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Effect of maternal asthma, inhaled glucocorticoids and cigarette use during pregnancy on the newborn insulin-like growth factor axis

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ABSTRACT

Background: Fetal growth varies in a sex-specific manner in response to maternal asthma during pregnancy, but the mechanisms are unclear.**Objective:** We examined the influence of maternal asthma severity and associated exposures, inhaled glucocorticoid treatment, maternal cigarette use, and fetal sex on fetal growth and placental function during pregnancy and on the newborn insulin-like growth factor (IGF) axis.**Study subjects and design:** Fetal growth was assessed in a prospective cohort of asthmatic and non-asthmatic women ($n = 145$). At delivery, umbilical vein plasma was collected from male ($n = 61$, controls $n = 16$ and asthmatic $n = 45$) or female ($n = 84$, controls $n = 22$ and asthmatic $n = 62$) fetuses. Cord plasma insulin-like growth factor (IGF) binding protein (BP)-1, IGFBP-3, IGF-1 and IGF-2 were measured by radio-immunoassay and ELISA.**Results:** Cord plasma IGF-1 was the main component of the neonatal IGF axis altered by asthma and cigarette use. IGF-1 was increased in the presence of mild asthma and a male fetus and decreased in the presence of a female fetus and maternal asthma with cigarette use. IGFBP-3 was also decreased in the female fetuses of pregnancies complicated by asthma and cigarette use. Inhaled glucocorticoid use for the treatment of asthma did not affect the IGF axis. The strongest overall predictor of female birth weight after accounting for asthma severity, inhaled glucocorticoid treatment and cigarette use was IGF-1. For males, the strongest predictor of birth weight was IGFBP-3.**Conclusion:** The data suggest male and female fetuses institute different strategies in response to adverse pregnancy conditions such as asthma and cigarette use.

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1. Introduction

Size at birth reflects fetal growth and substrate supply in utero and is associated with neonatal morbidity and mortality and longer term health outcomes [1]. Previously, we have reported that maternal asthma during pregnancy affects fetal growth and development in a sex-specific manner [2]. Female fetal growth was reduced and male growth was normal in the presence of maternal asthma that was not treated with inhaled glucocorticoids [2]. In the presence of a second stressful event such as an acute asthma exacerbation, the mean birthweight of the male fetus was reduced with an increased incidence of IUGR, preterm delivery or still birth

in males only [3]. In the presence of an exacerbation of asthma female growth did not change though the fetus was already small due to adjustments in growth in response to the pre-existing complication of asthma [3]. These studies and others [4,5] suggest that male and female fetuses respond differently to stressful events during pregnancy and these differences lead to alterations in birth weight. The mechanisms contributing to these sex-specific differences in responses to adverse exposures before birth are yet to be examined but may be mediated via changes in placental function [6]. Since the IGF axis is known to be a major contributing factor to fetal and placental development [1] it may contribute to the sex-specific differences in fetal growth observed in pregnancies complicated by asthma.

IGF-1 and IGF-2 are polypeptides with a sequence related to that of insulin [7], which have mitogenic properties, inducing somatic cell growth and proliferation [8,9]. Knockout and transgenic

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mice studies have demonstrated that IGF-1 and IGF-2 are required for optimal fetal and placental growth [10–13]. The actions of IGF-1 and IGF-2 are modulated by insulin-like growth factor binding proteins (IGFBP 1–6) [14]. IGFBP 1, 2, 4, 5 and 6 are present in low concentrations in adult plasma [15]. The predominant form, IGFBP-3 complexes with IGF-1 or 2 and an acid-labile subunit, the complex acting as a reservoir for IGFs in the circulation [14,16]. IGFBP-1 is dynamically regulated in human plasma, binding IGF-1 and -2 [14,17] with similar or greater affinity than either of the IGF receptors, thus preventing the IGFs from exerting their mitogenic actions [14]. Over-expression of IGFBP-1 in transgenic mice transiently reduces fetal growth in mid-gestation [18].

Numerous studies have found a positive relationship between circulating levels of IGF-1 in cord plasma and birth weight in normal term singleton infants [19–29]. In pregnancies complicated by intrauterine growth restriction (IUGR), umbilical cord blood IGF-1 is reduced compared to pregnancies with normal fetal growth [30–34]. The relationship between newborn plasma IGF-2 and birth weight is less clear with a positive correlation in term singletons in some [20,27], but not all studies [19,21] and no difference between normally grown and growth restricted neonates in another [30]. However, cord blood IGF-2 has been described to be positively correlated with placental weight [24], consistent with an important role in placental growth in a range of studies in other species [11–13,35].

The relationship between cord blood IGFBPs and fetal growth has also been examined. IGFBP-3 correlates positively with birth weight [21,25,27,36], while IGFBP-1 is inversely correlated with birth weight in term [22,29,37] or preterm infants [38,39]. Increased cord blood IGFBP-1 [40] and reduced IGFBP-3 have also been observed in IUGR neonates [31,32,40] consistent with IGFBP-3 providing a reservoir for IGFs and IGFBP-1 inhibiting IGF actions.

The human placenta produces IGF-1 and IGF-2 which may act as local growth regulators [41] with IGF-2 more highly expressed. IGF-2 is found throughout the chorionic villi, chorionic plate, basal plate and fetal membranes, while all IGFBPs are found in the decidua, with IGFBP-1 in greatest abundance [42,43]. During human pregnancy, IGFBP-1 may also be a major regulator of IGFs in the fetus and placenta, since it is the main product of the decidua, the main IGFBP in the amniotic fluid and a major binder of IGFs in fetal plasma [44–46].

Cortisol has been characterised as an important indirect regulator of fetal growth through its actions on IGFs and their binding proteins [1]. Cianfarani et al. [34] found that there was a positive correlation between IGFBP-1 and cortisol in human cord blood from appropriately grown fetuses. However, they could not demonstrate any such relationship in IUGR fetuses suggesting there are other factors influencing fetal growth in IUGR pregnancies [34]. Other studies report that glucocorticoids have varying effects on IGFBP-1 levels. For example, increased plasma IGFBP-1 concentrations have been reported following cortisol infusions in adults [15]. IGFBP-1 was increased in neonates following a stressful labour and delivery [47] and IGFBP-1 production was inhibited following dexamethasone treatment in fetal liver explants [48]. In the sheep fetus, IGF mRNA is decreased with cortisol treatment in the adrenal and liver [49,50]. These studies have shown IGF-1 and IGF-2 are down regulated by cortisol while IGFBP-1 concentrations are increased. Since we have previously observed sex-specific differences in the placental response to cortisol in pregnancies complicated by asthma, in this study we examined the relationship between maternal asthma, fetal cortisol and the IGF axis.

Fetal sex is known to affect fetal growth, with male fetuses on average being larger than female fetuses [51–53]. This difference may not be evident until after 30 weeks [51,54], but increases as gestation progresses [53] with a 150–200 g weight difference apparent by 38 weeks gestation [53,54]. The mechanisms which contribute to

the different growth patterns of male and female fetuses remain unclear. However, differences in growth regulation by the IGF axis may be involved. A recent study of 987 healthy singletons found that IGF-1 and IGFBP-3 concentrations in cord blood were higher in females than males [55]. No difference in cord blood IGF-2 between male and female neonates was reported, while growth hormone concentrations were higher in males than females [55]. Few other studies have examined sexually dimorphic patterns of IGF expression or production in the human fetus [19,28].

The present study therefore sought to determine the effect of maternal asthma on the IGF axis in relation to fetal sex and growth for the first time. Another common exposure in pregnancy that adversely affects fetal growth is cigarette use. Cigarette use is still common in the Australian community affecting 20–25% of pregnancies [56]. In pregnancy, cigarette use is known to be associated with several adverse outcomes including IUGR [57] and stillbirth [58]. Previous studies have reported that female birth weight relative to male birth weight was reduced in the presence of maternal cigarette use during pregnancy [59]. Pringle et al. [60] has reported decreased cord blood IGF-1 and IGFBP-3 concentrations with cigarette use. Previous studies indicate a combination of adverse factors may be a powerful stimulus for altered growth and development of the fetus during pregnancy and may affect males and females differentially. The present study sought to determine this possibility by examining the effect of maternal asthma on the IGF axis in relation to fetal sex and growth, and to identify whether cigarette smoking compounded this effect.

2. Experimental subjects

The study was approved by the Hunter Area Health Service and University of Newcastle Human Research Ethics Committees. Pregnant women were recruited at the John Hunter Hospital antenatal clinic during the first trimester ($n = 490$) and provided written informed consent for participation. The protocol for this study has been described in detail previously [2]. Those women with complications other than asthma such as pre-eclampsia, gestational diabetes, infection or preterm delivery were excluded from the analysis ($n = 121$). Birth weight and fetal sex were determined at birth. Within a subset of pregnancies, cord plasma was collected from the umbilical vein into heparinised tubes, from a subset of samples ($n = 145$, controls: $n = 38$, asthmatics: $n = 107$). Plasma was collected following centrifugation at 1000g for 15 min and stored at -20°C until further use.

3. Materials and methods

Clinical asthma severity was rated as mild, moderate or severe using the integrated severity score described in the Australian Asthma Management Guidelines [61], which closely approximate the National Heart, Lungs and Blood Institute Guidelines [62]. Proper inhaler use and compliance was assessed in the Asthma Management Service [63]. Cumulative, inhaled glucocorticoid dose was calculated for each trimester, and summarised as the mean daily dose of beclomethasone dipropionate (BDP) or equivalent used during pregnancy, where $1\text{ }\mu\text{g}$ BDP was considered equal to $1\text{ }\mu\text{g}$ budesonide or $0.5\text{ }\mu\text{g}$ fluticasone propionate [64]. For data analysis, the low, moderate and high dose groups were combined (glucocorticoid). Asthmatic women in all groups, including the no glucocorticoid group, used the inhaled β_2 agonist, salbutamol for symptom relief when required. Current smoking status was assessed by direct questioning at recruitment. All control subjects were non-smokers.

All women participating in the study were screened by ultrasound as part of the routine fetal assessment at 18 and 30 weeks of gestation. Fetal biparietal diameter, head circumference, abdominal

circumference and femur length were measured. Umbilical artery flow velocity waveforms were assessed by pulsed Doppler ultrasound [65] (Acuson XP4 Corporation, Mountain View, CA, USA).

Birth weight, length and head circumference were recorded at delivery. Birth weight centiles were calculated using www.gestation.net, which accounts for maternal weight, ethnicity, fetal sex and gestational age. Neonates born small for gestational age (SGA) were identified by a birth weight centile of less than 10th percentile. Length and head circumferences were converted to centiles using John Hunter Hospital growth charts. Gestational age was determined by date of the last menstrual period and 18 weeks ultrasound.

Cord plasma IGFBP-1 and IGFBP-3 were measured in umbilical vein plasma by radioimmunoassay (RIA) according to previously published protocols [66,67]. The IGFBP-3 assay utilised rabbit anti-serum R-100 against human IGFBP-3 at a final dilution of 1:100,000. Cortisol was measured in umbilical vein plasma using a commercial RIA kit (Orion Diagnostica, Espoo, Finland). The sensitivity of the cortisol assay was 5 nmol/l. Total cord plasma IGF-1 and IGF-2 were measured using a commercial ELISA (non-extraction IGF kits from DSL Inc., TX, USA) with sensitivity of 0.01 and 0.065 nmol/l, respectively. Samples were tested in duplicate and the intra-assay co-efficient of variation was less than 4%.

Results are presented as mean and standard error of the mean. Statistical analysis was performed using GraphPad Instat version 3.05 (GraphPad Software, San Diego, CA, USA) and SPSS software (SPSS Inc., Version 15.0, IL, USA). Levels of IGFBP-1 were transformed (by natural logarithm) to normalise the data set. Analysis of variance (ANOVA) was used to assess the effects of asthma and fetal sex on the IGF axis. A-priori post hoc analyses using ANOVA were conducted to further identify whether smoking or asthma treatment compounded the effects of asthma on the IGF axis. Tukeys post hoc test was used where indicated. Maternal and fetal characteristics were examined to explore the interactions between fetal sex, maternal asthma, inhaled glucocorticoid use and the presence and absence of cigarette use during pregnancy using ANOVA for normally distributed data and the Kruskal Wallis test otherwise. Pearson's correlations were used to assess associations between IGF axis, birth weight and cord plasma cortisol levels. Multiple linear regression was used to assess the effect of asthma severity, asthma treatment, smoking and cord plasma cortisol on IGF axis and birth weight centiles. Values are expressed as mean $\pm$ standard error of the mean (SEM). A p -value of <0.05 was considered significant.

4. Results

4.1. Maternal characteristics

Maternal characteristics in relation to asthma severity and fetal sex are shown in Table 1. Maternal age was reduced in both asth-

matic groups compared to controls [$F(2, 139) = 4.907$, $p = 0.009$]. Maternal FEV¹ was reduced in women with severe asthma compared to controls [$F(2, 112) = 3.290$, $p = 0.041$, post hoc test $p < 0.05$]. The percent predicted FEV¹ was reduced in moderate-severe women, compared to both mild asthmatics and controls [$F(2, 71) = 5.664$, $p = 0.005$, post hoc test $p < 0.05$]. Inhaled glucocorticoid dose in the third trimester of pregnancy increased with increasing asthma severity [$F(2, 138) = 32.060$, $p < 0.001$]. Maternal body mass index, BMI at 18 weeks gestation, gravidity or parity did not vary with asthma severity. Maternal parameters did not vary with fetal sex.

4.2. Fetal and neonatal characteristics

Fetal characteristics are presented in Table 2. Moderate-severe asthma reduced birthweight compared to controls [Fig. 1; $F(2, 139) = 2.929$, $p = 0.035$]. Head circumference centile was reduced in female compared to male neonates [Table 2; $F(2, 121) = 5.20$, $p = 0.024$], as was abdominal circumference at 18 weeks [Table 2; $F(1, 282) = 4.321$, $p = 0.039$].

Placental weight and placental blood flow as measured by systolic-diastolic ratio of the umbilical artery at 18 and 30 weeks gestation were also not altered by maternal asthma.

4.3. IGF axis

Birth weight centiles for the whole population positively correlated with cord plasma IGF-1 ($r = 0.375$, $p < 0.001$, $n = 89$) and IGFBP-3 concentrations ($r = 0.312$, $p < 0.001$, $n = 126$), and inversely with IGFBP-1 levels ($r = -0.346$, $p < 0.001$, $n = 124$).

Only non-smoking asthmatics (female $n = 40$, male $n = 36$) were used in analyses assessing the independent effect of asthma on the IGF axis, as there were no women who smoked in the cohort of the control group (female $n = 14$, male $n = 7$) who had IGF measurements taken. Subsequent multivariate analysis was then undertaken to identify the independent effects of smoking, asthma severity and asthma inhaled glucocorticoid treatment on IGF levels.

4.3.1. Effect of asthma and its treatment on newborn IGF axis and fetal growth

Cord plasma IGF-1 was affected by maternal asthma severity [$F(2, 87) = 4.681$, $p = 0.012$], with higher levels observed in those exposed to mild asthma but not moderate-severe asthma compared to controls (Fig. 1). Cord plasma IGF-2, IGFBP-1 and IGFBP-2 were unaffected by maternal asthma (Fig. 1).

To explore the effects of inhaled glucocorticoid treatment (nil, low, moderate and high) on the IGF axis, the severity levels (mild and moderate/severe) were analysed separately, as the require-

Table 1

Maternal characteristics of women in the control, mild and moderate-severe asthma groups, split by fetal sex.

	Female			Male		
	Control	Mild	Moderate-severe	Control	Mild	Moderate-severe
<i>n</i>	22	32	30	16	21	24
Maternal age	29.7 (1.1)	25.9 (0.9)*	23.9 (0.9)*	27.8 (1.3)	27.0 (1.1)*	26.7 (1.0)*
BMI (18 weeks)	26.7 (1.9)	27.2 (1.3)	26.2 (1.3)	27.3 (2.0)	29.6 (1.6)	28.8 (1.4)
Gravidity (median, min–max)	3, 1–10	2, 1–6	2, 1–5	2, 1–8	2, 1–5	2, 1–7
Parity (median, min–max)	1, 0–6	1, 0–4	0, 0–3	1, 0–6	1, 0–4	1, 0–6
Dose inhaled glucocorticoids (trimester 3)	0	224.5 (87.4)*	915.2 (90.3)**	0	238.1 (107.8)*	685.3 (103.1)**
FEV ¹	3.4 (0.2)	3.2 (0.1)	3.0 (0.1)*	3.3 (0.2)	3.2 (0.1)	3.0 (0.1)*
% Predicted FEV ¹	100.7 (4.0)	96.1 (3.3)	90.2 (2.6)**	98.6 (5.3)	105.5 (4.9)	89.6 (3.4)**
Vital capacity	3.8 (0.2)	3.7 (0.1)	3.8 (0.1)	3.9 (0.2)	3.9 (0.1)	3.7 (0.1)

BMI body mass index; FEV¹ forced expiratory volume at 1 s.

* Significantly different from controls.

** Significantly different from both controls and the mild asthma group.

Table 2

Fetal characteristics of male and females born to women in the control, mild and moderate-severe asthma groups, split by asthma severity.

	Female			Male		
	Control	Mild	Moderate-severe	Control	Mild	Moderate-severe
<i>n</i>	22	32	30	16	21	24
Gestational age	39.8 (0.3)	39.6 (0.3)	39.7 (0.3)	39.9 (0.4)	40.2 (0.3)	39.6 (0.3)
Birth weight	3519.3 (101.1)	3448.7 (83.6)	3285.8 (86.6)*	3627.2 (118.6)	3641.7 (103.5)	3432.1 (96.8)*
Birth weight centile	51.7 (6.1)	62.0 (5.1)	40.2 (5.2)	57.2 (7.1)	55.3 (6.2)	48.5 (5.8)
Cord blood cortisol (nmol/l)	223.4 (34.8)	238.6 (25.4)	259.2 (25.0)	233.6 (37.3)	213.3 (30.9)	240.5 (28.1)
HC centile	43.1 (28.3)**	37.0 (22.3)**	43.3 (32.6)**	52.7 (30.8)	58.4 (27.8)	48.4 (32.6)
Length centile	74.5 (7.3)	63.7 (5.9)	61.4 (5.3)	78.8 (8.4)	79.1 (6.8)	65.8 (6.1)
Apgar at 5 min	9.0 (0.1)	9.2 (0.1)	9.1 (0.1)	9.1 (0.1)	9.2 (0.1)	9.2 (0.1)
Placental weight	649.5 (28.6)	651.6 (22.9)	640.8 (26.5)	638.7 (35.0)	663.5 (29.4)	629.5 (26.5)
<i>18 weeks</i>						
HC	164.7 (4.1)	160.8 (3.3)	161.9 (3.0)	158.8 (5.2)	160.8 (4.5)	166.5 (3.4)
Femur length	30.0 (0.0)	29.9 (0.8)	29.5 (0.8)	29.6 (1.3)	30.4 (1.1)	31.3 (0.8)
SD ratio	3.9 (0.9)	4.6 (0.4)	5.0 (0.4)	3.7 (0.5)	3.7 (0.5)	4.3 (0.4)
Abdominal circumference	142.7 (3.7)**	140.9 (2.6)**	138.9 (3.0)**	146.5 (3.7)	150.5 (3.2)	141.6 (2.5)
<i>30 weeks</i>						
HC	295.4 (7.3)	275.9 (4.3)	278.7 (4.0)	280.2 (7.9)	284.3 (5.6)	280.9 (4.7)
Femur length	61.3 (2.1)	57.2 (1.2)	57.2 (1.1)	57.3 (2.2)	57.0 (1.6)	56.6 (1.3)
SD ratio	2.8 (0.2)	3.0 (0.1)	2.9 (0.1)	2.7 (0.2)	2.8 (0.2)	2.8 (0.1)
Abdominal circumference	263.3 (4.0)	260.1 (2.8)	265.4 (2.9)	270.6 (3.7)	262.5 (3.3)	268.4 (2.5)

SD = systolic:diastolic ratio.

* Significantly different compared to controls.

** Significantly different to males.

ment for glucocorticoids was dependent on asthma severity. In the mild asthma group, 66% ($n = 35$) of women used no inhaled glucocorticoid treatment for asthma, 13% ($n = 7$) used low dose, and 21% ($n = 11$) used moderate doses of inhaled glucocorticoids. In the moderate-severe asthma group, 18% ($n = 10$) used no inhaled glucocorticoid treatment, 17% ($n = 9$) used low dose, 39% ($n = 21$) used moderate dose while 26% ($n = 14$) used high doses of inhaled glucocorticoids for asthma treatment. The analyses in both the mild and moderate-severe asthmatic groups indicated no effect of inhaled glucocorticoid dose on cord plasma IGF-1, IGF-2, IGFBP-1 or IGFBP-3.

4.3.2. Effect of fetal sex and asthma IGF axis and growth

In males, cord plasma IGF-1 varied with asthma severity [$F(1, 40) = 3.960$, $p = 0.027$], with higher levels with exposure to mild asthma compared to both control and moderate-severe asthma ($p < 0.05$; Table 3). Male IGF-1:IGFBP-3 ratio was significantly increased with mild asthma compared to controls and moderate-severe asthma [$F(2, 31) = 3.52$, $p = 0.04$]. In females, cord plasma

IGF-1 did not vary with asthma severity. Cord plasma IGF-2, IGFBP-1 or IGFBP-3 did not vary with fetal sex (Table 3).

There were no correlations observed in the control groups between male or female birth weight centiles and any component of the IGF axis measured in cord plasma.

In pregnancies complicated by asthma, female birth weight centiles were significantly correlated with cord plasma IGF-1 concentrations ($r = 0.448$, $p = 0.004$, $n = 39$), and inversely associated with IGFBP-1 levels ($r = -0.455$, $p = 0.001$, $n = 30$). Male birth weight centiles were also positively associated with IGF-1 ($r = 0.454$, $p = 0.003$, $n = 36$) and negatively associated with IGFBP-1 levels ($r = -0.449$, $p = 0.014$, $n = 42$). In addition, IGFBP-3 levels were also positively associated with birth weight centiles in cord plasma from males ($r = 0.549$, $p < 0.001$, $n = 42$).

4.3.3. Effect of fetal sex, cigarette smoking and inhaled glucocorticoid treatment in pregnancies complicated by asthma on the IGF axis and growth

In asthmatic pregnancies, smoking was associated with reduced cord plasma IGF-1 in female fetuses [$F(1, 64) = 5.556$, $p = 0.021$], while male IGF-1 was unaffected by cigarette smoking (Fig. 2). All other components of the IGF axis were unaffected by cigarette use in both males and females (Fig. 2).

At birth, cigarette smoking was associated with elevated cord plasma cortisol levels [$F(1, 97) = 3.738$, $p = 0.042$], and reduced length centile [$F(1, 75) = 4.03$, $p = 0.048$] irrespective of fetal sex (Table 4). Birth weight, birth weight centiles, head circumference and placental weight were unaffected by smoking. Ultrasound growth data recorded at 18 and 30 weeks were also unaffected by maternal smoking during pregnancy (Table 4). The effect of the combined use of inhaled glucocorticoids with smoking in asthmatic pregnancies did not further alter any component of the IGF axis.

Birth weight centiles of female neonates of mothers with asthma who smoked during pregnancy were positively correlated with IGF-1 ($r = 0.597$, $p = 0.04$, $n = 14$) and negatively correlated with IGFBP-1 ($r = -0.563$, $p = 0.005$, $n = 22$). There were no correlations between any measured parameters in the presence of a male, maternal asthma and cigarette use.

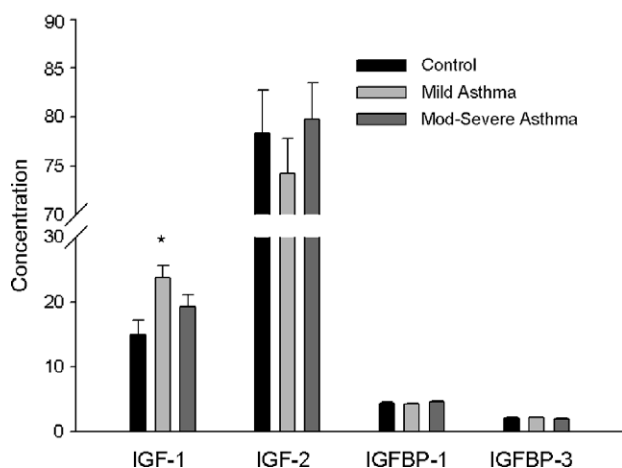
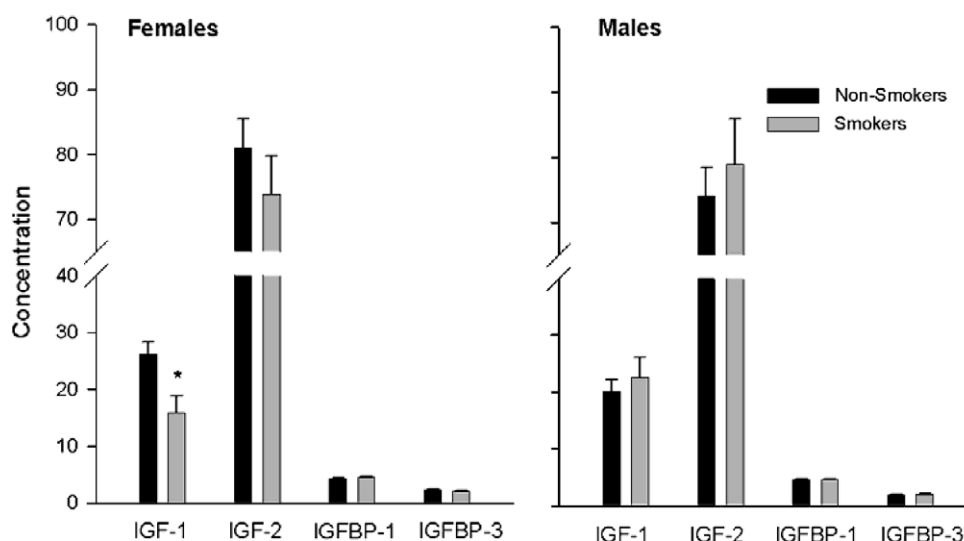


Fig. 1. Effect of asthma severity on the newborn cord plasma IGF axis. Control $n = 22$, mild asthma $n = 26$, moderate-severe asthma $n = 20$. Bars represent means + SEM.

Table 3

Cord blood IGF and IGFBP levels in males and females from control and pregnancies complicated by mild and moderate-severe asthma. Data represent mean (SEM).

	Female				Male			
	Control	Mild	Moderate-severe	<i>p</i>	Control	Mild	Moderate-severe	<i>p</i>
<i>n</i>	14	17	23		7	17	19	
IGF-1	17.714 (3.063)	23.076 (2.780)	20.821 (2.390)	0.437	13.611 (3.795)	23.894 (2.435)*	15.807 (2.304)	0.027*
IGF-2	80.93 (5.182)	81.295 (4.702)	71.109 (4.043)	0.181	72.646 (9.179)	67.853 (5.890)	80.994 (5.724)	0.284
IGFBP-1 (ln transformed)	4.331 (0.197)	4.184 (0.161)	4.581 (0.155)	0.208	4.820 (0.234)	4.537 (0.178)	4.526 (0.162)	0.547
IGFBP-3	2.178 (0.131)	2.396 (0.107)	2.152 (0.104)	0.224	2.118 (0.171)	1.900 (0.130)	1.896 (0.118)	0.522

* Significantly higher than both the control and moderate-severe asthma groups (Tukeys post hoc test, $p < 0.05$).**Fig. 2.** Effect of cigarette smoking in asthmatic pregnancies on IGF concentrations in cord plasma from female (left panel; non-smokers $n = 22$, smokers $n = 12$) and male fetuses (right panel; non-smokers $n = 23$, smokers $n = 9$). Bars represent means + SEM.**Table 4**

Fetal characteristics of male and females born to women with asthma, split by use of cigarettes during pregnancy. Data represent mean (SEM).

	Female		Male		<i>p</i>
	Non-smoker	Smoker	Non-smoker	Smoker	
<i>n</i>	38	23	33	12	
18 weeks					
HC	160.9 (3.0)	162.1 (3.6)	164.4 (3.3)	164.7 (5.3)	n.s.
Femur	30.0 (0.7)	29.3 (0.8)	31.4 (0.8)	29.8 (1.2)	n.s.
SD ratio	4.6 (0.3)	5.4 (0.5)	3.9 (0.4)	4.9 (0.9)	n.s.
Abdominal circ.	140.2 (2.4)	139.6 (3.9)	142.8 (2.5)	150.2 (3.8)	0.043 ^b
30 weeks					
HC	275.0 (3.8)	281.1 (4.8)	284.9 (4.3)	275.6 (7.0)	n.s.
Femur	56.8 (1.1)	57.8 (1.4)	57.3 (1.3)	55.5 (2.0)	n.s.
SD ratio	3.06 (0.1)	2.8 (0.1)	2.8 (0.1)	2.9 (0.2)	n.s.
Abdominal circ.	263.2 (2.5)	261.3 (3.9)	266.6 (2.5)	265.3 (4.0)	n.s.
At birth					
Weight	3451.9 (751)	3240.0 (94.6)	3525.9 (80.6)	3540.8 (133.2)	n.s.
Birth weight centile	52.1 (4.4)	36.3 (5.7)	53.1 (4.7)	47.9 (7.8)	n.s.
Cord blood cortisol (nmol/l)	222.0 (23.8)	292.2 (30.0)	218.9 (25.7)	251.4 (40.7)	0.042 ^a
HC centile	41.0 (4.9)	39.2 (6.3)	54.3 (5.1)	50.0 (8.3)	n.s.
Length centile	62.9 (5.1)	61.7 (6.5)	77.3 (5.2)	50.1 (10.2)	0.048 ^a
Placental weight	645.6 (20.1)	649.6 (27.6)	650.4 (21.9)	630.7 (34.4)	n.s.

HC = head circumference; SD = systolic to diastolic ratio; circ. = circumference; a = significance level for comparison smokers vs. non-smokers; b = significance level for comparison between males and females; n.s. not significant.

4.3.4. Effect of cortisol on male and female newborn IGF axis and growth

Sex specific associations between cortisol and the IGF axis were observed. In the cord plasma of females from control pregnancies, levels of cortisol were positively correlated with IGFBP-1 ($r = 0.70$, $p = 0.003$, $n = 16$) and not correlated with cord plasma IGF-1 or 2 or IGFBP-3.

In asthmatic pregnancies, cortisol was inversely associated with female birth weight centile ($r = -0.380$, $p = 0.003$, $n = 58$). Further, in these pregnancies, cortisol levels were directly and inversely related to IGFBP-3 levels ($r = -0.280$, $p = 0.43$, $n = 54$). In males, no association was observed between cortisol, the IGF axis and birth weight from either the control or asthmatic populations.

The combined use of inhaled glucocorticoids with smoking in asthmatic pregnancies was found to affect cortisol concentrations in cord plasma from females (Fig. 3). Increased cortisol concentrations were demonstrated in cord plasma from females with maternal smoking in the absence of inhaled glucocorticoid use [$F(4,67) = 3.233$, $p = 0.017$]. Maternal smoking with inhaled glucocorticoid use restored female cord plasma cortisol concentrations to control levels. Cord plasma cortisol levels did not statistically differ in males with maternal cigarette smoking and glucocorticoid use (Fig. 3). In the presence of cigarette use and asthma, there were no correlations between cortisol and the IGF axis or birth weight centile. Birth weight centile of neonates of cigarette users was negatively correlated with IGFBP-1 ($r = -0.563$, $p = 0.005$, $n = 34$) and positively correlated with IGF-1 ($r = 0.597$, $p = 0.004$, $n = 24$) and not correlated with IGF-2 or IGFBP-3.

4.3.5. The IGF axis and birth weight centiles

Hierarchical multivariate regression analysis was conducted to assess the independent contribution of smoking, glucocorticoid dose, asthma severity, IGF-1, IGF-2, IGFBP-1 and -3 and cortisol on male and female birth weight centiles. Smoking and asthma severity were entered as dummy variables (1 = non-smoking, 2 = smoking; severity: 1 = control, 2 = mild, 3 = moderate-severe).

Smoking, glucocorticoid use and asthma severity were entered at step one, and were found to jointly predict birth weight centiles in females [$R^2 = 0.278$, $F(3,40) = 4.746$, $p = 0.007$] but not males. Smoking ($\beta = -0.347$, $p = 0.011$) and glucocorticoid dose ($\beta = 0.423$, $p = 0.018$) were found to independently predict female birth weight centiles. These data indicate that smoking in the absence of glucocorticoid intake reduced female birth weight centiles, but combined smoking and glucocorticoid intake restored birth weight centiles, as seen in Fig. 4.

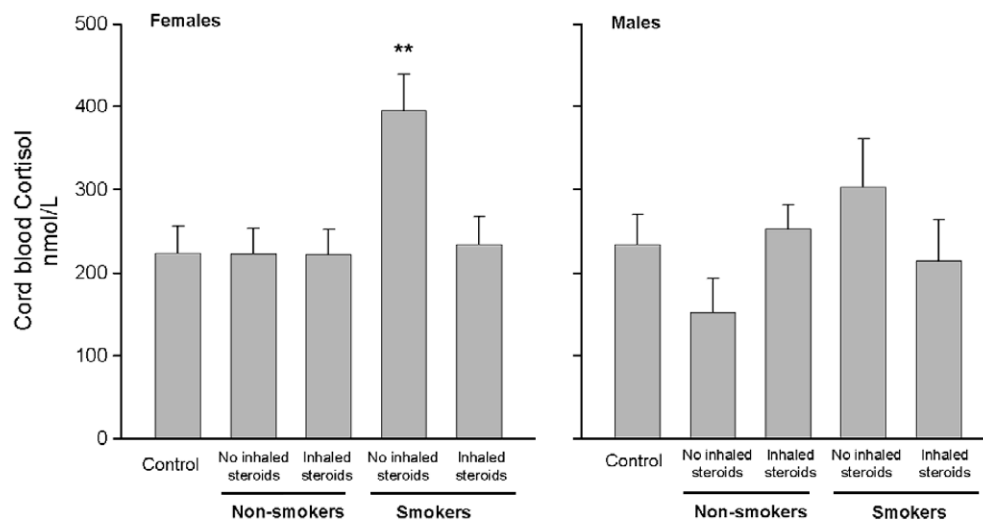


Fig. 3. Cord plasma cortisol concentrations in control and asthmatic groups in female (left panel) and male (right panel) neonates, split by smoking status and asthma treatment. Bars represent means + SEM. Control: females $n = 15$, males $n = 13$; non-smoker/no inhaled steroids: female $n = 17$, male $n = 10$; non-smoker/inhaled steroids: female $n = 18$, male $n = 20$; smoker/no inhaled steroids: female $n = 8$, male $n = 5$; smoker/inhaled steroids: female $n = 14$, male $n = 7$. * $p = 0.015$ (Tukeys post hoc) compared to all other female asthmatic and control groups.

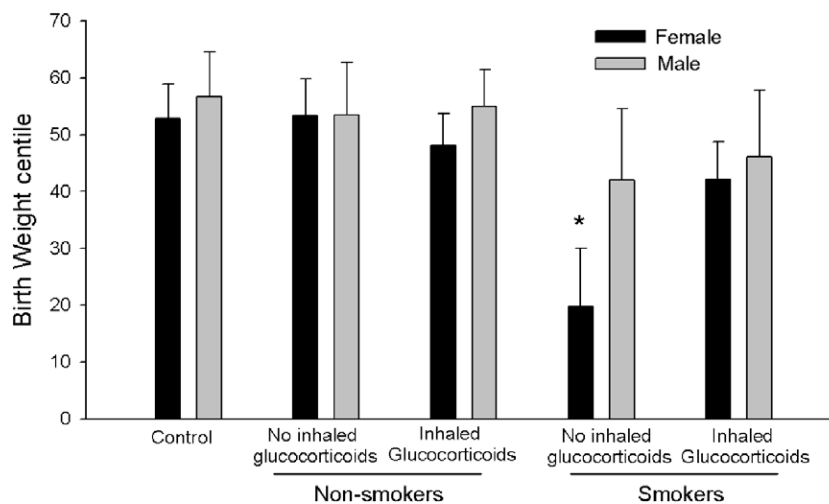


Fig. 4. Birth weight centiles in control and asthmatic groups in female (left panel) and male (right panel) neonates, split by smoking status and asthma treatment. Bars represent means + SEM. Control: females $n = 22$, males $n = 16$; non-smoker/no inhaled steroids: female $n = 19$, male $n = 12$; non-smoker/inhaled steroids: female $n = 19$, male $n = 21$; smoker/no inhaled steroids: female $n = 7$, male $n = 5$; smoker/inhaled steroids: female $n = 16$, male $n = 7$. * $p = 0.015$ (Tukeys post hoc) compared to all other female asthmatic groups.

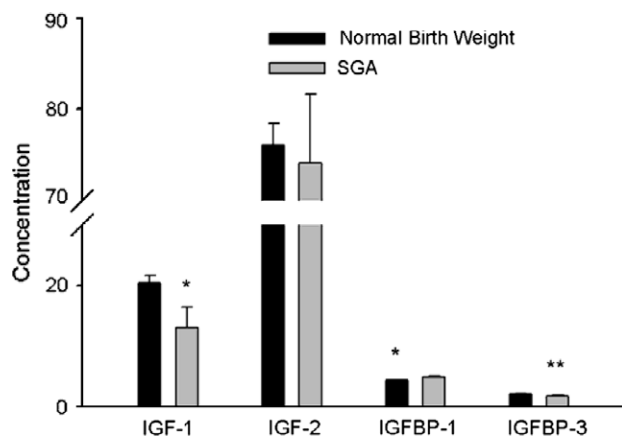


Fig. 5. Newborn cord plasma concentrations of IGF-1 and -2 and IGFBP-1 and -3 in infants born small for gestational age (less than the 10th birth weight centile; $n = 9$) compared to neonates of normal birth weight centile ($n = 79$). Bars represent means $\pm$ SEM. * $p < 0.05$, ** $p = 0.002$.

After entering IGF-1, IGF-2, IGFBP-1, IGFBP-3 and cortisol into the regression equation, controlling for the effects of the variables entered at step one, the regression equations predicting birth weight were significant for females [$R^2 = 0.267$, $F(5, 40) = 3.912$, $p = 0.006$] and males [$R^2 = 0.569$, $F(5, 33) = 2.677$, $p = 0.042$]. IGF-2 and IGFBP-1 were not significant predictors of birth weight centiles and were removed from the final analysis. The strongest significant independent predictor of birth weight in females, after controlling for asthma severity, smoking and glucocorticoid intake, was IGF-1 ($\beta = 0.334$, $p = 0.05$), while in males, IGFBP-3 was the only significant independent predictor of birth weight centile ($\beta = 0.406$, $p = 0.044$).

4.3.6. The IGF axis and SGA deliveries

Significantly lower levels of cord plasma IGF-1 and IGFBP-3 were demonstrated in neonates born SGA ($n = 9$) compared to those of normal birth weight ($n = 79$) (Fig. 5; $F(1, 123) = 4.163$, $p = 0.044$ and $F(1, 123) = 9.975$, $p = 0.002$, respectively). Further, significantly higher levels of IGFBP-1 were found in infants born SGA compared to those of normal birth weight [$F(1, 123) = 4.554$, $p = 0.035$]. IGF-2 did not change in SGA pregnancies relative to the control population. Neonatal sex was not found to interact with SGA to affect the levels of IGF-1 or IGF-2 or the measured binding proteins or the ratios.

5. Discussion

This study has identified sex-specific differences in the IGF axis in the presence of maternal asthma and in the presence of maternal cigarette use during pregnancy. Mild maternal asthma was associated with no change in neonatal birth weight relative to the control population and increased cord plasma IGF-1 in only newborn males. Both male and female birth weights were reduced in the presence of moderate-severe maternal asthma with no significant change in any component of the IGF axis when compared to the control population. This data suggests there are compensatory adjustments in the IGF axis in the presence of mild asthma and no adjustments to the IGF axis in the presence of moderate-severe asthma which may contribute to reduced growth in the male and female fetuses of this group of asthmatics. To ascertain if our findings were comparable to previous studies, we also examined the newborn IGF axis in all neonates identified in our cohort as small for gestational age at birth. We found reduced IGF-1 and IGFBP-3 and increased IGFBP-1 which is in agreement with previ-

ous studies [32,38]. Furthermore birthweight centiles in the entire population were positively associated with newborn IGF-1 and IGFBP-3 and negatively correlated with IGFBP-1 [23,29]. Overall these data indicate that IGF-1 remains the most significant factor contributing to increased birth weight and the newborn IGF axis appears to be differentially regulated depending on asthma severity and fetal sex.

Previous studies have identified that placentae of asthmatic women who were non-smokers had reduced placental vascular function in the presence of moderate and severe asthma and reduced umbilical SD ratio at 18 weeks gestation suggesting increased asthma severity induces increased vasoconstriction in the placental circulation [68]. Stereological studies of placentae from pregnancies complicated by asthma identified reduced capillary density and decreased capillary and villous length and area without significant changes in placental weight of pregnancies complicated by moderate-severe asthma [69]. The post hoc analysis of the placental morphology data indicated the observed changes in placental structure were attributable to male rather than female placentae of pregnancies complicated by asthma [69]. Reduced growth of male neonates in the presence of moderate-severe asthma may also be attributable to changes in placental morphological structure rather than significant alterations in the IGF axis.

Reduced female birth weight in the moderate-severe asthmatic group appears dependent on the relationship between cortisol and the IGF axis. We have previously demonstrated males and females were exposed to the same cord plasma concentrations of cortisol in response to maternal asthma but only the female neonate had reduced birth weight [2] suggesting that females are more sensitive to changes in glucocorticoid concentration than males. In this study we observed that cortisol was only correlated with female birth weight and the IGF axis. Cortisol did not appear related to any male parameters.

In our study cord plasma cortisol was positively correlated with IGFBP-1 in the control population of female newborns but not in the presence of asthma. Furthermore cortisol was negatively correlated with female birth weight centile and cord plasma IGFBP-3 in the presence of asthma but this relationship was not observed in the control population. These data suggest the IGFBP-1 has a greater impact on the regulation of fetal growth in normal pregnancy and in the presence of a complication such as maternal asthma, cortisol may affect female fetal growth via down regulation of IGFBP-3.

The addition of cigarette use to pregnancies complicated by asthma further changed the relationship between cortisol and the IGF axis in females. In the presence of maternal asthma and cigarette use, there was a reduction in IGF-1, IGFBP-3 and birth weight centiles and an increase in cord plasma cortisol in female neonates and not males. Female cord plasma cortisol was not correlated with any component of the IGF axis or with birth weight centile. Birth weight centile of females of mothers who had asthma and smoked was positively correlated with IGF-1 and negatively correlated with IGFBP-1 which are relationships comparable to the control population. Cigarette use during pregnancy has previously been shown to activate the fetal hypothalamic-pituitary adrenal axis with a rise in cortisol [70,71]. In non-asthmatic women who used cigarettes during pregnancy there was a significant decrease of cord plasma IGF-1 concentrations, no change in IGF-2 concentrations and decreased IGFBP-3 [60]. IGFBP-1 was not examined in this previous work [60]. The changes observed in our study are in agreement with this previous work but we have also identified there are additional considerations when assessing the IGF axis which include fetal sex and cortisol.

Interestingly, changes in fetal growth in the presence of asthma and cigarette use were not observed at the 18 or 30 weeks ultrasound and were not related to changes in placental umbilical ar-

tery SD ratios or placental weight. These data suggest the impact of asthma and smoking has its greatest effect on female fetal growth in the late third trimester and may not be mediated through reduced placental growth or reduced placental blood flow. Previous studies have shown that the effect of cigarette use on fetal growth was not measurable using mid-trimester ultrasound [72] and not associated with reduced placental weight [60]. Stereology studies have shown that smoking does affect placental structure in terms of villous composition and vasculature [73,74] which could in turn influence growth via reductions in nutrient and oxygen transfer.

We did not observe any effect of inhaled glucocorticoid use for the treatment of asthma on fetal growth or the IGF axis. The use of moderate to high doses of inhaled glucocorticoids for the treatment of non pregnant asthmatics are known to exert systemic effects by suppressing adrenal function [75] but it is not known if a similar effect occurs with pregnancy and inhaled glucocorticoid use. Markers of adrenal status in the fetus would suggest that inhaled glucocorticoid treatments do not cross into the fetal compartment to alter fetal adrenal function [2] and so the beneficial effects in terms of fetal growth observed with the use of inhaled glucocorticoid treatment of asthma relate to the inhibitory effects of these drugs on the maternal system in relation to adrenal function, lung function and systemic inflammatory pathways associated with maternal asthma. It is known that maternal betamethasone treatment for fetal lung maturation in pregnancies complicated by preterm labour can alter the IGF axis [76] but the dosage of betamethasone administered to women at risk of preterm delivery was 100 times greater than the average daily dose of inhaled glucocorticoid for the treatment of asthma and therefore the effect of betamethasone will be enormous in comparison to an asthma treatment on the IGF axis. We suggest there are no direct effects of inhaled glucocorticoid drugs on the IGF axis or fetal growth as the inhaled steroids do not cross the placenta. This may be due the inhaled steroid dose being too low to exert systemic effect on the fetus or it could be due to the multi-drug resistant protein, glycoprotein P which is known to shuttle synthetic steroids out of the placenta and back into the maternal circulation [77]. This has yet to be confirmed for pregnancies complicated by asthma.

The use of inhaled glucocorticoids for the treatment of asthma was protective against the growth inhibiting effects of cigarette use in asthmatic women pregnant with a female. This effect does not appear to be mediated by changes or adjustments in the IGF axis but may be due to no change in fetal cortisol concentrations with maternal inhaled glucocorticoid use during pregnancy. We suggest that inhaled glucocorticoid use suppresses maternal adrenal function resulting in no change in fetal cortisol concentrations with asthma. The effect of inhaled glucocorticoids on maternal adrenal function during pregnancy has yet to be determined.

In males, asthma, inhaled glucocorticoid use and cigarette use did not affect birth weight centile but length centile was reduced. The effects of asthma and smoking on neonatal length were apparent from measures of male fetal femur length at 30 weeks gestation. Placental blood flow and placental weight were not different between the groups suggesting the changes in male length were not due to changes in placental function or growth. Since asthma and inhaled glucocorticoid use, in the absence of cigarette use, did not affect neonatal length it is likely that cigarette use is the main contributing factor to changes in length. Cigarette usage during pregnancy in non-asthmatic women is known to be associated with a mean reduction of birth weight of approximately 200 g [69] decreased neonatal length and head circumference, no change in placental weight but increased umbilical artery SD ratios at 30 weeks gestation [60]. Our data suggest that asthma and cigarette use have different impacts on fetal growth in relation to the sex of the fetus.

The correlations between the IGFs or IGFBPs and growth indicated sex-specific regulation of fetal growth in pregnancies complicated by asthma may be controlled by the IGF axis. IGF-1 was decreased in cord plasma of females of mothers with asthma who used cigarettes and overall IGF-1 was the best predictor of female birth weight centile after controlling for the effects of smoking and asthma. This suggests IGF-1 has an important role in modulating female growth. The main factor that we measured in the IGF axis that may influence continued growth of the male fetus in the presence of asthma was IGFBP-3. From the multivariate analysis, taking into account all variables, we identified that IGFBP-3 was the main predictor of male birthweight. We also found that IGF-BP3 was positively correlated with male birthweight. Previous studies have demonstrated positive correlations between IGFBP-3 and birth weight, but these studies did not separate data based on fetal sex [25,36]. These data suggest IGFBP-3 may have a sexually dimorphic role by specifically promoting growth of the male fetus in the presence of maternal asthma.

The mechanism by which IGFBP-3 promotes fetal growth is unknown but may include pathways that are separate from its role as a reservoir for IGFs. Work conducted in breast epithelial cell lines indicates that IGFBP-3 can potentiate epidermal growth factor (EGF)-induced cell proliferation and survival [78] via increased phosphorylation of the EGF receptor and its associated signalling pathway [79]. EGF is a growth factor known to be positively correlated with fetal growth [80,81] but its actual role during pregnancy is yet to be elucidated. The continued growth of the male fetus in the presence of maternal asthma and cigarette use may be related to IGFBP-3 potentiation of EGF-induced cell proliferation.

Sex steroids are likely candidates involved in conferring the differences in growth in male and female fetuses. In adults, sex steroids are known to regulate IGF-1 and its binding proteins [82]. Estrogen supplementation in adult females increases serum IGFBP-1, decreases IGF-1 and increases binding of IGFBP-1 to IGF-1 therefore reducing bioavailability of IGF-1 [82]. In addition, testosterone has been demonstrated to increase IGFBP-3 and IGF-1 production in male foreskin fibroblasts in vitro [83,84] and in female lamb fetuses at day 65 of gestation [85]. Testosterone supplementation for 3 weeks in post menopausal women also increased circulating IGF-1 and IGFBP-3 [86]. In the human fetus the role of sex steroids in regulating the IGF axis have not been examined but previous studies have described sex-specific differences in IGF pathways and other factors associated with growth such as leptin and growth hormone in both the fetus [55,87,88] and neonate [89,90].

Our work confirms that a key determinant of fetal growth was IGF-1 which was positively correlated with birth weight in all neonates and was the strongest predictor of female birth weight after accounting for cigarette use, asthma severity and inhaled glucocorticoid treatment. IGFBP-3 was the strongest determinant of male birth weight centile and this suggests this particular binding protein may have its own anabolic effects separate from its role as a binding protein. The presence of a maternal complication such as asthma with or without cigarette use, has demonstrated there is a sex specific response of the fetal-placental unit to maternal complications and provides evidence that male and female fetuses have developed different strategies for coping with stressful pregnancies.

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