionnaire on pesticide exposure administered at school every 3 months during 2017-2019.

   The following are more detailed explanations of what each assessment will entail:

a) **Guardian Questionnaire:** A member of our study team will interview you to fill out a ±1hour questionnaire. You will be asked questions about general information about your child, his/her general medical health, genital health history, development, cell phone usage and lifetime environmental exposure to pesticides.

b) **Urine and hair samples:** Your child has to produce a urine sample (in privacy) voiding it into a plastic container and give it to the nurse. The nurse will also draw a few strands of hair or shave a small amount of hair from your child The samples will be analysed for the presence of pesticides.

c) **Blood sample:** A study nurse will draw 10 ml of blood from a vein on your child's arm. The blood will be analysed for reproductive hormone levels.

d) **Physical examination:** A nurse will assess your child's reproductive health and development by examining their genital area.

e) **Participant Questionnaire:** A member of our study team will administer a 20-minute questionnaire to your child. It has questions on whether they have a cell phone and about their experience with using cell phones and any other technical equipment linked to an internet source. There are a few questions on their leisure activities to determine their exposure to pesticides and electromagnetic fields (internet etc.) that we are studying.

f) **Behavioural Assessment:** This is a 30-40 minutes assessment to test brain functions like reaction and memory, to be administered by a member of our study team. Your child will be given a tablet, with a program that will ask them to follow instructions and respond through a touch-screen, similar to a computer game.

5. Risks and discomforts of the research

i. **From the blood tests:** A single needle stick will be felt when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure reproductive hormones and will be disposed of at the end of the study.

ii. **From the urine and hair samples:** There will be no discomfort as the urine sample is done privately by the participant themself in the toilet facility. Only a small amount of hair will be collected. The urine and hair samples will only be used to measure any evidence of metabolised pesticides and will be disposed of after this laboratory test.

iii. **From the physical examination:** This examination will have some discomfort for the participant as it requires them to reveal their genital area. However, this exam will be done in a private setting with the use of a curtained zone and in a professional manner by a nurse. In addition, the exam is observational and therefore will be done quite briefly.

iv. **From the questionnaires:** There are minimal risks associated with completing the questionnaires. The only risk is a loss of confidentiality about personal information but the data will be seen only by study personnel. All reports will present data in which individuals will not be identifiable by name but by their study number.

v. **From the behavioural assessment:** There is no risk in completing this assessment. It has been specifically adapted to accommodate children and their ability in this age group.

6. Expected benefits to you and others

i. A doctor/nurse will examine your child's reproductive health.

ii. Refreshments will be provided as compensation for the time spent participating in the study.

iii. This study on the reproductive health effects of pesticides will benefit children living in farming areas and those exposed from the environment. Steps can be taken to reduce or prevent exposure or the pesticides can be selected for further investigation and subsequent banning. The findings from the blood and the urine samples can be used to develop ways in which the amount of pesticides in your body can be monitored in people exposed such as yourself.

iv. The assessment on your child's neurobehavioral status will provide you with information about the child's functioning/coping in their daily activities for school tasks, home tasks and social interaction.

7. Costs from participation in the study.

The study is offered to you at no cost.

In the event, a problem is discovered and you wish to be seen by a doctor for it, we can recommend someone for you to see. However, the study cannot pay for these additional medical visits or treatments.

The University of Cape Town (UCT) has insurance cover for the event that research-related injury or harm resulting from your child's participation in the study. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the study. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that your child may sustain where the loss is caused by:

The use of unauthorised medicine or substances during the study.

- Any injury that results from your child not following the protocol requirements or the instructions that the study nurse may give.
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication.
- An injury that results from negligence on your child's part.

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses. An injury is considered study-related if, and to the extent that, it is caused by study activities. You must notify the study nurse immediately of any side effects and/or injuries during the study, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your child's injury came about because your child chose not to follow the instructions that your child was given while taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected.

8. Confidentiality of information collected

Study participants will not be personally identified in any of the study reports. The records will be kept confidential to the extent provided by law. The records, including any identification information, will be destroyed after the data collected has been fully analysed.

9. Documentation of the consent

One copy of this document will be kept together with our research records. A second copy will be given to you to keep.

10. Contact person

You may contact the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study:

Principal Investigator: Professor Mohamed Aqiel Dalvie Telephone #: 021 4066610

Researcher: Mr Wisdom Basera Telephone #: 082 5802776

Researcher: Mrs Shala Mhlanga Telephone #: 072 3308540

Ethics Administrator: Lamees Emjedi Telephone #: 021 4066338

Nature of participation

The participation in this project is voluntary (assent from your child) subsequent to your consent, you may refuse your child to participate or withdraw from the study at any time without penalty or loss of benefits to which you may otherwise be entitled.

Consent of the Parent/Guardian

I have read the information given above. I understand the meaning of this information. I hereby consent my child, _____ to participate in the study.

Printed name of Parent/Guardian	Signature	Date

Printed name of Researcher	**Signature**	**Date**

Printed name of Witness	**Signature**	**Date**

Date: _____

APPENDIX 10: Permission letter for parent

Date: _____/_____/_____

Dear Parent/Guardian

Re: An epidemiological cohort study of school-going children investigating reproductive and neurobehavioral effects due to environmental pesticide and cell phone use exposure in the Western Cape, South Africa

We would like to ask for your permission to include your child in the important study above conducted by the University of Cape Town.

This study will investigate the reproductive and neuro-behavioural health effects that pesticides and radiation from cell phone usage may have on children. This will be of benefit to people who make use of cell phones and those exposed to pesticides in the environment that can be absorbed through the skin, breathed in and ingested through contaminated drinking water. Your child will undergo free medical testing and will benefit educationally from participation in the study.

This is a 3-year study, starting in 2017 and ending in 2019. In the 1st year, you will be required to complete a questionnaire at your home on your child's demographic details, health and pesticide exposure and your child will be required to perform the following tests at school: produce a urine and blood sample, undergo a physical examination of the genital area; perform a neurobehavioural test on a computer and complete a short questionnaire on pesticide exposure and cellphone use. These tests will be repeated in 2019. The tests will cause minimal disruption as they will last for only 2 hours at most. Additionally, a urine sample will be collected from your child and a short questionnaire on pesticide exposure administered at school every 3 months during 2017-2019.

Please note children at the school will be randomly selected to participate in the study and that your child might not be selected to participate. Can you please indicate in the note attached if you give permission for your child to participate in the study?

The results of the study would help in further planning in reducing environmental exposures in rural areas in the Western Cape. and the impact it has on children's health and development.

The survey has the approval of the Department of Education and Research Ethics Committee of the University of Cape Town.

Yours sincerely

Associate Professor MA Dalvie (Principle Investigator)

Cell phone number: 0827863781

APPENDIX 11: Child assent form

The Western Cape Pesticides and Cellphone use study

Introduction

Introduction

Hi [child's name]! My name is_____ and I would now like to talk to you about your health. Before I begin, I want to assure you that we have your parent or guardian's permission to approach you. You now have the right to refuse to participate, after I explain to you what we want to do.

1. **Title of the research project**

Reproductive and neurobehavioral effects due to environmental pesticide exposure and cell phone use in the Western Cape, South Africa

2. **Purpose of the research**

People have done research on the pesticides that farmers use to protect their crops from insects and how they affect our health. There is very little research done in SA on how these pesticides are harmful to children, so with your help, our study will be one of the very few done so far. Through The University of Cape Town, we are going to be looking at 2 important areas of health that may be affected from being exposed to the pesticides used on the farm and EMF through cell phone usage. This will help other children living in farming areas who are exposed to pesticides by ensuring that farmers cannot use those harmful chemicals. Pesticides can spread from the environment by the wind that disperses it to drinking water, to skin and may be breathed in. Cell phones are also another area with little research done so far. so we hope to find out more on the effects of mobile phones through this study.

3. **Description of the research project**

This is a 3-year study. The study will be done in the 1st year, 2017 and then again in the 3rd year, 2019. In each of these years, we will need you for one day to do some tests and answer some questionnaires. In between the 2 years, a nurse will visit you every 3 months for a urine sample, hair sample and a short questionnaire on pesticide exposure related activities.

If you agree to participate, you will be asked to complete:

a) Questionnaire:

I want you to know that the answers you give me to the questions I ask about your health and cell phone usage will be private and we won't share your answers with other kids or

with your parents. Only project members of this study will see the answers and they will use these answers to help you improve your health. There are no right or wrong answers to these questions I will ask you. We want to know how you feel. Also, if you do not want to answer one particular question or if you want to stop at any time and not answer any more questions, you can do that by telling me you don't want to continue. Nothing will happen to you if you decide not to answer these questions. But your participation is important and will help us understand health problems in children and this will help other children who might have similar health problems in the future.
This is a 10-15 minute questionnaire, administered by a member of our study team. It has questions on whether you have a cell phone and about your experience with using cell phones and any other technical equipment linked to an internet source.

There are a few questions on your leisure activities to determine your exposure to pesticides and cell phone use (internet etc.) that we are studying.

b) Urine and hair sample: We will collect a urine sample and a hair sample from you to test for chemicals.
c) Blood sample: A nurse will draw a small blood sample from you to check the level of your hormones.
d) Physical examination: A nurse will do a very brief body assessment by examining your genital area.
e) Behavioural Assessment: This is a 30-40 minutes assessment to test your brain functions like reaction and memory, to be administered by a member of our study team. You will be given a tablet, with a program that will ask you to follow instructions and respond through a touch-screen, similar to a computer game.

4. **Confidentiality of information collected**

Your name will not appear in any reports on this study. The records of questionnaires, assessments, blood samples, urine samples and examination, will be kept completely confidential at the University of Cape Town and will be seen only by our study team.

5. **Contact person**.

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study. You may also contact these persons for questions related to your child's rights or any injury you may feel is related to the study.

Principal Investigator: Professor Mohamed Aqiel Dalvie Telephone#: 021 4066610

Researcher: Mr Wisdom Basera Telephone#: 082 5802776

Researcher: Mrs Shala Mhlanga Telephone#: 072 3308540

Ethics Administrator: Lamees Emjedi Telephone#: 021 4066492

6. **Assent for your participation**

The information above has been read to me. I understand the meaning of this information

Dr./Mr./Ms. _____has offered to answer any questions concerning the study. By signing this form, I agree to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

_____

_____

Printed name of child Signature, Mark, or Thumb Print

_____

_____

Interviewer's name (Print) Signature

_____

_____

Witness (Print) Signature

DATE: _____

APPENDIX 12: All Questions used in the sub-study

Source	Type of variable and question
	Asthma-related Questions
Parent/ Guardian Questionnaire	**5.1** Has your **child** ever had wheezing or whistling in the chest at any time in the past?
	5.2 If yes (to **5.17**), has your **child** had wheezing or whistling in the chest in the past 12 months?
	5.3 If yes **(**to **5.17)** how many attacks of wheezing has the **child** had in the past 12 months?
	5.4 In the past 12 months, how often on average, has your **child's** sleep been disturbed due to wheezing?
	5.5 In the past 12 months, has the wheezing ever been serious enough to limit your **child's** speech to only one or two words at a time between breaths?
	5.6 Has your **child** ever had asthma?
	5.7 In the past 12 months, has your **child's** chest sounded wheezy during or after exercise?
	Controls and confounders
	5.8 What was the duration of pregnancy (gestational age) at the birth of the child? *(Hint: refer to Road to Health card if available)*
	5.9 What was the **child's** birth weight (grams)? *(Hint: refer to Road to Health card if available)*
	5.10 What was the **child's** birth length (cm)? *(Hint: refer to Road to Health card if available)*
	5.11 What was the **child's** birth head circumference (cm)? *(Hint: refer to Road to Health card if available)*
	5.11.1 Obesity/Overweight
	5.11.2 Foetal Alcohol Syndrome (FAS)
	5.11.3 Tuberculosis
	5.11.4 Any Lung infections
	5.32 Does anyone in the biological family have any of the following allergic diseases? (Tick all that apply)
	9.16 Did the mother smoke during pregnancy?
	9.17 Does anyone in the household currently smoke or ever smoked at home?
Child	Age

Questionnaire	1.2. Are you currently living on a farm?
	1.1.2. Please specify the name of the farm?
	1.5. Have you ever tried to smoke? (If participant has said they only tried once, then tick never; otherwise, we want to recall regular smokers) Hint: show cigarette amount cheat sheet
	1.18. Have you ever tried to smoke? (If participant has said they only tried once, then tick never; otherwise, we want to recall regular smokers) Hint: show cigarette amount cheat sheet
Physical Examination	Height (cm)
	Weight (kg)
	Birth weight (kg)

APPENDIX 13: Author instructions

The Journal of Allergy and Clinical Immunology: In Practice

https://www.jaci-inpractice.org/

Author Information (https://www.jaci-inpractice.org/content/authorinfo)

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

Introduction

JACI: *In Practice* publishes clinically impactful articles on the spectrum of conditions treated by allergist-immunologists in their practice: *food allergy, respiratory disorders* (including asthma, allergic and nonallergic rhinitis/rhinoconjunctivitis, nasal polyps, chronic sinusitis, cough, chronic obstructive pulmonary disease (COPD), allergic bronchopulmonary aspergillosis (ABPA), and hypersensitivity pneumonitis), *drug allergy, insect sting allergy, anaphylaxis, dermatologic disorders* (including atopic dermatitis, contact dermatitis, urticaria, angioedema, and hereditary angioedema [HAE]), *immunodeficiency, autoinflammatory syndromes, eosinophilic disorders,* and *mast cell disorders*. **The Journal emphasizes cutting edge practical clinical information for practitioners** that they can use in everyday practice or will help them acquire new knowledge or skills they can directly apply to patient care. Mechanistic or translational studies without immediate or near future clinical relevance and animal studies are not within the scope of the Journal.

Please Note: When selecting a title for your paper, please consider the following guidelines:

Keep the title succinct: Limit it to 12 words or fewer.

- Communicate a single subject or idea in the title.
- Construct the title around the article's key words.
- Include the specific symptom, condition, intervention, mechanism, or function of the paper's central focus.
- Mention any defining population, age, or gender that distinguishes the work.
- Use terms that are specific rather than general (e.g., "penicillin" rather than "betalactam antibiotic") and include terms that clarify (e.g., "fractional exhaled nitric oxide" rather than "airway inflammation").
- Avoid using strong words (such as "robust," "innovative," "significant," "vigorous," and "aggressive"), as they may suggest exaggerated or unwarranted claims.
- Use wit carefully and appropriately; be informative first and clever second. Although a universally understood pun can work well to attract interest, ensure that it will not confuse or mislead the reader.
- The titles of papers accepted for publication in *The Journal of Allergy and Clinical Immunology: In Practice* may be revised for improved clarity and appeal to the readership. Such revision will have final approval by the authors.

Article types

The *Journal* will consider publication of several types of manuscripts:

A. Original articles. These articles should describe fully, but as concisely as feasible, the results of original clinical research. Original Articles should not exceed **3,500** words, not including the abstract, figure legends, and references. Each figure legend should be held to **60** words or less. Each Original Article may be accompanied by a total of no more than **8** graphic presentations (tables and/or figures).

Original Articles should include:
1. Title page. The first page of the manuscript should be a title page, containing the following items:

- A brief, clear title.
- The list of authors, including their full names, highest academic degrees, and institutional affiliations. **Please note:**
- The name, address, telephone number, and email address of the author who should be contacted regarding the manuscript *following its publication*. Note: A different author may be designated as the Corresponding Author in the submission system for the duration of the submission and review processes.
- Email addresses should be provided for all authors.
- A declaration of all sources of funding for the research reported in the manuscript. Note regarding National Institutes of Health (NIH)-sponsored research: JACI: *In Practice*'s publisher, Elsevier, facilitates author posting in connection with the posting request of the NIH (referred to as the NIH "Public Access Policy"). For more information about PubMed Central, please visit http://www.ncbi.nlm.nih.gov/pmc/about/faq/.
- Word count for the Abstract and word count for the text.

2. Abstract. The abstract should be no longer than **250** words. It should summarize the results and conclusions concisely. Tabular data should not be included and acronyms/abbreviations should be avoided or spelled out fully. Abstracts should be structured as follows:

- **Background:** What is the major problem that prompted the study?
- **Objective:** What is the purpose of the study?
- **Methods:** How was the study done?
- **Results:** What are the most important findings?
- **Conclusion:** What is the most important conclusion drawn?

In addition to written Abstracts, the *Journal* will also consider Visual Summaries. Visual Summaries should be submitted with the manuscript and will undergo peer review. Please note that these are not guaranteed for acceptance, even if the manuscript is accepted.

3. Highlights box. Each Original Article will be accompanied by a *highlights box* that provides answers (no longer than **35** words each) to the following questions:

1. What is already known about this topic?
2. What does this article add to our knowledge?
3. How does this study impact current management guidelines
4. Key words. A list of up to 10 key words should follow the Highlights Box.

5. Abbreviations. Provide a list of any abbreviations/acronyms and their definitions following the key words. Only standard abbreviations are to be used. If you are uncertain whether an abbreviation is considered standard, consult *Scientific Style and Format* by the Council of Science Editors or the AMA's *Manual of Style*. A laboratory or chemical term or the name of a disease process that will be abbreviated must be spelled out at first mention, with the acronym or abbreviation following in parentheses. This policy should be followed for both the abstract and manuscript separately.

6. Text. The manuscript should be written in clear and concise English. The text should be organized into the following sections: **Introduction, Methods, Results,** and **Discussion**. Each section should begin on a new page. The generic terms for all drugs and chemicals should be used.

- In studies involving human subjects, a statement describing approval by the appropriate Institutional Review Board is required.

7. Acknowledgments. General acknowledgments for consultations, statistical analyses, and the like should be listed at the end of the text, including full names of the individuals involved. However, as noted above, acknowledgment of funding should be listed on the title page.

8. References. It is the Editors' expectation that authors will perform a comprehensive search of the literature to

gather the most current articles relative to the subject matter. Guidelines for formatting references can be found below.

B. Clinical Communications. Clinical Communications are brief reports of clinical or laboratory observations or case series. Single case reports will only be considered if they demonstrate a novel, impactful insight, rather than simply an educational point. Clinical Communications are limited in scope, and without sufficient depth of investigation to qualify as Original Articles. Like Original Articles, these manuscripts are subject to peer review.

In case report submissions, authors should include a statement in the manuscript confirming that informed consent was obtained from the patient (or caregiver if the patient is a dependent) to publish the case report along with all accompanying visual elements. Additionally, all identifying details of the patient should be omitted if they are not essential.

A Clinical Communication must:
(1) Be brief. A Clinical Communication should not exceed **1,000** words, not including the figure legend(s) and references. The figure legend(s) should be held to **60** words or less. Please note: Clinical Communication manuscripts that are determined to exceed these limits will be returned to the authors for shortening prior to review.
(2) Have a short, relevant title.
(3) Have a complete title page (see above section A1).
(4) Provide 1-2 sentences (maximum **40** words) that summarize the clinical implications and importance of the report to be used in a *Clinical Implications box* published at the beginning of the article.
(5) Have no more than **9** references.
(6) List the references as complete bibliographic citations following the end of the letter body.
(7) Be limited to a total of **2** figures and/or tables. (An additional **2** figures or tables may be placed in the article's Online Repository)
(8) Not have references in the Online Repository.

C. Images in Allergy. Images in Allergy articles focus on pictures (eg, of physical examination findings, cutaneous eruptions, allergens, radiographs, rhinoscopy findings, etc.) that intrinsically impart important clinical information that the allergist-immunologist should visually recognize to provide optimal care. Ideally, the image will provide characteristic features that are unique to a particular diagnosis. They are accompanied by a brief description, limited to **500** words, that elaborates upon the unique features of the image and their relationship to diagnosis or management of clinical disease, possibly related to a specific case presentation. Up to 2 references may be included.

In case report submissions, authors should include a statement in the manuscript confirming that informed consent was obtained from the patient (or caregiver if the patient is a dependent) to publish the case report along with all accompanying visual elements. Additionally, all identifying details of the patient should be omitted if they are not essential.

D. Correspondence and Replies. Correspondence concerning articles recently published in *JACI: In Practice* will be considered for publication and accepted based on their pertinence, their scientific quality, and available space in the *Journal*. If the correspondence is considered acceptable, a response will be requested from the authors of the referenced *JACI: In Practice* article. Upon review and approval by the Editor, the Correspondence and relevant Reply will both be published together.
Both Correspondence and Reply manuscripts must:
(1) Be no longer than **500** words.
(2) Have a short, relevant title, distinct from the title of the referenced article. Please note that all Replies should have the title "Reply to [First author's name]."
(3) Have a complete title page (see above section A1).
(4) List the references as complete bibliographic citations at the end of the letter with the *Journal* article being discussed as the first reference. The total number of references should be no more than seven. Replies should include as two of the first references the Correspondence to which they are responding and the published article that initially started this conversation.
(5) Have no more than **one** graphic presentation (table or figure).
(6) Begin with the salutation "To the Editor:" and close with the author's name(s), academic degree(s), institution(s), and location(s).

E. Review articles. Review articles published in the Journal are invited by the Editors. Proposals for review articles may be emailed to the Editorial Office (InPractice@aaaai.org), but current space constraints do not usually allow for the acceptance of unsolicited review manuscripts. Specific guidelines for review articles will be provided to authors when needed.

F. Rostrum articles. Opinion articles about subjects of particular interest and/or debate may be accepted for peer review after preliminary review by the Editor. Proposals for rostrum articles may be emailed to the Editorial Office (InPractice@aaaai.org); they will be evaluated based on level of interest, novelty, and the current needs of

the *Journal*. Specific guidelines for Rostrum articles will be provided to authors upon request.

G. Practice Options From Beyond Our Pages. This feature is focused on identifying, critiquing, and placing into context research studies that have the potential to change our clinical practices. Published studies beyond the pages of the *Journal of Allergy and Clinical Immunology: In Practice* and the *Journal of Allergy and Clinical Immunology* that have a high likelihood of changing practice **NOW** should be the focus of submissions in this series. Articles to consider are meta-analyses, randomized double-blind placebo-controlled trials, effectiveness studies, new diagnostic breakthroughs, etc.

Who should submit: Allergy-Immunology Fellows-In-Training partnered with faculty members. Authors do not require an invitation to submit. Submission does not guarantee publication. Suggestions for revisions may be made before the contribution is considered acceptable.

Practice Options From Beyond Our Pages should have the following characteristics:
(1) Be **1,000** words or less.
(2) The title should be a succinct description of the major topic and the potential practice change.
(3) The manuscript text should be arranged in the following format:
(a) *Reference*: The study that is being reviewed.
(b) *Background*: The authors should clearly state the current clinical practice and/or guideline and how this study has the potential to change the current practice.
(c) *Methods*: Summary of the methods used in the study that is being reviewed.
(d) *Results*: Summary of the main results. (Possibly include a small table. Please note that permissions would need to be obtained for any tables reproduced from the original study).
(e) *Critical appraisal*: The authors should discuss any major limitations of the study and how they influence the potential to translate the findings into practice. Comparisons with previous studies that addressed similar practice questions should be considered and appropriately cited in a reference list at the end of the manuscript.
(f) *Recommendation*: The authors should briefly state the recommended practice change.

H. Practice Pearls.This is a feature that promotes sharing of clinical wisdom among practicing allergist-immunologists. A **Practice Pearl** is something that helps an allergist-immunologist practice more safely, effectively, timely, efficiently, equitably, or in a more patient-centered, way. A **Practice Pearl** is generally not a case report of a very unique situation and is not based on a formal study, but is rather a solution to a practical challenge that is developed by the submitter and can be applied by allergist-immunologists to help many patients.

Submissions should be structured into two sections: (1) Practice Challenge and (2) Practice Solution. Submissions should be no longer than **300** words and inclusion of up to two illustrations (figures or tables) and two references are optional. Audio and video online supplements are encouraged. Submissions will be peer-reviewed prior to acceptance.

I. Case Studies in Health Disparities. This feature is a case report with particular attention given to highlighting any social determinants of health (SDOH) as described by Healthy People 2030 (https://health.gov/healthypeople/objectives-and-data/social-determinants-health) that are relevant to and have an impact on the case. Salient features of the case should be integrated into the discussion and clarify the specific SDOH(s) relevant to allergy/immunology clinical care. The case should also be used to provide practical actionable steps that a clinician could apply with similar future patients.

In case report submissions, authors should include a statement in the manuscript confirming that informed consent was obtained from the patient (or caregiver if the patient is a dependent) to publish the case report along with all accompanying visual elements. Additionally, all identifying details of the patient should be omitted if they are not essential.

Case Studies in Health Disparities must:
(1) Be no longer than 1000 words.
(2) Have no more than two visual elements (tables or figures).
(3) Have no more than 9 references.
(4) Have a final section of "Practical Tips" based on the case that clinicians could implement in their practices.

J. Topics in Quality Improvement and Patient Safety. The content of this feature includes quality and safety measurement techniques, requirements, and recommendations as well as quality and safety improvement processes and outcomes in various aspects of the field of Allergy/Immunology. Health equity is a component of health care quality and thus a relevant topic for this feature. Articles may be either Original Articles or Review Articles, which can be submitted without an invitation.

Original Article submissions of Topics in Quality Improvement and Patient Safety

The formatting for the *Original Articles* in this feature is as described above for Original Articles. Specific potential

research topics include:

- Development, adaptation, and/or implementation of innovative thinking, strategies, and practices in improving quality and safety in health care.
- Development, validation and/or assessment of clinical tools for quality improvement.
- Research studies of new methodologies or novel applications of methodologies on the effectiveness of improvement interventions.
- Research advances and field applications in areas of patient safety including adverse events, system modifications that are barriers to error, and the impact of regulatory changes on healthcare delivery.
- Research advances in understanding and addressing modifiable reasons for health inequities and interventions aimed at reducing health disparities.

Review Article submissions of Topics in Quality Improvement and Patient Safety

The formatting for *Review Articles* in this feature is as follows:

The overall length of the manuscript should be limited to 3000 words (not counting the title page, abstract, key words, abbreviations, and references) and should include relevant figures and tables. Please also include in the manuscript file a conflict of interest disclosure statement, an unstructured abstract of approximately 200 words, as well as a list of keywords and abbreviations used in your manuscript.

Review articles for this feature should summarize information pertaining to relevant aspects of quality and safety, such as: process improvement, quality measurement, safety culture, adverse event reporting, root cause analysis, health equity, and patient experience. The articles should provide practical guidance for the allergy/immunology clinician.

Feature Box (for review articles):

This feature discusses quality and safety measurement techniques, requirements, and recommendations as well as quality and safety improvement processes and outcomes in various aspects of the field of Allergy/Immunology. It is coordinated by Kimberly Blumenthal and Nicholas Rider.

Notes about this new feature

- Original Articles would be under the TOC sub-header "***Topics in Quality Improvement and Patient Safety***"
- A new Collection would be added for this feature.
- The first article in this series will be accompanied by an Editorial by Drs Blumenthal and Rider.
    - Both will be published S300 not released as an article in press).
- After the first article, the subsequent articles will be released as S5 uncorrected proofs).

SUBMISSION

Submission to this journal online (through https://www.editorialmanager.com/inpractice/default.aspx) and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail. For instructions regarding how to use the submissions site, please visit https://service.elsevier.com/app/answers/detail/a_id/116.

Submit your article

Please submit your article via https://www.editorialmanager.com/inpractice/default.aspx.

Peer Review Process

All manuscripts are reviewed by at least one editor. Manuscripts may be rejected by the editor(s) without peer review due to being out of the journal scope or if they are determined to be low priority based on considerations such as novelty, validity, generalizability, and clinical impact. Manuscripts warranting further consideration are sent for peer review. Most manuscript are evaluated by at least 2 reviewers, and a biostatistical reviewer is

requested when appropriate. The peer review process is single-blind with the identity of the reviewers concealed from the authors. The editor(s) make decisions regarding the acceptability of the manuscripts (accept, revise, decline) based on the reviewer recommendations, including confidential comments to the editors and comments to the authors. Appeals may be considered by contacting the editorial office (inpractice@aaaai.org).

Before You Begin

Ethics in publishing

Please see our information on Ethics in publishing.

Human studies and consent

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

In case report submissions, authors should include a statement in the manuscript confirming that informed consent was obtained from the patient (or caregiver if the patient is a dependent) to publish the case report along with all accompanying visual elements. Additionally, all identifying details of the patient should be omitted if they are not essential.

Conflict of Interest

All authors must disclose all financial relationships for themselves and their immediate family/significant others. The Journal requires all authors to acknowledge, on the title page of the manuscript, all funding sources that supported their work and any commercial associations that might pose a conflict of interest. These include consultant arrangements, speakers' bureau participation, stock or other equity ownership, patent licensing arrangements, support such as financial or materials grants for research, employment, or expert witness testimony. Further information can be found at https://www.elsevier.com/conflictsofinterest and at https://service.elsevier.com/app/answers/detail/a_id/286/supporthub/publishing.

The Corresponding Author is responsible for obtaining each author’s statement and all authors should see and approve the complete disclosure before submission to the Journal.

Permission to reuse previously published material/informed consent releases

If applicable, authors of manuscripts submitted to *JACI: In Practice* must provide the Editorial Office with proof of permission to reuse any previously published material that has appeared in another publication. Because articles appear in both the print and online versions of the journal, wording in the permissions form/release should specify "permission to publish in all forms and media." Written permission to reuse the specified material can be uploaded with the manuscript submission or forwarded to the Editorial Office by email (InPractice@aaaai.org) or fax (319-467-7583). Acceptance of a manuscript is conditional upon receipt of permission. Additionally, in the case of photographs of identifiable persons, it is required that the author obtain written consent from said person. Confirmation of this consent will be requested at the time of submission.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

JACI: *In Practice* allows submissions that have been posted on preprint servers. Upon publication in our journal, authors are required to update their preprint article with a link to the final published version.

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If a preprint is used as a reference, it should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided. View Elsevier's Preprint Policy. **Please note** that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints (eg, on a preprint server) will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting Race and Ethnicity

JACI: *In Practice* encourages the reporting of race and ethnicity in all clinical studies unless the information is not available. Reporting of race and ethnicity should not be considered in isolation but should be accompanied by reporting and discussion of intersecting sociodemographic and social determinant factors. The following guidance is provided to standardize and optimize the reporting of race and ethnicity in the *Journal* and is based on updated guidance in the *AMA Manual of Style*.*

- "The names of races, ethnicities, and tribes should be capitalized, such as eg, African American, Alaska Native, American Indian, Asian, Black, Cherokee Nation, Hispanic, Kamba, Kikuyu, Latino, and White."*
- The term "White" should be used instead of "Caucasian" except when referring to people from the Caucasus region in Eurasia.
- "The term *minorities* should not be used when describing groups or populations because it is overly vague and implies a hierarchy among groups."* Other terms such as *underserved populations, underrepresented populations, marginalized/historically marginalized*, or *historically excluded* may be used as more accurate and descriptive terminology.
- "Racial and ethnic terms should not be used as a noun form (eg, avoid Asians, Blacks, Hispanics, or Whites)."* The adjectival form should be used instead (eg, Asian women, Black patients, Hispanic children, or White participants), which follows AMA style regarding person-first language.
- Do not use the term race/ethnicity but use the term *race and ethnicity* instead.
- Provide an explanation of how participant race and ethnicity was classified "and the source of the classifications used (eg, self-report or selection, investigator observed, database, electronic health record, survey instrument)."*
- Provide an explanation of how participant race and ethnicity was classified and the source of the classifications used (eg, self-report or selection, investigator observed, database, electronic health record, survey instrument).
- "Specific racial and ethnic categories are preferred over collective terms, when possible."* Define what categories are included in groups labeled as *other*. "The terms *multiracial* and *multiethnic* are acceptable in reports of studies if the specific categories these terms comprise are defined or if the terms were predefined in a study or database to which participants self-selected."*
- "Categories should be listed in alphabetical order in text and tables.
- "Race and ethnicity categories of the study population should be reported in the Results section of the manuscript."*
- When appropriate, outcomes should be stratified by race and ethnicity.
- In the Discussion, comment on the overall representatives of the clinical study regarding race and ethnicity and discuss the relevance of any underrepresentation to the condition being studied.

For studies focusing on health disparities by race or ethnicity, the following additional guidance is provided:

- Provide a conceptual model to convey the relationships between race and/or ethnicity with other variables being tested. Original literature relevant to the conceptual model should be referenced when applicable.
- Self-identification is the gold standard for identification of race and ethnicity with write-in and/or ability to select multiple categories.
- Unless the study design allows more specific and/or precise race and ethnicity identification, use the 2020 U.S. Census classifications established with guidance from the Office of Management and Budget: American Indian or Alaska Native; Asian; Black or African American; Native Hawaiian or Pacific Islander, White; and whether of Hispanic, Latino/a/-x or Spanish origin (ethnicity).
- Provide justification in the context of the scientific question when including race or ethnicity as a covariate in risk-adjustment models.
- Test and report the results from analyses according to the proposed conceptual framework.
- Interpret data according to the proposed conceptual model.

Reporting Sex and Gender

- The term *sex* should be used when reporting biological factors and *gender* should be used when reporting gender identity or psychosocial/cultural factors.
- The methods used to obtain information on sex, gender, or both (eg, self-reported, investigator observed or classified, or laboratory test) should be explained in the Methods section.
- The sex and/or gender distribution of study participants should be reported in the Results section.
- When appropriate, outcomes should be stratified by sex and/or gender.
- In the Discussion, comment on the overall representatives of the clinical study regarding sex and/or gender and discuss the relevance to the condition(s) being studied.

Authorship

As recommended by the International Committee of Medical Journal Editors (ICMJE) (ICMJE | Recommendations | Defining the Role of Authors and Contributors), in order to be included in the list of authors on a manuscript, an individual must have done all of the following: (1) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafted the article or reviewed it critically for important intellectual content; (3) given final approval of the version to be published; and (4) agreed to be accountable for all aspects of the work.

The Journal of Allergy and Clinical Immunology: In Practice (JACI: *In Practice*) does not allow "ghostwriting" or uncredited authorship. All writers of a manuscript should be clearly identified.

First authorship

It is assumed that the author in the first position of the manuscript's author list has taken primary responsibility for the presentation and content of the article and is designated as having *first authorship*. In the less common circumstance in which two authors have shared equally in this primary responsibility, they may be designated as having *co-first authorship*. This will be identified in the manuscript with an asterisk next to their names in the author list and a footnote saying, "these authors have contributed equally and are designated to have co-first authorship." First authorship may not be shared by more than two authors.

Corresponding authorship

Upon submission, one (and only one) author is designated as the Corresponding Author. All correspondence from the Journal regarding the manuscript will go to that author. The Corresponding Author is expected to share information with the other authors as appropriate. At the time of publication, the name and email address of the Corresponding Author will appear in a footnote on the first page of the article. A different author may be designated as the post-publication Corresponding Author simply by providing that author's name, mailing address, and email address on the title page of the manuscript at the proof stage.

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Authors are expected to carefully consider the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. The rationale for any addition or deletion of author names in the authorship list after submission will need to be provided to the Journal editor by the Corresponding Author. If the manuscript has already been published, any requests for changes to authorship approved by the editor will result in a corrigendum.

Reporting clinical trials

Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with International Committee of Medical Journal Editors recommendations. **NOTE: CLINICAL TRIALS MUST REGISTER AT OR BEFORE THE ONSET OF PATIENT ENROLLMENT.** The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration. For any questions, please contact the Editorial Office at inpractice@aaaai.org.

JACI: *In Practice* offers expedited reviews for qualifying randomized controlled clinical trials. An expedited review will provide an initial decision within 14 days. To qualify for expedited review, randomized clinical trials must be 1) deemed to be novel, generalizable, and clinically impactful by the editors, 2) registered with ClinicalTrials.gov or a similar acceptable registry, and 3) accompanied by the CONSORT checklist, final trial protocol, and Statistical Analysis Plan (SAP).

Special instructions regarding statistical analyses and reporting

1. METHODS: Reporting on Statistical Methods. The Consolidated Standards of Reporting Trials (CONSORT) statement is a set of guidelines for reporting on the methods and results of randomized and nonrandomized medical research studies.

The first CONSORT statement provides a checklist of items that should be included in a manuscript that reports the results of a randomized clinical trial (RCT). Items 7 through 12 of the checklist are relevant to the statistical methods section for a manuscript submitted to *JACI: In Practice* based on a RCT. Thus:

• With respect to item 12, the statistical methods and commercial software should be cited.
• Item 7 and item 12 of the checklist are relevant to the Statistical Methods section of a manuscript submitted to *JACI: In Practice* based on a nonrandomized study. Thus:

2. RESULTS.
Items 13 through 19 of the CONSORT checklist describe items that are important to the Results section for a manuscript submitted to *JACI: In Practice* based on a RCT (some of the items might not be relevant if the study is nonrandomized). Thus:

2A. Results: Descriptive Statistics at Baseline
If the distribution for a continuous variable is approximately normally distributed, then report either
• the sample mean and the sample standard deviation or
• the sample mean and the 95% confidence interval for the population mean.
If the distribution for a continuous variable is known (or suspected) to be nonnormal, then report either
•the sample median and the sample interquartile range or
• the sample median and the sample first and third quartiles.
Many blood and urine measurements are log-normally distributed-i.e., the logtransformed variable is approximately normally distributed. If the distribution for a continuous variable is known (or suspected) to be lognormal, then an alternative to sample medians and quartiles is to report either
• the sample geometric mean (calculate as the exponentiation of the sample mean of the natural log-transformed data) and the sample coefficient of variation or
• the sample geometric mean and the 95% confidence interval.
If the distribution of the variable is categorical, then report the raw numbers and the percentages for the categories. Do not use more than three digits for the percentages-i.e., 79% or 79.3% are fine, but 79.32% is not. Statistical tests, along with reported *P* values, for comparing groups at baseline are not necessary unless there is a strong reason to include them.

2B. Results: Outcomes
• Every *P* value should be reported using two digits after the decimal point. If each of the first two digits after the decimal point is zero, then a third digit can be used. If each of the first three digits after the decimal point is zero, then simply report $P < .001$.
• If the *P* value is close to the level to be used for claiming a statistical significance or if each of the first two digits after the decimal point is zero, then a third digit can be used. For example, if the significance level is 0.05, then $P = .046$ or $P = .054$ can be reported. Nonsignificant results (e.g., where the *P* value is > 0.05) should be accompanied by *P* values; it should not simply be stated that they are nonsignificant (NS).

• *P* values alone are not sufficient to report the results of statistical tests. *JACI: In Practice*'s readers need to see the magnitude of the effects via point estimates and 95% confidence intervals for the group comparisons.

An estimate of odds ratios and relative risks (and their corresponding confidence interval estimates) should not exceed two digits beyond the decimal point.

2C. Results: Primary Outcomes, Multiple Comparisons, and *Post Hoc* Comparisons
• Prespecified primary outcome/analysis should be identified, as well as any prespecified secondary, subgroup, and/or sensitivity analyses. Additional analyses considered during the course of the prespecified analyses or after the study was completed should be identified as post hoc. For analyses of more than one primary outcome, corrections for multiple testing should generally be used. For secondary outcomes, address multiple testing or consider such analyses as exploratory and interpret them as hypothesis-generating. For secondary and subgroup analyses, there should be a description of how the potential for type I error due to multiple comparisons was handled, for example, by adjustment of the significance threshold. In the absence of some approach, these analyses should generally be described and interpreted as exploratory.
2D. Results: Missing Data
• Report losses to observation, such as dropouts from a clinical trial or those lost to follow-up or unavailable in an observational study. If more than 10% of participants are excluded from analyses because of missing or incomplete data, provide a supplementary table that compares the observed characteristics between participants with complete and incomplete data. Consider multiple imputation methods to impute missing data and include an assessment of whether data were missing at random.

Adherence to other key guidelines

JACI: In Practice endorses the following guidelines and encourages authors to make every attempt to conform to their recommendations:

Allergen Nomenclature

The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergen(s), the systematic name of the allergen must be approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior to manuscript publication. To avoid the risk of delay of publication, authors are encouraged to apply for a new allergen name using the posted submission form at the WHO/IUIS Allergen Nomenclature website (http://www.allergen.org) before manuscript submission. The systematic nomenclature consists of the first three letters of the taxonomic genus of the allergen source, followed by a space; the first letter of the species epithet, followed by a space; and an Arabic numeral usually indicating the chronological order in which the allergen was described. For example, the first allergen to be purified from the house dust mite, Dermatophagoides pteronyssinus, is named "Der p 1." Further examples of the systematic allergen nomenclature for over 500 allergens can be found at :http://www.allergen.org. The submissions to the Allergen Nomenclature Sub-Committee will be kept confidential until publication if requested by the authors."

STROBE statement for observational studies
When preparing observational reports, we encourage authors to review the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) Statement, available at www.strobe-statement.org.

PRISMA guidelines for systematic reviews and meta-analyses
For meta-analysis of RCTs, we encourage authors to consult the recommendations of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement, available at www.prisma-statement.org.

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For reports of diagnostic studies, we recommend the STARD (Standards for Reporting of Diagnostic Accuracy) Statement, available at www.stardstatement.org.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

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Preparation

Basic formatting

The title page, abstract, key words, abbreviations, text, acknowledgments, references, and figure legends should be included in a single file (.doc or .docx format). Tables and their legends may be included at the end of the same file (after the reference list and figure legends, if applicable). Alternatively, tables and their legends can be loaded as a separate Tables file.

The generic terms for all drugs and chemicals should be used.

Figures should be uploaded each as separate Figure files, with the figure legends placed in the manuscript file, after the reference list. Tables can either be placed in the manuscript file, after the reference list and figure legends (if applicable), or uploaded as a separate Tables file. Please see the Artwork section for specific formatting information for Figures. Tables need to be created using Microsoft Words Tables function, and uploaded a .doc file(s).

All sections should be double-spaced. On each page, the page number should appear in the upper right corner. Begin numbering with the title page as page 1. Be sure to display line numbers (1, 2, 3, and so forth) in the left margin of the manuscript. The line numbering should be continuous throughout the entire manuscript, from the title page through final page (i.e., do not begin numbering from 1 again at the top of each page).

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References

It is the Editors' expectation that authors will perform a comprehensive search of the literature to gather the most current articles relative to the subject matter.

References should follow "Vancouver style." See the examples below, or http://www.nlm.nih.gov/bsd/uniform_requirements.html for more information. Manuscripts in preparation, personal communications, and other unpublished information should not be cited in the reference list but may be mentioned in the text in parentheses. Citing abstracts as references is strongly discouraged. An abstract should only be included as a reference if the evidence it provides is important to the manuscript and exists nowhere else in citable form. Abstracts that are included in the reference list must be bolded so that reviewers can easily identify them and evaluate their appropriateness. The references must be identified in the text by superscript Arabic numerals and numbered in consecutive order as they are mentioned in the text. The list of references, in numeric sequence, should be typed at the end of the article. In the submitted version of the manuscript, references should not appear as footnotes or endnotes, and if you have used a program such as EndNote or Reference Manager to create them, the links between the reference numbers and the citations must be removed using the following steps:

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Please note that inclusive page numbers are required. List **all** authors' names when there are six or fewer; when there are seven or more, list the first **six** before adding "et al."

Examples of Reference Formatting

Journal article:
Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood leukaemia in Europe after Chernyobyl: 5-year follow-up. Br J Cancer 1996;73:1006-12.

Book:
Ringsven MD, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

Chapter in a book:
Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: Pathophysiology, Diagnosis, and Management. 2nd ed. New York: Raven Press; 1995:465-78.

Internet resource:
US positions on selected issues at the third negotiating session of the Framework Convention on Tobacco Control. Washington, DC: Committee on Government Reform; 2002. Available at: http://www.house.gov/reform/min/inves_tobacco/index_accord.htm. Accessed March 4, 2002.

Preprint:
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Bloss CS, Wineinger NE, Peters M, et al. A prospective randomized trial examining health care utilization in individuals using multiple smartphone-enabled biosensors. *bioRxiv*. Preprint posted online October 28, 2015. doi: 10.1101/029983.

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• Construct the title around the article's key words.
• Include the specific symptom, condition, intervention, mechanism, or function of the paper's central focus.
• Mention any defining population, age, or gender that distinguishes the work.

• Use terms that are specific rather than general (e.g., "penicillin" rather than "betalactam antibiotic") and include terms that clarify (e.g., "CXCR4" rather than "chemokine receptors").
• Avoid using strong words (such as "robust," "innovative," "significant," "vigorous," and "aggressive"), as they may suggest exaggerated or unwarranted claims.
• Use wit carefully and appropriately; be informative first and clever second. Although a universally understood pun can work well to attract interest, ensure that it will not confuse or mislead the reader.
• The titles of papers accepted for publication in the *Journal of Allergy and Clinical Immunology: In Practice* may be revised for improved clarity and appeal to the readership. Such revision will have final approval by the authors.

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Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

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Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

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If no funding has been provided for the research, please include the following sentence:

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Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

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• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the printed version.
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TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
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TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
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Figure legends should be listed in the manuscript file, on a separate page after the tables. They should not appear in the figure files. The figure legend will be included when sizing the figure and its length must therefore be taken into consideration. The figure title should appear at the beginning of each legend. The legends themselves should be succinct (no more than 60 words), identifying the data or subject being presented, but not explaining methods or results.

Tables

If tables appear in the manuscript, they must be included in the electronic submission. They may be placed within the manuscript file or loaded as separate files (in .doc or .docx format). Tables should supplement, not duplicate, the text; they should be on separate pages, one table per page, and should be numbered with Roman numerals in order of mention. A brief title should be provided directly above each table. Any abbreviations should be defined at the bottom of the table. When creating a table, use the wordprocessing program's table formatting feature; otherwise, use only tabs (not spaces) to align columns.

Preprint references

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

Reference style

It is the Editors' expectation that authors will perform a comprehensive search of the literature to gather the most current articles relative to the subject matter. Citing abstracts as references is strongly discouraged. An abstract should only be included as a reference if the evidence it provides is important to the manuscript and exists nowhere else in citable form. Abstracts that are included in the reference list must be bolded so that reviewers can easily identify them and evaluate their appropriateness.

References should follow "Vancouver style." See the examples below,
or http://www.nlm.nih.gov/bsd/uniform_requirements.html for more information. Manuscripts in preparation, personal communications, and other unpublished information should not be cited in the reference list but may be mentioned in the text in parentheses. Citing abstracts as references is strongly discouraged. An abstract should only be included as a reference if the evidence it provides is important to the manuscript and exists nowhere else in citable form. Abstracts that are included in the reference list must be bolded so that reviewers can easily identify them and evaluate their appropriateness. The references must be identified in the text by superscript Arabic numerals and numbered in consecutive order as they are mentioned in the text. The list of references, in numeric sequence, should be typed at the end of the article. In the submitted version of the manuscript, references should not appear as footnotes or endnotes, and if you have used a program such as EndNote or Reference Manager to create them, the links between the reference numbers and the citations must be removed using the following steps:

(1) Using the "Select All" feature (Ctrl-A for PCs. Cmd-A for Macs), highlight the entire text of the file, including the references.
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(3) Save. This will remove the links (permanently) without disturbing the reference numbers or the citations. It is recommended that you save one copy of your manuscript with the EndNote links in place (for your reference) and one copy of your manuscript without the EndNote links (for submission purposes).

Please note that inclusive page numbers are required. List all authors' names when there are *six* or fewer; when there are seven or more, list the first six and add "et al."

Examples of Reference Formatting

Journal article:
Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood leukaemia in Europe after Chernyobyl: 5-year follow-up. Br J Cancer 1996;73:1006-12.

Book:
Ringsven MD, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

Chapter in a book:
Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Internet resource:
US positions on selected issues at the third negotiating session of the Framework Convention on Tobacco Control. Washington, DC: Committee on Government Reform; 2002. Available at: http://www.house.gov/reform/min/inves_tobacco/index_accord.htm. Accessed March 4, 2002.

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