

Original article

Maternal testosterone in pregnancy and atopic outcomes in childhood

Background: In mice, androgens downregulate Th2 cytokine responses, but whether androgen levels during pregnancy might influence the development of allergy in the offspring has not been studied.

Methods: In the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based birth cohort of 14 541 pregnancies, we related maternal blood total testosterone during pregnancy, measured in a subset of the cohort, to allergic outcomes in the offspring, including asthma, hayfever, eczema ($n = 543$) and wheezing ($n = 532$) at 69–81 months, and atopy (positive skin prick test to *Dermatophagoides pteronyssinus*, cat or grass, $n = 386$) and blood total immunoglobulin E (IgE; $n = 314$) at 7 years. We used logistic and linear regression to analyse binary outcomes and log-transformed IgE, respectively, controlling for potential confounders.

Results: Maternal testosterone was negatively associated with total IgE in boys [adjusted geometric mean ratio (GMR), per doubling of testosterone, 0.33 (0.20–0.55), $P = 0.000038$ ($n = 168$)], but not in girls [GMR 1.04 (0.53–2.06), $P = 0.91$ ($n = 146$)], P -value interaction 0.0086. The effect in boys was even stronger in the absence of maternal atopic disease. Testosterone was not associated with skin test positivity or atopic disease in either sex.

Conclusions: Higher testosterone levels in pregnancy are associated with lower IgE production in boys.

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Evidence is accumulating to suggest that the prenatal environment plays an important role in the aetiology of childhood atopy and asthma. Maternal exposures in pregnancy, which have been implicated, include tobacco smoke, antibiotics, paracetamol, infections and other complications, and nutrition (1–8). Cytokine production by the fetoplacental unit is thought to provide a key mechanism by which the prenatal environment influences maturation of the fetal immune system and risk of atopy after birth (9).

The relation between maternal sex hormone levels in pregnancy and atopic outcomes in the offspring has been little studied, although *in vivo* studies of adult mice have shown that androgens can reduce allergy. In particular, castration promotes, and testosterone inhibits, production of antigen-specific immunoglobulin E (IgE) in males (10), and dehydroepiandrosterone (DHEA), the precursor of sex steroids and a weak androgen, lowers total IgE

levels in males (11) and females (12). However, effects in adults may not be the same as those during critical periods of early development. Prenatal testosterone exposure is known to have a powerful programming influence on physical, neural and behavioural sex differences (13, 14), and could also influence sexual dimorphism in immune development (15, 16). In the case of allergic immune responses, boys tend to have a higher prevalence of allergen sensitization and higher total IgE levels than girls (17, 18). We have explored whether maternal blood testosterone level in pregnancy is negatively associated with allergy in the offspring.

Methods

The Avon Longitudinal Study of Parents and Children (ALSPAC; 19, 20) is a prospective study of 14 541 pregnancies. Women were

enrolled as early in pregnancy as possible, on the basis of an expected date of delivery between 1 April 1991 and 31 December 1992, and place of residence within the three Bristol-based health districts of the former county of Avon, UK. It was estimated that 85–90% of eligible pregnant women were enrolled. There were 14 062 live births (January 1991 to February 1993), and 13 971 infants survived to 1 year. The study is closely monitored by its own Ethics and Law Committee (21, 22) and by four local research ethics committees.

During pregnancy, maternal blood was taken, and plasma aliquots were stored, initially at -20°C , and subsequently at -70°C , and when the offspring were three and a half years of age, information was collected from their mothers about gender role behaviour, using the Preschool Activities Inventory (PSAI) questionnaire. As part of a study of the influence of prenatal testosterone exposure on gender role behaviour, PSAI scores were used to select a subset of stored maternal plasma samples for 336 boys and 331 girls in six groups of approximately similar size: boys and girls with extremely masculine scores, boys and girls with extremely feminine scores, and boys and girls randomly selected from the remaining children (23). Total testosterone and sex hormone-binding globulin (SHBG) were measured (nM) in 1998 in these samples, using an automated chemiluminescence assay (Chiron Diagnostics Corp., East Walpole, MA, USA) and the DELFIA fluoroimmunoassay (Wallac, Inc., Gaithersburg, MD, USA), respectively. The timing of the samples ranged from week 6 of gestation to week 38, with 75% of samples being taken between weeks 8 and 24, the period of testosterone surge in male fetuses (24).

When the children were 81 months old, mothers were asked: 'Has he/she had any of the following in the past 12 months: Asthma; Eczema; Hayfever?' (Data collected at 91 months suggest that, of mothers who responded positively to the question about asthma at 81 months, and subsequently gave a clear answer to the following question at 91 months: 'Has a doctor ever actually said that your study child has asthma?', 95% responded positively to this later question too.) Mothers were also asked: 'In the past year has he/she had any periods when there was wheezing with whistling on his/her chest when he/she breathed?' At 7 years, the child underwent allergen skin prick testing and venepuncture. Atopy was defined as a positive reaction (any detectable weal) to *Dermaphagoides pteronyssinus*, cat or grass (after subtracting positive saline reactions from histamine and allergen weals, and excluding children unreactive to histamine), and serum total IgE (kU/l) was measured by fluoroimmunoassay using the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Statistical methods

Statistical analysis was carried out using the STATA statistical package 9.1 (25). We measured associations of maternal levels of total testosterone, the ratio of total testosterone/SHBG, and SHBG during pregnancy, with atopic outcomes, in boys and girls separately. Hormone data were complete for 667 children. Of these, information was complete on atopy for 386, on total IgE for 314, on asthma, hayfever and eczema for 543, and on wheezing for 532. We used logistic regression to measure odds ratios (ORs) for atopy and the other atopic outcomes, and linear regression on the logs to measure geometric mean ratios (GMRs) for IgE (using Huber variances to define confidence intervals), per doubling of maternal hormone level (defined by entering the binary logarithm of the exposure into the model). We used a propensity score method for continuous exposures (26) to control for potential confounding variables, and weightings inversely proportional to sampling probability based on a combination of gender and decile of PSAI score

within gender. For each combination of exposure, outcome and gender, two models were used, namely an unadjusted model (in which the ORs or GMRs ratio were relative to a single baseline odds or GM) and a propensity-adjusted model (in which the ORs or GMRs were assumed to be common to six exposure-propensity groups, each with its own baseline odds or GM).

The propensity groups for each combination of exposure and gender were defined using the following potential confounders: mother's age group at birth of the child (under 25, 25–29, 30–34 and 35+ years), gestational age group when the blood sample was taken (0–9, 10–19, 20–29 and 30+ weeks), gestational age group at birth (<37, 37–40 and 41+ weeks), child's birth weight group (under 2500, 2500–2999, 3000–3499, 3500–3999, 4000+ g and unknown), maternal parity group (0, 1, 2+ and unknown), multiple pregnancy (nonmultiple or multiple), head circumference group (under 33, 33–34.99, 35–36.99 and 37+ cm and unknown), maternal body mass index (BMI) group based on self-reported prepregnancy height and weight (under 18.5, 18.5–24.99, 25–29.99 and 30+ kg/m^2 and unknown), maternal education level (below O level, O level only, A level or above and unknown), maternal housing tenure (owned/mortgaged, council rented, noncouncil rented and unknown/other), maternal ethnic origin (white, non-White and unknown), maternal tobacco smoke exposure group during pregnancy (unknown, unexposed, passive only and 1–9, 10–19 and 20+ cigarettes/day), maternal paracetamol exposure at 20–32-week gestation (never, some days, most/all days and unknown), maternal asthma history (yes and no), maternal rhinoconjunctivitis history (yes and no), maternal eczema history (yes and no), and unknown maternal disease history (yes and no). *Post hoc*, we controlled additionally for alcohol intake in pregnancy at two time points (units/week at 8 and 32 weeks) and during two time periods (glasses per day/week in first 3 months and last 2 months), as this exposure has been linked to maternal testosterone levels in pregnancy (27) and to atopic outcomes in the offspring (28, 29). For each of the six combinations of the three exposures (testosterone, SHBG and testosterone : SHBG ratio) and two genders, we defined a propensity score for each subject as the predicted Y-value for that subject from a regression model, in which the log of the exposure was the dependent variable and the confounders were the categorical predictors, and defined six propensity groups as the sextiles of the propensity score.

As secondary analyses, we stratified by maternal atopic disease history (presence of any of the three atopic conditions) and smoking in pregnancy, to explore possible effect modification by these factors. We also repeated the analyses with the sexes combined, and tested for interactions with gender. Finally, in view of the large number of ORs and GMRs measured, we used the Simes procedure (30), controlling the false discovery rate at 0.05, to discover a subset of effect estimates that could be considered statistically significant, given the multiple statistical tests carried out.

Results

In mothers of boys ($n = 336$) and girls ($n = 331$), the median (minimum, maximum) total testosterone level was 3 (1, 8.5) and 3 (1, 9) nM, respectively. The 80th/20th interpercentile ratio (IPR), as a measurement of variability in testosterone level, was 2.0. This ratio compares the bottom of the top quintile with the top of the bottom quintile. Consequently, a ratio of two, corresponding to a per-doubling OR or GMR, means that a doubling in testosterone level covers an appreciable fraction of the

range of testosterone. The median (minimum, maximum) SHBG level was 238 (44, 852) nM in mothers of boys and 250 (18, 804) nM in mothers of girls. The IPR was 2.4 and 1.9, respectively. Amongst boys and girls with complete hormone data the prevalence of atopy was 24.2% and 18.2%, respectively, and the GM IgE was 78.08 and 56.63.

Table 1 shows the relations of potential confounding factors to maternal testosterone and SHBG levels in pregnancy, by sex of offspring. Testosterone levels were higher in younger mothers, and testosterone and SHBG levels were higher later in pregnancy when the blood sample was taken, regardless of the sex of the offspring. When the offspring were male, higher testosterone levels were associated with greater levels of smoke exposure in pregnancy and with lower birth weight, and SHBG levels were higher in nulliparous and better educated mothers and in multiple pregnancies. When the offspring were female, testosterone levels were higher, and SHBG levels lower, in mothers with higher prepregnancy BMI.

When analyses were restricted to those with complete data on atopic outcomes, maternal total testosterone, SHBG and the testosterone : SHBG ratio were all unrelated to atopy in boys and girls (Table 2). In contrast, a doubling in total testosterone was associated with a highly significant reduction in total IgE of two-thirds in boys [GMR 0.33 (95% CI: 0.20–0.55), $P = 0.000038$], although no relation was seen in girls (Table 2). Controlling additionally, *post hoc*, for alcohol intake in pregnancy strengthened this association in boys [GMR 0.23 (0.13–0.43), $P = 5.3 \times 10^{-6}$]. There was also a negative association between SHBG and IgE in boys only. The testosterone : SHBG ratio was not related to IgE in either sex (Table 2). If we included total testosterone and SHBG simultaneously in the model for IgE in boys, the effect estimate for testosterone was unchanged, although even more significant [GMR 0.32 (95% CI: 0.21–0.48), $P = 1.6 \times 10^{-7}$], but the effect of SHBG was weakened [GMR 0.53 (0.32–0.90), $P = 0.019$]. In the absence of maternal atopic disease, the relation between total testosterone and IgE in boys became even stronger [GMR 0.15 (0.07–0.32), $P = 2.6 \times 10^{-6}$; P -value for interaction with maternal atopy 0.0043], and a negative association was seen between the testosterone : SHBG ratio and atopy in boys [OR 0.22 (0.08–0.61), $P = 0.0034$]. When we combined the sexes, the overall association between total testosterone and IgE was still significant [propensity-adjusted GMR 0.59 (0.38–0.90), $P = 0.014$], and there was evidence of a significant interaction with gender [ratio of adjusted GMRs for IgE (boys/girls) 0.32 (0.14–0.75), $P = 0.0086$].

Total testosterone was not significantly associated with other atopic outcomes. Whilst some effect estimates suggested positive, rather than negative associations, the confidence intervals were very wide and could not exclude negative effects. There was weak evidence in boys for a positive association between SHBG and eczema and

hayfever, and for a negative association between the testosterone : SHBG ratio and hayfever (Table 3). The latter association was stronger in the absence of maternal atopic disease [OR 0.26 (0.10–0.71), $P = 0.008$]. When the Simes procedure was carried out on the 36 adjusted gender-specific ORs and GMRs, the association between total testosterone and IgE in boys remained significant, being below a corrected critical P -value of 0.0014.

Discussion

We have found that a higher maternal level of testosterone in pregnancy is strongly associated with lower total IgE levels in boys. The P -values suggest that this novel observation is unlikely to have arisen by chance, even considering the number of statistical comparisons undertaken.

In keeping with our prior report on this data set (23), other studies have confirmed that maternal testosterone levels increase as pregnancy progresses (31, 32) and are lower in older mothers (33, 34). The positive relations of testosterone with smoking and BMI are also consistent with previous studies (35, 36). Given that we controlled for a large number of potential confounders, it seems unlikely that an 'unknown' confounder would be sufficiently strongly associated with maternal testosterone and IgE in the offspring to explain the main finding, although this possibility cannot be excluded. Similarly, as we only studied a small subsample of the whole cohort, which had been selected partly on the basis of behavioural characteristics, we have to consider whether this might have biased our findings. However, we weighted the analyses to take account of the sampling strategy, and for the negative association between testosterone and IgE to have arisen spuriously through selection bias, maternal testosterone would have to be very strongly *positively* associated with IgE in boys amongst cohort members who were not studied. One would expect biological effects of testosterone to be more strongly associated with free hormone than with total hormone levels. Whilst the testosterone : SHBG ratio has been used as a proxy estimate of free testosterone, it is generally agreed that it is a poor surrogate measure, especially in pregnancy, because the binding capacity of SHBG is saturated. We are therefore not surprised that associations with the ratio were not stronger than relations with total testosterone.

To our knowledge, the only study which has examined the possible relation between maternal sex hormones and allergy in the offspring, measured progesterone and estradiol in early pregnancy, and found no relation with asthma or atopic disease in the children at 5–6 years of age. However, neither maternal testosterone, nor objective markers of allergy in the children, was measured (37).

As reported previously for this data set (23), maternal testosterone levels did not differ according to sex of the offspring. This has been replicated by others (32, 38), and

Table 1. Associations of geometric mean maternal testosterone and SHBG levels in pregnancy with potential confounding factors, by sex of offspring

Confounder	Boys			Girls		
	N	Testosterone (nM)	SHBG (nM)	N	Testosterone (nM)	SHBG (nM)
Mother's age group (years)						
<20	6	2.51	277.85	9	3.13	204.49
20–24	42	3.21	240.01	40	2.80	291.89
25–29	133	2.81	225.07	131	2.88	241.56
30–34	121	2.56	225.57	104	2.70	222.13
35+	34	2.69	202.90	47	2.41	239.45
Gestational age group at sampling (weeks)						
0–9	73	2.80	138.06	53	2.81	169.55
10–19	190	2.62	228.90	194	2.64	226.90
20–29	36	3.00	350.05	30	2.87	327.03
30+	37	3.12	358.99	54	3.06	341.91
Gestational age group (weeks)						
<37	17	2.78	202.57	17	3.01	214.47
37–40	234	2.74	229.02	219	2.70	235.34
41+	85	2.77	220.93	95	2.82	253.74
Birth weight group (g)						
<2500	15	3.16	172.08	15	2.43	184.55
2500–2999	39	2.89	238.87	50	2.97	230.26
3000–3499	103	2.82	242.25	119	2.73	244.21
3500–3999	123	2.63	227.51	110	2.64	239.70
4000+	55	2.69	200.84	34	3.03	256.44
Unknown	1	2.00	179.00	3	2.82	324.75
Parity group						
0	134	2.85	255.22	153	2.82	255.43
1	127	2.73	207.63	103	2.67	220.51
2+	65	2.55	207.39	65	2.71	238.22
Unknown	10	2.98	212.49	10	2.78	211.86
Multiple pregnancy						
No	327	2.74	221.73	321	2.75	239.29
Yes	9	2.84	418.93	10	2.74	240.86
Head circumference group (cm)						
<33	13	3.31	203.37	21	3.03	222.10
33–34.99	100	2.68	243.42	123	2.73	235.15
35–36.99	123	2.73	224.55	101	2.85	265.83
37+	29	2.66	173.31	5	2.45	216.40
Unknown	71	2.81	231.67	81	2.60	221.26
Maternal BMI group (kg/m ²)						
<18.5	13	3.05	206.30	15	2.55	274.95
18.5–24.99	221	2.68	229.19	224	2.73	243.39
25–29.99	52	2.94	233.47	51	2.87	203.94
30+	13	3.09	204.81	16	3.09	231.35
Unknown	37	2.68	208.42	25	2.65	268.39
Mother's education level						
<O level	71	2.83	187.03	76	2.76	243.59
O level	104	2.73	217.23	112	2.64	237.03
A level+	148	2.70	252.23	135	2.82	238.94
Unknown	13	3.01	237.22	8	3.16	238.41
Housing tenure						
Owned/mortgaged	245	2.68	232.72	267	2.75	238.38
Council rented	41	2.92	193.00	26	2.73	219.78
Noncouncil rented	28	2.68	202.21	24	2.69	255.43
Unknown/other	22	3.26	244.43	14	2.82	270.72
Mother's ethnic origin						
White	315	2.74	224.73	320	2.76	240.14
Non-White	8	2.88	238.43	4	2.61	201.86
Unknown	13	2.91	237.98	7	2.48	226.32
Prenatal tobacco exposure (maximum in pregnancy)						
Not exposed	147	2.57	221.38	156	2.73	245.84
Passive only	109	2.82	234.36	98	2.75	237.18
Mother 1–9/day	28	2.90	214.44	31	2.77	235.91

Table 1. Continued

Confounder	Boys			Girls		
	N	Testosterone (nM)	SHBG (nM)	N	Testosterone (nM)	SHBG (nM)
Mother 10–19/day	35	2.99	243.29	25	2.73	261.38
Mother 20+/day	14	3.22	194.23	18	3.04	189.96
Unknown	3	3.11	185.41	3	2.29	177.77
Paracetamol at 20–32-week gestation						
Never	184	2.71	228.83	183	2.79	249.46
Sometimes	126	2.78	219.59	133	2.68	227.57
Most days/daily	5	2.83	195.66	2	2.39	220.43
Unknown	21	2.82	241.37	13	2.97	226.49
Maternal asthma history						
No	307	2.74	223.63	309	2.75	240.72
Yes	29	2.85	246.82	22	2.69	220.66
Maternal rhinoconjunctivitis history						
No	260	2.72	224.22	272	2.77	240.44
Yes	76	2.83	230.13	59	2.65	234.29
Maternal eczema history						
No	300	2.74	225.84	282	2.75	237.89
Yes	36	2.83	223.12	49	2.73	247.81
Maternal disease history unknown						
No	321	2.74	226.41	324	2.75	238.98
Yes	15	2.88	207.70	7	2.90	256.41

SHBG, sex hormone-binding globulin; BMI, body mass index.

suggests that testosterone does not cross the placenta from fetus to mother, and that maternal blood levels reflect maternally derived testosterone. However, there is debate as to whether maternal testosterone crosses the placenta to the fetus, and the extent to which maternal testosterone levels correlate with fetal levels may not be strong (39). In contrast, levels of testosterone in amniotic

fluid are mainly influenced by fetal production and hence do differ according to the sex of the fetus, and may therefore give a better estimate of fetal sex hormone exposure (32, 38). We therefore propose that maternal testosterone may *indirectly* influence IgE in the offspring through downregulation of maternal and fetoplacental Th2 cytokine production, and that the altered cytokine

Table 2. ORs and GMRs (with 95% CIs) for atopy and IgE at 7 years, per doubling of maternal blood total testosterone, SHBG and the testosterone : SHBG ratio during pregnancy

	N	OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
<i>Atopy</i>					
Testosterone (nM)					
Boys	194	1.14 (0.45–2.90)	0.78	0.96 (0.32–2.86)	0.94
Girls	192	1.41 (0.60–3.32)	0.43	1.19 (0.46–3.12)	0.72
SHBG (nM)					
Boys	194	1.64 (0.83–3.23)	0.16	1.39 (0.50–3.87)	0.53
Girls	192	0.85 (0.41–1.76)	0.67	1.32 (0.40–4.34)	0.64
Testosterone : SHBG ratio					
Boys	194	0.73 (0.40–1.33)	0.3	0.80 (0.40–1.61)	0.54
Girls	192	1.31 (0.69–2.50)	0.41	1.16 (0.50–2.69)	0.73
	N	GMR (95% CI)	P-value	Adjusted GMR (95% CI)	P-value
<i>IgE (kU/l)</i>					
Testosterone (nM)					
Boys	168	0.39 (0.23–0.65)	0.00037	0.33 (0.20–0.55)	0.000038
Girls	146	0.81 (0.41–1.62)	0.55	1.04 (0.53–2.06)	0.91
SHBG (nM)					
Boys	168	0.65 (0.42–1.02)	0.063	0.44 (0.25–0.78)	0.0054
Girls	146	0.94 (0.54–1.66)	0.84	0.97 (0.57–1.66)	0.92
Testosterone : SHBG ratio					
Boys	168	0.94 (0.63–1.42)	0.78	0.82 (0.49–1.37)	0.45
Girls	146	0.94 (0.57–1.53)	0.79	0.81 (0.52–1.26)	0.35

SHBG, sex hormone-binding globulin; IgE, immunoglobulin E; OR, odds ratio; CI, confidence interval; GMR, geometric mean ratio.

Table 3. ORs (with 95% CIs) for atopic disease outcomes at 81 months, per doubling of maternal blood total testosterone, SHBG and the testosterone : SHBG ratio during pregnancy

	N	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
<i>Asthma</i>					
Testosterone (nM)					
Boys	270	0.73 (0.32–1.66)	0.46	0.55 (0.18–1.70)	0.3
Girls	273	1.51 (0.44–5.24)	0.51	1.55 (0.46–5.22)	0.48
SHBG (nM)					
Boys	270	1.58 (0.84–2.97)	0.16	1.96 (0.51–7.52)	0.33
Girls	273	1.45 (0.91–2.31)	0.12	1.98 (0.69–5.67)	0.2
Testosterone : SHBG ratio					
Boys	270	0.65 (0.42–1.01)	0.056	0.59 (0.26–1.36)	0.22
Girls	273	0.93 (0.52–1.65)	0.8	1.05 (0.49–2.28)	0.89
<i>Wheezing + whistling</i>					
Testosterone (nM)					
Boys	265	2.49 (0.83–7.50)	0.1	3.08 (0.84–11.36)	0.091
Girls	267	2.83 (0.81–9.92)	0.1	2.24 (0.51–9.81)	0.28
SHBG (nM)					
Boys	265	2.41 (0.87–6.66)	0.091	3.93 (0.79–19.51)	0.094
Girls	267	1.34 (0.83–2.17)	0.24	2.09 (0.61–7.24)	0.24
Testosterone : SHBG ratio					
Boys	265	0.85 (0.57–1.28)	0.44	0.91 (0.51–1.61)	0.74
Girls	267	1.26 (0.71–2.24)	0.43	1.34 (0.54–3.34)	0.52
<i>Eczema</i>					
Testosterone (nM)					
Boys	270	2.19 (0.74–6.51)	0.16	1.73 (0.57–5.21)	0.33
Girls	273	1.52 (0.60–3.85)	0.37	1.64 (0.72–3.73)	0.23
SHBG (nM)					
Boys	270	1.28 (0.60–2.74)	0.52	2.32 (0.99–5.46)	0.054
Girls	273	1.50 (0.76–2.98)	0.24	1.47 (0.71–3.06)	0.3
Testosterone : SHBG ratio					
Boys	270	1.12 (0.54–2.33)	0.76	0.96 (0.48–1.89)	0.9
Girls	273	0.91 (0.53–1.58)	0.74	0.93 (0.50–1.74)	0.83
<i>Hay fever</i>					
Testosterone (nM)					
Boys	270	0.85 (0.23–3.13)	0.81	0.70 (0.19–2.62)	0.59
Girls	273	0.99 (0.16–6.08)	0.99	1.04 (0.13–8.34)	0.97
SHBG (nM)					
Boys	270	2.72 (1.18–6.27)	0.019	4.17 (1.27–13.73)	0.019
Girls	273	0.38 (0.15–0.97)	0.043	0.44 (0.14–1.37)	0.16
Testosterone : SHBG ratio					
Boys	270	0.50 (0.28–0.91)	0.022	0.44 (0.22–0.90)	0.024
Girls	273	2.11 (0.84–5.28)	0.11	2.33 (0.70–7.67)	0.17

SHBG, sex hormone-binding globulin; OR, odds ratio; CI, confidence interval.

milieu, in turn, influences maturation of the fetal immune system in favour of Th1 responses and lower IgE after birth (9). If this were the case, one might expect to see a negative association between maternal testosterone and atopy in the mother. Whilst we found no significant relation between testosterone and reported maternal atopic disease, we could not examine relations with maternal atopy more definitively, as neither allergy skin prick testing, nor IgE or cytokine measurements had been performed in the mothers. One might anticipate, however, that effects of maternal testosterone on the cytokine milieu would be more pronounced in the absence of maternal atopic disease, as maternoplacental cytokine production is likely to be less markedly Th2 skewed and over-riding in this situation, and this is what we found.

Support for a downregulating effect of testosterone on Th2 cytokines comes from studies of adult mice which have shown that DHEA *in vivo*, and the metabolite of testosterone, dihydrotestosterone, *in vitro*, reduce levels and expression of interleukin (IL)-4, IL-5, IL-6 and IL-10 (11, 12, 40, 41). Data are conflicting on the effects of these androgens on the Th1 cytokines interferon- γ and IL-2 (12, 40, 41).

We were surprised to find a strong relation between maternal testosterone and IgE in boys and not in girls. A previous study in this cohort showed a positive relationship between maternal testosterone in pregnancy and male-typical gender role behaviour (as measured by PSAI score) in girls (23), a finding consistent with evidence that testosterone promotes male-typical neural,

behavioural and physical development in a wide variety of mammalian species (13, 14). Based on this established role of testosterone in sexual differentiation, and the fact that boys have higher IgE than girls, we might have predicted that higher maternal testosterone would be associated with higher, rather than lower, levels of IgE. Therefore, our findings do not seem to have resulted from the effects of testosterone on processes involved in sexual differentiation. To explain the intriguing sex difference, we speculate that maternal and fetoplacental cytokines may have a greater influence on the immune system of males than females. In support of this hypothesis, we examined the association between maternal atopic disease and childhood IgE in the whole cohort and found a much stronger positive relation in boys than in girls (*P*-value for interaction 0.018). This is in keeping with the observation in another birth cohort that maternal atopic disease was associated with an increased risk of early persistent wheezing in boys, but not in girls (42), although maternal atopy was not a stronger predictor of childhood wheeze in boys than in girls in a New Zealand birth cohort (43).

In contrast to the strong relation with total IgE in boys, total testosterone was not associated with atopy or atopic disease. Whilst these discordant results might seem contradictory, there are examples of other risk factors, such as prenatal paracetamol exposure (7), and large head circumference at birth (44, 45), which are related to total IgE, but not to atopy. On the other hand, in this relatively small sample, we had less statistical power to detect modest effects on binary outcomes, as reflected by the wide confidence intervals around the effect estimates for atopy and atopic disease, than we did to detect effects on total IgE, which was a continuous variable.

In conclusion, we have found a strong negative relation between maternal testosterone in pregnancy and total IgE

in male offspring, and propose that the underlying mechanism may involve downregulation of maternal and fetoplacental Th2 cytokines, which in turn influences maturation of the male fetal immune system. However, given the absence of association with atopy and atopic disease, the apparent gender-specific nature of the relation with total IgE, and that these findings come from a small selected subset of the ALSPAC cohort, we regard these findings as preliminary, and are interpreting them with caution. It would be of interest to confirm whether they are generalizable and can be replicated in other birth cohorts. Further understanding of these observations may come from investigating relations of testosterone, oestrogen, other hormones and cytokines, to atopic outcomes after birth.

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