

Maternal Asthma Is Associated with Reduced Female Fetal Growth

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Asthma during pregnancy is associated with a low birth weight, although the mechanisms contributing to this outcome remain unknown. The relationship between maternal asthma and its treatment, placental function, fetal sex, and low birth weight was examined to establish the effect of asthma on fetal growth. Glucocorticoid intake by women with asthma was assessed throughout pregnancy. The placenta was collected after delivery, and 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) activity was measured. Fetal cortisol and estriol were measured in the umbilical vein plasma at delivery. Those with asthma were compared with a nonasthmatic control group. In women with asthma who did not use inhaled steroids and were pregnant with a female fetus, we observed significantly reduced birth weights, whereas male birth weights were unaffected. The presence of a female fetus was associated with significantly increased maternal circulating monocytes, significantly reduced placental 11 β -HSD2 activity and fetal estriol, and a trend toward elevated fetal plasma cortisol. This study provides evidence that in pregnancies complicated by asthma there is a fetal sex-specific effect on the maternal immune system with adverse effects on placental function and female fetal growth.

Keywords: asthma; pregnancy; placenta; fetal growth; 11 β -hydroxysteroid dehydrogenase type 2

The prevalence of asthma in Western societies is increasing (1, 2). Inflammatory diseases during pregnancy such as asthma (3–8), malaria (9, 10), rheumatoid arthritis (11), inflammatory bowel disease (12, 13) and systemic lupus erythematosus (14) are characterized by poor pregnancy outcomes, including low birth weight. Low birth weight predisposes neonates to an increased risk of developing diseases such as hypertension (15, 16), heart disease (17), and diabetes (18) in adult life. In addition, the maternal–fetal environment during pregnancy may be an important factor in the development of atopy and asthma in childhood (19, 20). The mechanisms causing low birth weight in women with asthma are currently unknown; however, alterations in placental function, asthma severity, or treatment may be contributing factors (21). Inflammation in the mother may also play a role in fetal growth regulation, as elevated maternal serum levels or increased placental gene expression of inflammatory cytokines

such as tumor necrosis factor- α , interleukin (IL)-8 (10, 22, 23), IL-18 (24), and macrophage colony-stimulating factor (25) have previously been associated with intrauterine growth restriction.

The placenta plays an important role in controlling fetal growth by supplying nutrients and oxygen from the mother. The placenta also prevents the transfer of large concentrations of maternal cortisol to the fetus. The placental enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) performs this function, acting as a barrier by metabolizing cortisol to inactive cortisone, thereby preventing excess maternal cortisol from reaching the fetus, where it may inhibit fetal growth. Previous studies have demonstrated reduced 11 β -HSD2 enzyme activity in intrauterine growth restriction (26).

This study has been a detailed examination of the relationships between mother, placenta, and fetus in pregnancies complicated by asthma, with the aim of understanding what factors are important for normal fetal growth in this disease state. Some of the results have been previously reported in the form of abstracts (27, 28).

METHODS

The study was approved by the Hunter Area Health Service and University of Newcastle Human Research Ethics Committees. Pregnant women with and without asthma were recruited in the John Hunter Hospital antenatal clinic during the first trimester after a previously described protocol (21). Clinical asthma severity was rated as mild, moderate, or severe using the integrated severity score described in the Australian Asthma Management Guidelines (29), which closely approximate the National Heart, Lungs, and Blood Institute Guidelines (30). Proper inhaler use and compliance was assessed in the Asthma Management Service. Cumulative, inhaled glucocorticoid dose was calculated for each trimester and summarized as the mean daily dose of beclomethasone dipropionate or equivalent used during pregnancy, where 1 μ g of beclomethasone dipropionate was considered equal to 1 μ g of budesonide or 0.5 μ g of fluticasone propionate (31). Subjects with asthma were grouped based on glucocorticoid dosage: no glucocorticoid use, low-dose glucocorticoid (less than 400 μ g/day), moderate-dose glucocorticoid (400–1,500 μ g/day), and high-dose glucocorticoid (more than 1,500 μ g/day). For most data analysis, the low-, moderate-, and high-dose groups were combined (glucocorticoid). Women with asthma in all groups used the inhaled β_2 agonist salbutamol for symptom relief when required.

A full blood count was measured in maternal blood from samples collected in early pregnancy (less than 20 weeks) and late pregnancy (more than 30 weeks).

Fetal biparietal diameter, head circumference, abdominal circumference, and femur length were measured at 18 and 30 weeks of gestation by ultrasound. Birth weight and head circumference were recorded at delivery, and centiles were calculated using John Hunter Hospital intrauterine growth charts (32), based on gestational age determined by the date of the last menstrual period and an 18-week ultrasound. The placenta and cord blood were collected after delivery from a subset of patients.

Placental 11 β -HSD2 activity was measured as previously described (33) by the conversion of ^{3} H-cortisol to ^{3} H-cortisone in placental microsomes after a 15-minute incubation at 37°C with a saturating concentration of cortisol (5 μ M). Cortisol and unconjugated estriol were measured in

(Received in original form March 13, 2003; accepted in final form September 3, 2003)

Supported by National Health and Medical Research Council of Australia (Grant No. 252438), the Asthma Foundation of NSW, the NSW Department of Health, Hunter Medical Research Institute, a National Health and Medical Research Council Dora Lush (Biomedical) Postgraduate Research Scholarship (V.M.), and an Arthur Wilson Memorial Scholarship from the Royal Australian and New Zealand College of Obstetricians and Gynaecologists Research Foundation (V.C.).

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Am J Respir Crit Care Med Vol 168, pp 1317–1323, 2003

Originally Published in Press as DOI: 10.1164/rccm.200303-3740C on September 18, 2003

Internet address: www.atsjournals.org

umbilical vein plasma using commercial radioimmunoassay kits (Orion Diagnostica, Espoo, Finland; and DSL, Webster, TX, respectively). The sensitivity of the cortisol assay was 5 nM and of the estradiol assay was 0.03 ng/ml.

Results are presented as means $\pm$ SEM. Statistical analysis was performed using GraphPad Instat version 2.04a (GraphPad Software, Inc., San Diego, CA) and Stata version 7 (Stata Corporation, College Station, TX). Analysis of variance (ANOVA) and the nonparametric equivalent were used where appropriate. Graphical methods were used to test distributional assumptions. When comparing two groups with a normal distribution, the Student's *t* test was used to compare means, whereas the Mann-Whitney test was used to compare medians of two groups where data were not normally distributed. A *p* value of less than 0.05 was considered significant. A multivariate analysis was performed using Stata version 7. Generalized linear latent and mixed models and generalized estimating equations were used for repeated-measures data (34, 35). Outcomes were adjusted for asthma severity, cumulative inhaled glucocorticoid intake, fetal sex, and smoking.

RESULTS

Maternal Characteristics

Pregnant women with asthma (*n* = 138) and pregnant women without asthma (control, *n* = 44) were recruited during the first trimester. Clinical assessment divided the women with asthma into 62 with mild asthma, 28 with moderate asthma, and 48 with severe asthma. Women with asthma were classified based on inhaled glucocorticoid intake during pregnancy as no glucocorticoid or glucocorticoid. Clinical characteristics separate from asthma were similar in all groups (Table 1). When fetal and placental data were analyzed according to asthma severity ratings, there were no significant differences between the groups. Therefore all data are presented on the basis of the glucocorticoid intake classification.

FEV₁ was lower in the groups with asthma (no glucocorticoid, 3.19 ± 0.07 L, *n* = 45; glucocorticoid, 3.02 ± 0.05 L, *n* = 90) compared with the control group (3.25 ± 0.10 L, *n* = 24, ANOVA, *p* = 0.05). The maternal FEV₁:VC ratio was significantly lower in women of the glucocorticoid group who were pregnant with a female fetus compared with women in the no-glucocorticoid group who were pregnant with a female fetus (Mann-Whitney test, *p* = 0.015; Table 1). However, among women pregnant with a male fetus, there was no significant difference in the maternal FEV₁:VC between the glucocorticoid and no-glucocorticoid groups (Mann-Whitney test, *p* = 0.345; Table 1).

Fetal Growth

There were no significant differences in fetal biparietal diameter, head circumference, abdominal circumference, or femur length between any groups at either 18 or 30 weeks of gestation (ANOVA, *p* > 0.05).

The birth weight of female neonates in the no-glucocorticoid group was significantly reduced compared with females in the control and glucocorticoid groups (Kruskal-Wallis nonparametric ANOVA, *p* = 0.027; Table 2). The birth weight centile of female neonates was also significantly reduced in the no-glucocorticoid group (34.5 ± 4.7 , *n* = 22) compared with females in the control group (54.2 ± 6.5 , *n* = 15) and the glucocorticoid group (49.0 ± 4.1 , *n* = 47, ANOVA, *p* = 0.047; Figure 1A). Within the no-glucocorticoid group, there was a high proportion of small-for-gestational-age (less than 10th centile) female neonates (18.2% compared with 0% in the control group and 12.8% in the glucocorticoid group). Female neonates in the no-glucocorticoid group had a head circumference centile that was similar to their birth weight centile, but was not significantly different from head circumference centile of females in the control group or the glucocorticoid group (ANOVA, *p* = 0.710; Table 2). Most women in the no-glucocorticoid group who had a female fetus had mild asthma (82%; Table 1). Those with mild asthma who did not use inhaled glucocorticoids had significantly smaller female neonates (birth weight centile, 36.6 ± 5.3 , *n* = 18) than those with mild asthma who did use inhaled glucocorticoids (birth weight centile, 56.1 ± 6.1 , *n* = 14, unpaired *t* test, *p* = 0.021). Ponderal index (birth weight/length³ $\times$ 100) was not significantly different between groups (ANOVA, *p* = 0.156; Table 2). In addition, placental weight did not differ between any groups (ANOVA, *p* = 0.824; Table 2).

No similar effects on growth were observed in male fetuses (Table 2 and Figure 1B). Male birth weight was not significantly different between the control group, the no-glucocorticoid group, or the glucocorticoid group (ANOVA, *p* = 0.192). Male neonates from those with mild asthma were of similar size regardless of glucocorticoid use (birth weight centile, 66.2 ± 5.9 , *n* = 16, no glucocorticoid versus 64.9 ± 6.9 , *n* = 14, glucocorticoid).

There was a significant positive correlation between maternal lung function, expressed as the FEV₁:VC ratio, and neonatal birth weight for females in the no-glucocorticoid group only (*r* = 0.505, *n* = 19, *p* = 0.028, excluding one preterm delivery; Figure 2). This relationship was not observed for males in the no-glucocorticoid group (*r* = 0.14, *n* = 21, *p* = 0.553), or for any other groups (*p* > 0.05, data not shown). Overall, there

TABLE 1. MATERNAL CHARACTERISTICS

	Classification of Inhaled Glucocorticoid Intake During Pregnancy					
	Female Fetus			Male Fetus		
	Control	No Glucocorticoid	Glucocorticoid	Control	No Glucocorticoid	Glucocorticoid
Total number of subjects	21	22	47	23	24	45
Asthma severity		18 Mild 0 Moderate 4 Severe	14 Mild 10 Moderate 23 Severe		16 Mild 5 Moderate 3 Severe	14 Mild 13 Moderate 18 Severe
Maternal FEV ₁ (l)	3.24 ± 0.16 (<i>n</i> = 11)*	3.20 ± 0.11 (<i>n</i> = 21)	3.02 ± 0.08 (<i>n</i> = 44)	3.26 ± 0.13 (<i>n</i> = 13)*	3.17 ± 0.08 (<i>n</i> = 24)	3.02 ± 0.07 (<i>n</i> = 45)
Maternal FEV ₁ :VC	0.88 ± 0.03 (<i>n</i> = 11)	0.85 ± 0.01 (<i>n</i> = 20)	0.79 ± 0.01 (<i>n</i> = 39) [†]	0.83 ± 0.01 (<i>n</i> = 13)	0.82 ± 0.01 (<i>n</i> = 21)	0.81 ± 0.01 (<i>n</i> = 41)
Maternal body mass index	24.6 ± 1.5 (<i>n</i> = 15)	25.2 ± 1.0 (<i>n</i> = 21)	27.3 ± 1.1 (<i>n</i> = 40)	28.1 ± 1.6 (<i>n</i> = 17)	28.5 ± 2.0 (<i>n</i> = 22)	28.1 ± 1.1 (<i>n</i> = 40)
Gravidity	2.9 ± 0.6 (<i>n</i> = 21)	2.1 ± 0.3 (<i>n</i> = 22)	2.6 ± 0.2 (<i>n</i> = 47)	2.9 ± 0.4 (<i>n</i> = 23)	2.8 ± 0.4 (<i>n</i> = 24)	2.1 ± 0.2 (<i>n</i> = 45)
Parity	1.3 ± 0.3 (<i>n</i> = 21)	0.6 ± 0.2 (<i>n</i> = 22)	1.0 ± 0.2 (<i>n</i> = 47)	1.5 ± 0.3 (<i>n</i> = 23)	1.0 ± 0.2 (<i>n</i> = 24)	0.8 ± 0.1 (<i>n</i> = 45)

Values given are mean $\pm$ SEM.

* *p* < 0.05 compared with asthma groups.

[†] *p* < 0.05 compared with no-glucocorticoid group.

TABLE 2. FETAL AND NEONATAL CHARACTERISTICS

	Classification of Inhaled Glucocorticoid Intake During Pregnancy					
	Female Fetus			Male Fetus		
	Control	No Glucocorticoid	Glucocorticoid	Control	No Glucocorticoid	Glucocorticoid
Gestational age, weeks	38.9 ± 1.0 (n = 21)	39.0 ± 0.6 (n = 22)	39.2 ± 0.2 (n = 47)	39.6 ± 0.4 (n = 23)	39.6 ± 0.4 (n = 23)	38.9 ± 0.4 (n = 45)
Birth weight, g	3527.7 ± 85.8 (n = 15)	3094.5 ± 120.0 (n = 22)*	3375.5 ± 89.9 (n = 47)	3601.9 ± 166.2 (n = 16)	3701.5 ± 88.9 (n = 24)	3446.5 ± 88.0 (n = 43)
Head circumference at birth, cm	34.5 ± 0.3 (n = 15)	33.9 ± 0.3 (n = 21)	34.3 ± 0.2 (n = 46)	34.8 ± 0.4 (n = 16)	34.9 ± 0.3 (n = 23)	34.7 ± 0.3 (n = 43)
Head circumference centile at birth	44.0 ± 6.8 (n = 15)	34.0 ± 5.4 (n = 21)	44.7 ± 4.2 (n = 46)	57.2 ± 6.7 (n = 16)	56.8 ± 5.7 (n = 23)	55.0 ± 4.1 (n = 43)
Ponderal index	2.55 ± 0.06 (n = 14)	2.51 ± 0.08 (n = 21)	2.65 ± 0.04 (n = 42)	2.58 ± 0.05 (n = 15)	2.49 ± 0.05 (n = 22)	2.52 ± 0.04 (n = 42)
Placental weight, g	641.7 ± 28.6 (n = 12)	606.4 ± 28.0 (n = 12)	601.2 ± 36.8 (n = 17)	610.6 ± 51.4 (n = 11)	653.6 ± 42.0 (n = 15)	644.8 ± 31.0 (n = 22)

Values given are mean ± SEM.

*p < 0.05 compared with control and glucocorticoid groups.

was no relationship between FEV₁/VC and birth weight when examining all of the mothers with asthma (n = 121).

Some women in our study were smokers (25%), and smoking has been reported to contribute to low birth weight (36). However, we found no significant differences in birth weight between smokers and nonsmokers within any groups. When examining female neonates of smoking mothers, those from the no-glucocorticoid group had a birth weight of 3,033.3 ± 118.6 g (n = 6), whereas female neonates of smoking mothers from the glucocorticoid group had a birth weight of 3,240.0 ± 184.8 g (n = 14). This difference was not significant (Mann-Whitney test, p = 0.444). Female neonates from nonsmoking mothers had a birth weight of 3,117.5 ± 160.7 g (n = 16) in the no-glucocorticoid group and a birth weight of 3,433.0 ± 101.5 g (n = 33) in the glucocorticoid group, indicating that the absence of glucocorti-

coid use in those with asthma was associated with reduced female birth weight regardless of maternal smoking.

Using multivariate analysis, there was a significant reduction in female fetal growth in women using no glucocorticoids (p = 0.037) and subjects with mild asthma (p = 0.02). There was no significant effect of smoking or asthma severity on birth weight, and sex still remained the determining factor.

Placental Function

There was a significant reduction in placental 11β-HSD2 enzyme activity in placentas from female fetuses in the no-glucocorticoid group (2.60 ± 0.33 nmol cortisone/mg protein/hour, n = 7) compared with placentas from females in the control group (4.96 ± 1.02 nmol/mg/hour, n = 6) or the glucocorticoid group (6.88 ± 0.59 nmol/mg/hour, n = 26, ANOVA, p = 0.002; Figure 3A). Placental 11β-HSD2 activity was not significantly different between males in the control, no-glucocorticoid, or glucocorticoid groups (ANOVA, p = 0.420; Figure 3B). Placental 11β-HSD2 activity was significantly lower in placentas collected from males compared with females within the glucocorticoid group (Mann-Whitney test, p = 0.0016) and when the control and glucocorticoid groups were combined (Mann-Whitney test, p = 0.001).

Placental 11β-HSD2 activity was not significantly altered by maternal smoking. 11β-HSD2 activity for smokers was 4.63 ± 0.76 nmol/mg/hour (n = 16) compared with 5.06 ± 0.40 nmol/mg/hour (n = 49) for nonsmokers (Student's *t* test, p = 0.597). There was no significant difference in placental 11β-HSD2 activity between smokers and nonsmokers within any of the no-glucocorticoid or glucocorticoid male/female subgroups (Student's *t* test, p > 0.05, data not shown).

Multivariate analysis indicated that 11β-HSD2 activity was associated with sex and glucocorticoid intake (p = 0.008) but was not affected by maternal asthma severity or smoking.

Mean fetal cortisol concentrations in the umbilical vein at delivery were higher in female fetuses from the no-glucocorticoid group (279.8 ± 56.3 nM, n = 13), as expected from reduced placental 11β-HSD2 activity. However, these were not significantly different from values in the control group (214.2 ± 20.2 nM, n = 12) or the glucocorticoid group (224.2 ± 22.9 nM, n = 25, Kruskal-Wallis ANOVA, p = 0.881). Male cortisol concentrations were also not significantly different between the control (193.2 ± 27.7, n = 11), no-glucocorticoid (287.8 ± 59.6, n = 15), or glucocorticoid groups (281.4 ± 41.8, n = 25, Kruskal-Wallis ANOVA, p = 0.624).

Fetal estriol concentrations were significantly reduced in females from the no-glucocorticoid group (417.6 ± 76.2 nM, n = 8) compared with females from the control group (994.2 ± 309.3 nM, n = 7) and the glucocorticoid group (872.1 ± 84.9 nM, n = 20,

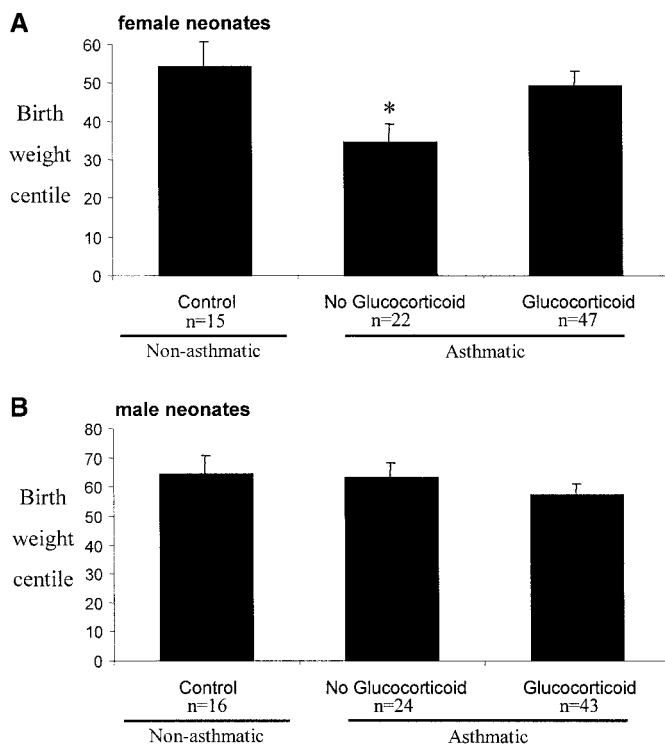


Figure 1. Neonatal birth weight centile in pregnancies complicated by asthma. Mean birth weight centile ± SEM for female neonates and for male neonates. *p = 0.047 (analysis of variance [ANOVA]).

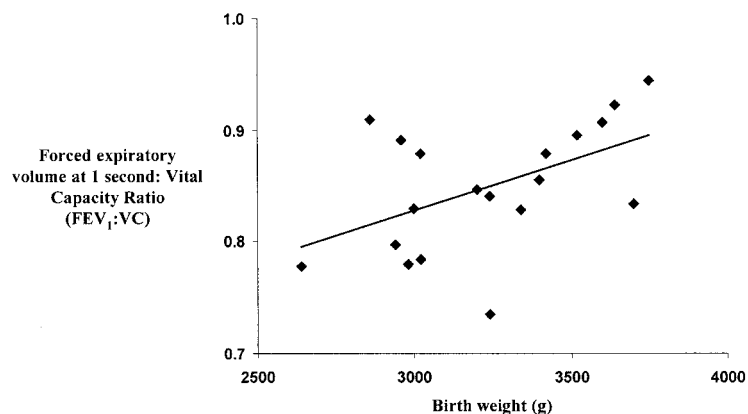


Figure 2. Neonatal birth weight in relationship to maternal lung function in subjects with asthma that did not use inhaled steroids during pregnancy. The correlation between birth weight (g) and maternal FEV₁:VC is shown for women with asthma in the no-glucocorticoid group pregnant with a female fetus ($r = 0.505$, $n = 19$, $p = 0.028$).

Figure 4A, Kruskal-Wallis ANOVA, $p = 0.007$). There was no significant difference in male estriol concentrations between groups (Kruskal-Wallis nonparametric ANOVA, $p = 0.308$; Figure 4B).

Maternal Inflammatory Pathways

To investigate whether changes in placental function and fetal growth may be due to increased maternal inflammation associated with changes in asthma, we examined drug intake by women with asthma who used inhaled glucocorticoids during pregnancy. Women treated with moderate or high doses of inhaled glucocorticoids who were pregnant with a female fetus ($n = 41$) significantly increased their inhaled glucocorticoid use during pregnancy from 917 ± 99 $\mu\text{g/day}$ in the first trimester to $1,350 \pm 111$ $\mu\text{g/day}$ in the third trimester (paired nonparametric test, $p = 0.0002$). However, subjects with asthma using moderate or high doses of inhaled glucocorticoids who were pregnant with a male fetus ($n = 30$) did not significantly alter their inhaled

glucocorticoid use during pregnancy, using 930 ± 106 $\mu\text{g/day}$ in the first trimester and $1,080 \pm 103$ $\mu\text{g/day}$ in the third trimester (paired nonparametric test, $p = 0.176$).

To investigate inflammatory pathways in the no-glucocorticoid group, maternal white blood cell counts were examined. The maternal monocyte count significantly increased from early gestation ($0.58 \pm 0.04 \times 10^9$ per L, $n = 22$) to late gestation ($0.80 \pm 0.08 \times 10^9$ per L, $n = 7$) in women with asthma in the no-glucocorticoid group who were pregnant with a female fetus (unpaired t test, $p = 0.020$). In addition, the percentage of white blood cells that were monocytes significantly increased from early to late pregnancy ($6.2 \pm 0.4\%$, $n = 22$, to $7.6 \pm 0.4\%$, $n = 7$) in the no-glucocorticoid group in women pregnant with a female fetus (Mann-Whitney test, $p = 0.020$) and in late pregnancy was significantly higher than the other groups (Kruskal-

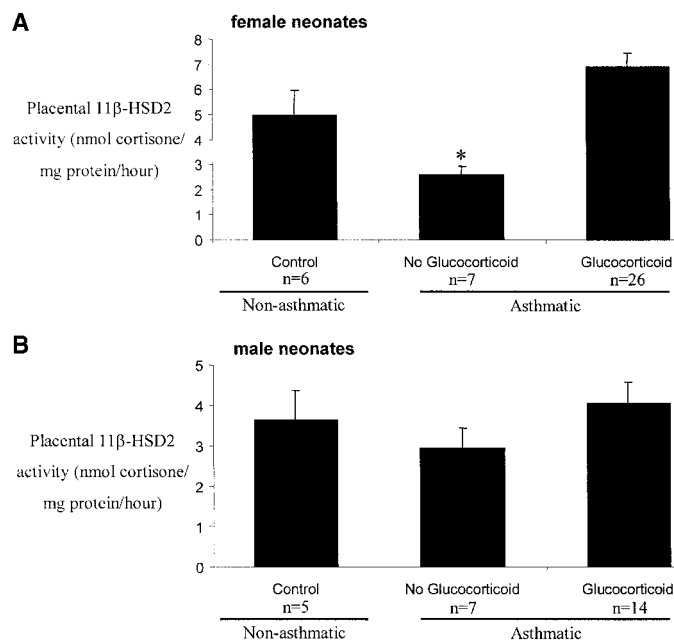


Figure 3. Placental 11 β -HSD2 enzyme activity in pregnancies complicated by asthma. Enzyme activity is presented as nmol cortisone formed per mg protein per hour. Values are mean $\pm$ SEM for female placentas and for male placentas. * $p = 0.002$ (ANOVA).

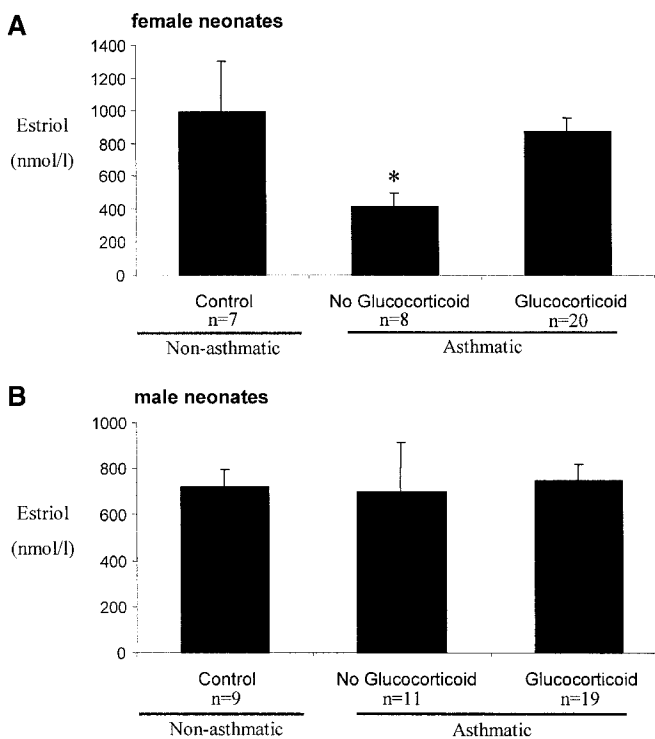


Figure 4. Fetal umbilical vein estriol concentrations at delivery. Fetal estriol concentrations (nM) are depicted as mean $\pm$ SEM in female cord blood and male cord blood. * $p = 0.007$ (Kruskal-Wallis nonparametric ANOVA).

Wallis ANOVA, $p = 0.017$). There was no significant change during pregnancy in maternal monocyte count in any other group, including the women with asthma who did not use glucocorticoids and were pregnant with a male fetus ($0.56 \pm 0.05 \times 10^9$ per L, $n = 14$, in early pregnancy to $0.50 \pm 0.05 \times 10^9$ per, $n = 8$, in late pregnancy, unpaired t test, $p = 0.370$). Other white blood cell counts were also examined, including lymphocytes, neutrophils, eosinophils, and basophils. There were no significant differences in any of these parameters between women pregnant with a male or female fetus in the no-glucocorticoid or glucocorticoid groups ($p > 0.05$, data not shown). Eosinophil counts were significantly higher in all of the groups with asthma compared with the control group (less than 20 weeks, Kruskal-Wallis ANOVA, $p < 0.0001$). However, there was no significant increase in eosinophil counts as pregnancy progressed or any difference between males and females of the no-glucocorticoid or glucocorticoid groups ($p > 0.05$, data not shown).

DISCUSSION

This study examined the effects of asthma on endocrine and immune relationships between the mother, placenta, and fetus and their role in the control of fetal growth during human pregnancy. We have demonstrated that the female fetus has a different effect from the male fetus on the maternal immune system during pregnancy, with an upregulation of inflammatory pathways observed in some women with asthma pregnant with a female fetus. These changes were observed in women with very mild asthma who had been medically advised not to use inhaled glucocorticoids. Alterations observed in maternal asthma in the presence of a female fetus may be directly involved in the changes in placental function, which included a reduction in placental 11 β -HSD2 activity and a trend toward increased cord blood cortisol. These changes in placental function were associated with reduced fetal growth and adrenal function in females. We propose that maternal inflammation is the key to alterations in female fetal growth in this setting, as the use of inhaled glucocorticoids by pregnant women with asthma was protective. The male fetus appeared to be insensitive to the effects of inflammation in the mother, with no changes in placental function or growth observed in male fetuses. We conclude that the female fetus has an adverse effect on maternal asthma, which when not treated with inhaled glucocorticoids results in reduced fetal growth.

Recent data in asthmatic pregnancies suggests that women pregnant with a female fetus have increasing asthma severity as gestation progresses (37, 38) and an increase in the incidence of complications such as pre-eclampsia or preterm delivery (39). We found that inhaled glucocorticoid intake by women with asthma using moderate or high doses significantly increased in late pregnancy when women were pregnant with a female fetus, suggesting an upregulation of inflammation associated with asthma as gestation progressed. Such changes in maternal systemic inflammation in the presence of a female fetus may also be involved in the increased risk of developing pre-eclampsia or preterm labor in asthmatic pregnancies. In the women with asthma who were medically advised not to use inhaled steroids because they were assessed as having a very mild disease, we observed a significant rise in the number of circulating monocytes as gestation progressed when they were pregnant with a female fetus. This supports the concept that increased maternal inflammation is associated with reduced female fetal growth in this group.

Monocytes, the precursors to macrophages, are important inflammatory mediators in asthma, via their interactions with Th2 lymphocytes, eosinophils, and mast cells within the asthmatic airway. Alveolar macrophages from patients with mild asthma

are highly activated, as demonstrated by the presence of cell wall antigens required for recognition by CD4⁺ lymphocytes (40). Previous studies have demonstrated that coculture of CD4⁺ T cells with peripheral blood monocytes from atopic subjects with asthma results in enhanced production of IL-4 and IL-5 (41, 42). In addition, monocytes interact with airway smooth muscle cells *in vitro*, inducing collagen degradation through the induction of matrix metalloproteinase 1, 2, and 9 (43). Monocytes release numerous cytokines, including tumor necrosis factor- α (44–46), IL-1 β (44, 46), IL-6 (46), and granulocyte macrophage colony-stimulating factor (44, 45). In our study, both the number and the percentage of monocytes in the maternal circulation of those with asthma who did not use steroids and had a female fetus increased during gestation, suggesting that there was a specific upregulation of this leukocyte, rather than simply an overall increase in white blood cell numbers. We noted that eosinophil numbers were also higher in early pregnancy in mothers with asthma compared with those without asthma, which is in agreement with previous studies in nonpregnant adults (47). However, unlike monocytes, the eosinophil count did not differ significantly between mothers pregnant with a male and female fetus in the no-glucocorticoid group nor did it increase as gestation progressed. These data suggest that in the absence of inhaled glucocorticoid use and in the presence of a female fetus, cytokines derived from circulating monocytes may be primarily responsible for alterations in placental function, which result in reduced female fetal growth.

Our study indicates that in pregnant women with asthma there is reduced female fetal growth when no inhaled glucocorticoids are used. This occurred regardless of asthma severity or maternal smoking. Female birth weight and head circumference were both reduced to the 34th centile, and ponderal index was normal, suggesting symmetrical growth restriction. Schatz and colleagues have previously demonstrated that poor maternal lung function, indicated by lower maternal FEV₁, was associated with a greater incidence of birth weights in the lower quartile and asymmetric growth restriction (48). We also found a relationship between maternal lung function (FEV₁:VC) and neonatal birth weight among females from the no-glucocorticoid group, suggesting that reduced lung function may be a contributing factor to reduced birth weight in this group. However, the lung function of mothers in the glucocorticoid group was significantly worse than that in the no-glucocorticoid group (with a female fetus present), and yet no changes in female fetal growth were observed in this group. In addition, the use of glucocorticoids by women with mild asthma was associated with female birth weight centiles comparable to control nonasthmatics. These data suggest that inflammation rather than alterations in lung function itself is a major component of the mechanism contributing to low birth weight in asthmatic pregnancies. Our data indicate that women with asthma with a relatively mild inflammatory disease, who were medically recommended not to use inhaled glucocorticoids, had significant changes in placental function and symmetrically reduced fetal growth.

Changes in female fetal growth may be mediated by decreased placental 11 β -HSD2 activity and the antimitogenic effects of cortisol. Fowden and colleagues described that the cortisol surge toward late gestation in sheep was coincident with the slowing down of growth, reflected by a decrease in the increment of crown-rump length growth (49). Significant reductions in placental 11 β -HSD2 activity have previously been observed in intrauterine growth restriction placentas (26). Multiple doses of betamethasone, a steroid that is not metabolized by placental 11 β -HSD2, administered to women at risk of preterm delivery, resulted in a 9% reduction in neonatal birth weight and 4% reduction in neonatal head circumference (50). In our study, there was a 12% reduction

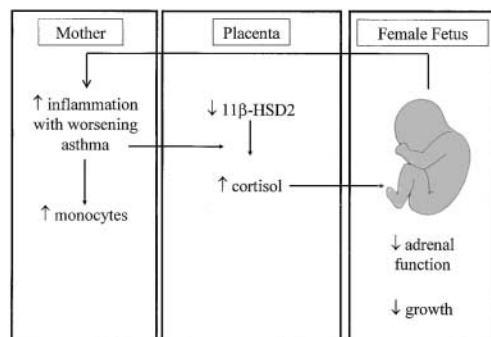


Figure 5. Proposed mechanisms involved in reducing female fetal growth in pregnancies complicated by asthma. 11 β -HSD2 = 11 β -hydroxysteroid dehydrogenase type 2.

in female birth weight, which equated to an approximately 500-g mean difference in size compared with female neonates from mothers who did not have asthma. This is far greater than fetal growth reductions previously reported for smoking mothers, which average 200 g (36, 51). These data indicate that increased circulating concentrations of bioactive cortisol in the presence of decreased 11 β -HSD2 activity results in reduced symmetric growth of the female fetus.

Increased fetal exposure to glucocorticoids in animal models has previously been associated with alterations in fetal hypothalamic–pituitary–adrenal axis development and long term changes into adulthood. Pregnant guinea pigs exposed to synthetic glucocorticoids have altered female fetal hypothalamic–pituitary–adrenal function associated with increased hippocampal mineralocorticoid and glucocorticoid receptor expression (52). In rats, inhibition of placental 11 β -HSD2 activity is associated with altered stress responses in offspring (53) and long-term changes such as delays in the development of puberty in females (54). Our human study showed suppression of the female fetal hypothalamic–pituitary–adrenal axis in the presence of maternal asthma and reduced placental 11 β -HSD2 activity, as demonstrated by reduced umbilical vein concentrations of estriol, a derivative of fetal adrenal dehydroepiandrosterone sulfate (55). These data suggest that despite similar levels of cortisol in cord blood from males and females of mothers with asthma not treated with steroids, the females of this group are more sensitive to changes in placental cortisol metabolism. This observation supports previous clinical data demonstrating a greater response to synthetic glucocorticoid treatment for lung maturation in female fetuses at risk of preterm delivery (56, 57) and suggests that the female fetus may be more sensitive to changes in cortisol concentration.

In summary, our study of women with asthma during pregnancy has demonstrated that there is a relationship between fetal sex and maternal asthma during pregnancy, with women pregnant with a female fetus showing evidence of increased inflammation in late pregnancy. Figure 5 outlines our proposed mechanism for reduced female fetal growth in pregnancies complicated by asthma. We speculate that an unknown factor derived from the female fetus alters maternal immune function. This factor could be a sex steroid hormone or a novel protein originating from the fetus. Previous studies indicate that female sex hormones alter cytokine release from macrophages (58). We propose that fetally derived factors could also be involved in an alteration of monocyte/macrophage phenotype or function in the mother. An upregulation of maternal inflammation may be directly involved in the reduction of placental cortisol metabo-

lism by 11 β -HSD2 and ultimately in altering the development of the female fetal hypothalamic–pituitary–adrenal axis and reducing female fetal growth (Figure 5).

Our results have several important clinical and scientific implications. We have demonstrated that the use of inhaled steroids by women with mild asthma was beneficial for the growth of female fetuses, by controlling maternal systemic inflammation. In addition, the female fetus itself influenced the course of maternal asthma through pregnancy. Scientifically, this study has contributed to understanding the mechanisms regulating fetal growth in human pregnancy. Placental 11 β -HSD2 activity is a key component of this mechanism through its control of cortisol concentrations reaching the fetus. The female fetus was particularly sensitive to alterations in cortisol exposure and the downstream effects of this included reduced fetal adrenal function and reduced growth. These changes in fetal hypothalamic–pituitary–adrenal axis development and growth potentially expose these female neonates to an increased risk of developing diseases in later life through altered fetal programming. By examining the endocrine and immune relationships between mother, placenta, and fetus during asthmatic pregnancies, this study has provided strong evidence for a detrimental effect of maternal inflammation on placental function and female fetal growth and development.

Conflict of Interest Statement: V.E.M. has no declared conflict of interest; P.G.G. has no declared conflict of interest; W.B.G. has no declared conflict of interest; T.Z. has no declared conflict of interest; R.S. has no declared conflict of interest; A.M.B. has no declared conflict of interest; C.G.K. has no declared conflict of interest; V.L.C. has no declared conflict of interest.

Acknowledgment: The authors thank the staff of the Antenatal Clinics and Delivery Suite at the John Hunter Hospital for their assistance in subject recruitment and placenta collection.

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