AMERICAN JOURNAL OF HUMAN BIOLOGY 17:178–194 (2005)

Original Research Article

Growth Patterns of the Heart and Kidney Suggest Inter-organ Collaboration in Facultative Fetal Growth

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ABSTRACT Maternal smoking during pregnancy has been associated with a number of negative sequelae among offspring, including elevated postnatal blood pressure. While animal studies have described organ level alterations with smoke exposure, human data have been more limited. Thirty-four healthy maternal/fetal pairs (24 nonsmokers, 10 smokers) participated in a longitudinal growth study from the thirteenth week of pregnancy to document fetal kidney and heart growth trajectories and morphology. Curve fitting followed by a mixed model for repeated measures identified significantly different growth patterns in kidney width, thickness, length, and volume growth with exposure: the smoke-exposed fetal kidney was wide and thick compared to the unexposed kidney during the second and early third trimester, declining to proportionately thin kidneys for length and width subsequently. Cardiac growth in width and volume followed a reverse pattern: a surge in cardiac volume occurred after 30 weeks with acceleration in cardiac width, resulting in a heart that was wide for length and for fetal weight. Smoke exposure altered fetal growth in size and timing of the heart and kidneys during midgestation, with changes in organ morphology suggesting compensatory growth. These are the first data providing anatomical evidence of altered renal/cardiac volume relationships that may provide a mechanism to previously reported sequelae of in utero smoke exposure. They suggest that cell-level adaptive responses to hypoxia and/or chemical insults are operative and illustrate the importance of longitudinal ultrasound to directly assess the organ-level growth response of the human fetus to a prenatal stress, in lieu of relying on birth outcome measures. Am. J. Hum. Biol. 17:178–194, 2005.

More than half a century ago, it was reported that cigarette smoking during pregnancy increases the fetal heart rate (Sontag and Wallace, 1935). Subsequently, it was noted that smoke exposure is associated with intrauterine growth retardation and low birth weight (Davies and Abernethy, 1976; Naeye, 1981), and many investigations have clearly established that prenatal maternal smoking has multiple negative effects for the developing fetus (USDHS, 1990; DiFranza et al., 2004). More recent studies have linked smoking during pregnancy with longer-term health sequelae in child and adult offspring, including asthma (Jaakkola and Gissler, 2004), obesity (Vik et al., 1996; Power and Jefferis, 2002; von Kries et al., 2002; Wideroe et al., 2003), and type 2 diabetes (Montgomery and Eckbom, 2002), as well as elevated blood pressure in some samples (Morley et al., 1995; Beratis et al., 1996; O'Sullivan et al., 1996; Williams and Poulton, 1999; Blake et al., 2000). These findings have been interpreted as specific examples of how an intrauterine milieu leading to low weight at birth may have lasting influence on offspring physiology and health.

The mechanisms through which fetal in utero experience organizes organ-level functional morphology in humans are unclear. It has been reported that low birth weight or intrauterine growth retarded fetuses have smaller kidneys by late gestation (Konje et al., 1996; Silver et al., 2003). More specifically, some studies have noted fewer nephrons (Hinchcliffe et al., 1992; Beech et al., 2000; Manalich et al., 2000), while others have not (Haycock, 2001; Jones et al., 2001). Questions have been raised concerning how to interpret risks associated with the wide range of human nephron number (Kett and Bertram, 2004).

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Received 21 September 2004; Revision received 7 December 2004; Accepted 7 December 2004

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajhb.20109

Overall, the findings of nephron deficits in animal models of intrauterine growth restriction (Merlet-Benichou et al., 1994; Bassan et al., 2000; Bauer et al., 2003; Langley-Evans et al., 2003), have suggested an anatomical basis for elevated blood pressure found among humans born small or to a mother poorly nourished during pregnancy (Barker, 1995; Ingelfinger, 2004). Alternative animal models have documented intrauterine growth restricted rats exhibiting increased heart mass relative to body weight, renal dysfunction, and subsequent increased systolic blood pressure (Battista et al., 2002), while the spontaneously hypertensive rat model illustrates both renal and cardiac alterations during fetal growth (Lewis et al., 1997).

It is presently unknown whether organ-level responses occur in the human fetus developing in the context of maternal smoking. As smoking rates among women and young adults rise worldwide (http://www.tobacco.who.int; http://www.cdc.gov/tobacco; Cnattingius, 2004), accompanied by no appreciable decline in pregnancy-related smoking (Ebrahim et al., 2000), there is a need to investigate the prenatal organ-level responses of the fetus to maternal smoking (DiFranza et al., 2004). It has been previously documented that smoke exposure during fetal development is a toxic environment due to smoke-induced carboxyhemoglobin and chronic hypoxia (Harrison and Robinson, 1981; Socol et al., 1982), erratic oxygen delivery with episodic nicotinic-mediated alterations in heart rate and cardiovascular tone (Sindberg Eriksen and Marsal, 1987), and toxic chemical constituents.

This report used real-time ultrasonographs to investigate gestational age-specific effects of exposure to maternal smoking during pregnancy on two organs of the cardiovascular system—the heart and kidneys—in the growing fetus. While several previous studies have identified general trends in fetal body reduction associated with prenatal smoke exposure (Jeanty et al., 1987; Goldenberg et al., 1993; Vik et al., 1996; Bernstein et al., 2000; Zaren et al., 2000), to our knowledge no study has yet reported patterns of organ level alterations using weekly fetal human longitudinal data. These data were not collected as part of an epidemiological investigation but were part of a clinical inquiry aimed to document time intensive growth patterns of the fetus. The present report is a post-hoc analysis based on the postulation that the functional demands imposed upon the developing fetal heart and kidneys by growth in the context of prenatal maternal smoking would be reflected in distinct organ growth patterns. The results suggest mechanisms by which organ growth may contribute to facultative fetal adaptive strategies more broadly as well as those that may relate to previous epidemiologic reports of maternal prenatal smoking sequelae.

Study sample and measurement protocol

Thirty-four healthy maternal/fetal pairs (24 nonsmokers and 10 smokers) participated in a longitudinal fetal growth study at the Hôpital Erasmus in Brussels with a weekly measurement protocol from 13 gestational weeks. This was not a matched case-control sample. All subjects provided informed consent, were volunteers from the health care profession, and of middle class socioeconomic background. While relatively homogenous in health status, occupation, and socioeconomic level, specific information about diet, exercise, or other behavioral confounders that may have affected fetal growth were not collected. As the sample size in this study is insufficient to have included these important epidemiologic variables as analytic covariates, the mixed-model statistical approach used here addresses individual-level effects such as these by inclusion of the subject as a random effect. All women had unremarkable medical and pregnancy histories. Gestational ages were calculated as time since last menstrual period, confirmed by ultrasound measurement of crown–rump length between 8 and 10 weeks (Robinson, 1973). Fetal sex was recorded for 27 subjects (60% males).

Thirteen fetal body parameters were directly measured once at each visit (Table 1) with transabdominal ultrasound by the same individual (P.J.) using calipers and a Toshiba SAL 10A with a frame freeze and a 2.4-MHz transducer according to methods published in detail elsewhere (Jeanty et al., 1982a, 1982b, 1984a, 1984b). This report focuses on the kidney and heart data, with kidney measurements that were initiated at 19 gestational weeks and heart measurements at 13 weeks.

Kidney length was measured in a longitudinal section of the fetus, either parasagittal or coronal depending on the fetal position. The anteroposterior and transverse diameters were obtained in an axial section of the kidney at the widest level as observed by scanning up and down in the kidney. Only the most proximal kidney to the transducer was

TABLE 1. Ultrasonographic measurements taken (P.J.) by techniques previously described (Jeanty et al., 1982a,b; 1984a,b; 1986)

	Body parameters directly measured by ultrasonography
Head	Biparietal diameter
	Occipital—frontal diameter
Torso	Transverse abdominal diameter
	Anterior–posterior abdominal diameter
Diaphyseal limb lengths	Humerus
	Ulna
	Femur
	Tibia
Heart	Longitudinal cardiac diameter
	Transverse cardiac diameter
Kidneys	Longitudinal kidney length (greatest length)
	Transverse diameter (greatest width, 90° to length)
	Anterior–posterior diameter (greatest thickness at point width taken)

measured to avoid shadowing from the spine. Previous studies have reported no significant prenatal renal size differences based on laterality (Hsieh et al., 2000; Yu et al., 2000).

Cardiac diameters were obtained outside the myocardium by previously published methods (Jeanty et al., 1986) in which the best four-chamber view was attained from a transverse section of the fetal thorax. The longitudinal diameter of the heart was measured from the apex to the highest aspect of the interatrial septum, and the transverse diameter was the largest possible diameter perpendicular to the longitudinal diameter. The measurements were obtained at various times across the cardiac cycle on all subjects, with the aim of providing an average size during the cardiac cycle.

Both kidney and cardiac volumes were calculated using a predictive equation that estimates an ellipsoid (Jeanty et al.,1984b; Hsieh et al., 2000). A validation study using direct measurements of autopsy specimens found this technique to produce estimates that were highly correlated with direct measurement ($r = 0.96$) (Jones et al., 1983; Sampaio, 1995).

Estimated fetal weight (EFW) was calculated according to the method of Aoki (1990), which estimates fetal weight (EFW) from biparietal diameter (BPD), abdominal area (AA), and femur length (FL):

$$\mathrm{EFW} = (1.25647 \times \mathrm{BPD}^3) + (3.50665 \times \mathrm{AA} \times \mathrm{FL}) + 6.3.$$

This has been reported to be an approach with high validity and small systematic bias in a previous validation study with European subjects (Chien et al., 2000).

The mothers categorized as smokers in this sample were those who smoked prior to pregnancy and continued for the duration. Smoking was ascertained by maternal report according to number of cigarettes smoked daily, coded as none ($n = 24$), <20 (one pack of cigarettes, $n = 3$), and >20 cigarettes per day (more than one pack of cigarettes, $n = 7$). Biochemical profiles of smoke exposure were not collected. Previous research has documented significant increases in fetal cord serum cotinine and thiocyanate levels as the result of maternal self-reported daily smoking (Nafstad et al., 1996; Klebanoff et al., 2001) In this analysis, smoking was analyzed as a categorical variable delineating smokers from nonsmokers. There is no reason to suspect lack of reporting among the women in this study. Their self-reported smoke exposure has been previously identified to be associated with altered body growth among the smoke-exposed fetuses in this sample (Lampl et al., 2003), consistent with the presence of chronic hypoxia and/or chemical insults associated with maternal smoking as shown in other samples (Newnham et al., 1990; Vik et al., 1996; Zaren et al., 2000; Hanke et al., 2004).

Parental height, prepregnancy weight, age, and parity were obtained on 32 subjects (23 nonsmoking and 9 smoking).

Statistical analysis

After confirming normality, two-tailed *t*-tests were used to test for the nonequivalence between mean levels of potential confounding parental characteristics by smoking status (Table 2).

In order to test the hypothesis that smoke exposure altered the growth patterns of fetal heart and kidney, a sequence of exploratory data analyses followed by curve fitting was undertaken. This approach was used because there were no previously published reports of cardiac and renal growth patterns for data collected at weekly intervals. For both cardiac and kidney volume, the raw data for each group (smokers and nonsmokers) were fitted by a linear function, and nonlinear functions including polynomials of second to fifth degree,

TABLE 2. Parental characteristics of sample

Parental characteristics[a]	Total sample (SD)	Nonsmokers (SD)	Smokers (SD)	*P* value
Mother's height (cm)	165.4 (6.33)	164.2 (5.78)	168.3 (7.05)	0.10
Mother's weight (kg)	59.4 (8.36)	58.13 (5.71)	62.8 (12.8)	
Mother's BMI (kg/m^2)	20.85 (4.38)	21.57 (1.95)	19.0 (7.65)	
Father's height (cm)	180.3 (6.63)	179.3 (6.21)	182.7 (7.42)	0.10
Father's weight (kg)	78.0 (6.60)	76.78 (7.18)	81.1 (3.48)	
Father's BMI (kg/m^2)	23.3 (4.66)	23.89 (2.07)	21.8 (8.32)	
Primagravida	25%	25%	22%	
Mother's age (years)	25.7 (3.39)	25.6 (3.61)	25.9 (2.93)	

[a]Parental data were available for 32 individuals (23 unexposed, 9 exposed).

symmetric and asymmetric sigmoidal curves (Gompertz and logistic function), and exponential models. These are functions commonly used to model a number of fetal and infant growth dimensions and were suggested by the exploratory analyses as competitive descriptors. The goal of this analysis was to statistically identify the best longitudinal trend in these data with the null hypothesis that smoke exposure would not distinguish growth patterns of heart and kidney dimensions.

Goodness-of-fit criteria included best R^2 values together with a check for randomness of residuals as assessed by runs' tests. A runs' test checks how well any mathematical form captures the raw data points, with a good model fit leaving only randomly distributed residuals about the trend line. Model comparisons by smoke exposure involved sum-of-squares *F*-test and Akaike and Bayes information criteria specifically investigating whether or not the equation parameters were equivalent with the null hypothesis that one curve fit both groups.

Further statistical approaches were employed to estimate the magnitude of the smoke exposure effect on organ growth, information not available from the curve fits. A mixed model for repeated measures with smoke exposure and gestational age included as fixed effects and subject as the random effect was used to test for the time-specific differences evidenced in the curves (*xtreg*, STATA 8). This approach accommodated imbalance in the data due to variable number of repeated measures and unequal intervals between measurements. In addition, subject specific effects such as sex, developmental timing, maternal effects, or other potential biological confounders not explicitly identified were considered in the individual random effects component. These analyses were applied to periods of rapid organ growth identified from the growth pattern analyses after the organ measurements were tested for goodness-of-fit to linear assumptions. No significant runs were identified for any variable across the intervals investigated.

Statistical significance was accepted at $P \leq 0.05$, and trends in the data were considered evident at $P \leq 0.10$. All analyses were conducted with version 8 of the STATA statistical package (2003). Graphical representation of significant results are shown as means by exposure at 23, 27, and 32 gestational weeks of age ($\pm$10 days) where each subject is represented once (Figs. 2, 5, and 6).

RESULTS

After testing for normality, the sample birth outcomes are presented in Table 3, where the median birth weight, length, and mean ponderal index ranged between the 40th and 50th percentiles of sex-specific reference standards (Kuczmarski et al., 2000). The small sample size of subjects with recorded birth outcome variables (6 smoke-exposed and 21 unexposed) was insufficient for meaningful statistical distribution analysis among the exposed fetuses, and for comparison of exposure effects after controlling for gestational age and sex. Therefore, birth weights were compared with a national sample combined sex gestational age-based birth

TABLE 3. Birth outcome[a]

	Sample	Nonsmokers
Birth weight (g)	3340 (3160–3700)	3300 (2975–3690)
Birth length (cm)	51 (50–51)	51 (49–52)
Ponderal index (g/cm^3)	2.6 (0.18)	2.6 (0.2)
Birth gestational age (days)	275 (8)	274 (8.8)

[a]Birth outcome data were available for 27 individuals (21 unexposed, 6 exposed). Median and interquartile range are shown for birth weight and length; mean (sd) are shown for ponderal index and age at birth.

weight reference recently developed and useful here (Oken et al., 2004). No obvious skewing was evident between exposed and unexposed infants (Fig. 1). A previous study reported birth weight adjusted for maternal weight as about 2% less with smoke exposure (Grove et al., 2001). This was also the case in the present sample. Birth length and ponderal index of smoke-exposed fetuses were not distinctive from unexposed neonates (Table 3).

Ultrasound measurements of fetal femur length and head and abdominal diameters of sample subjects corresponded well with reference standards of similar genetic background across gestation (Guihard-Costa et al., 1995), with study medians corresponding to the 50th percentile for head diameters and femur length and the 60th percentile for abdominal circumference. All fetal subjects were thoroughly examined by a pediatrician at birth and found to be clinically healthy.

All births were term (mean gestational age 275 days), and there was no intrauterine growth retardation assessed by weight for gestational age. There were no indications that this sample was biased in any meaningful way. No significant differences in maternal age or parity were found. There was a trend for parental heights to be greater among the smoking sample ($P = 0.10$). Smoke exposure was associated with significantly different organ growth patterns, reflecting different growth rates and morphology.

Renal growth patterns

Renal volumes among the unexposed fetuses were close to previously reported values from other samples (Lawson et al., 1981; Sagi et al., 1987) with late gestation values, in line with published neonatal values (Schmidt et al., 2004). The kidney volume/fetal weight ratio, a previously published value and used here for reference, showed a pattern of only modest change over the study interval for the unexposed sample (Fig. 2). This finding is consistent with Gloor et al. (1997), further suggesting that this smaller sample was not biased by comparison with previous reports.

The curve-fitting procedures identified that the longitudinal growth patterns of kidney measurements were significantly different by exposure for all measured parameters and estimated renal volume. Overall, the smoke-exposed fetal kidney was relatively wide and thick compared to the unexposed kidney during the second and early third trimesters, declining subsequently to proportionately thin kidneys for length and width during the late third trimester.

Specifically, a variable slope sigmoidal function (three-parameter logistic) well described growth of kidney volume and thickness for all subjects (Fig. 3, Table 4). The fitted functions

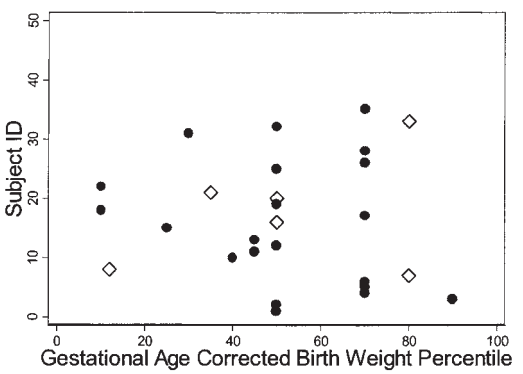


Fig. 1. Birth weight. Birth weight percentiles for gestational age among exposed (open squares) and non-exposed fetuses by subject.

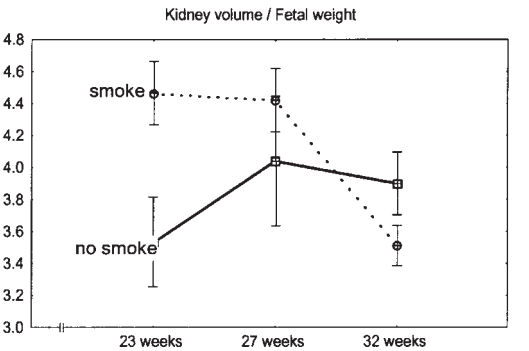
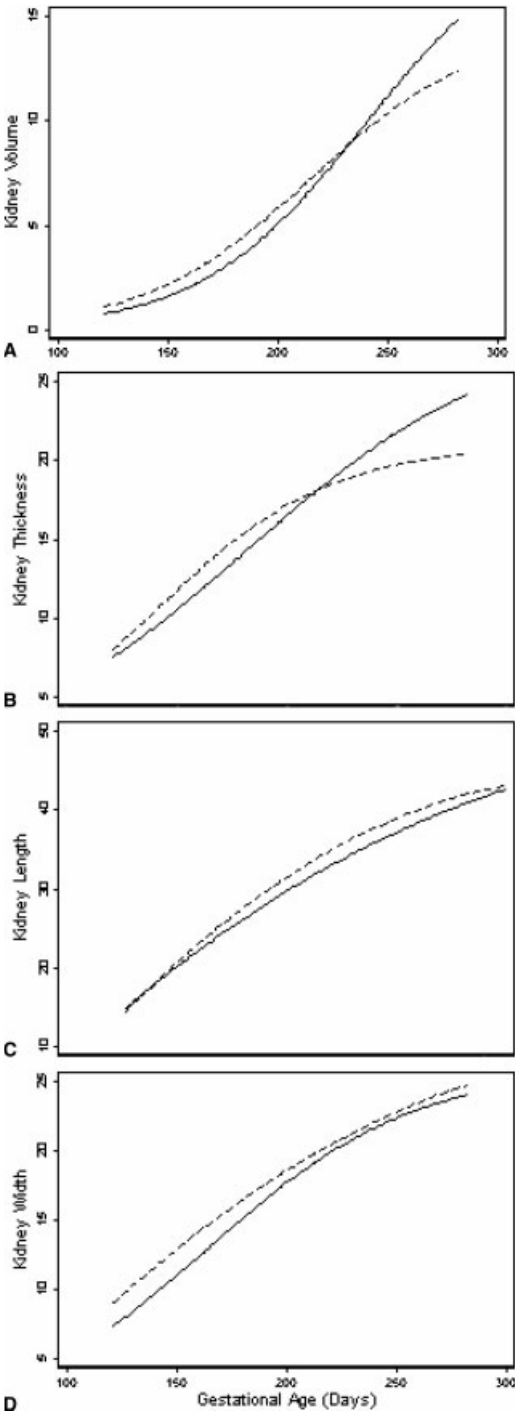


Fig. 2. Ratio of kidney volume to fetal weight (mean cm/g, ± SEM), calculated as per the text. The smoke-exposed kidneys (dotted line) grew significantly more rapidly in early gestation and are significantly larger than the non-smoke-exposed (solid line) by 23 weeks of age. A significant decline in growth rate of the smoke-exposed kidneys after 27 weeks results in smaller kidneys relative to fetal weight by 32 weeks. Sample size constrains statistical significance, which would require more than 100 subjects per study group based on these observations. Between 27 and 32 weeks of age, the non-smoke-exposed fetuses' kidney volume increased by 60% compared to 33% for the smoke-exposed. This may have significant consequences as it is the period of maximum nephrogenic development, which is nearly complete by birth in humans. There is little significant change in growth rate for the non-smoke-exposed fetuses over the entire study interval, with renal volume keeping steady pace with fetal weight.



were compared and the parameter estimates were statistically different by exposure. The null hypothesis of a single curve fitting both groups was rejected for each variable. Kidney length was best described by a second-degree polynomial with significantly different parameter estimates by exposure and the identical curve hypothesis was rejected. Growth in kidney width was best described by a three-parameter logistic for unexposed fetuses and a second-degree polynomial characterized the growth pattern of the exposed fetal kidney.

The mixed models linear approximation quantifying the strength of the exposure effect identified a 1- to 2-fold positive effect on the slopes for kidney thickness and width growth from 130 to 210 days of age, respectively, indicating more rapid growth between ~18.5 and 30 weeks (Table 5). Kidney length was greater throughout gestation. Thesedimensional growth rates resulted in a significant increase in the slope of estimated renal volume growth (a more rapid growth rate) with smoke exposure until 210 days of age (30 gestational weeks). Controlling for the length and width of their kidneys, smoke-exposed fetuses experienced a negative slope in kidney thickness after 220 days (~31 weeks).

Thus, the significant results identified that smoke exposure altered the timing of kidney growth, accelerating measured thickness, width and calculated volume before 30 weeks, followed by declining values after 31 weeks. This resulted in an altered morphology of the fetal kidney: relative to length, a proportionately thick kidney during the second and early third trimesters developed to a proportionately thin kidney by late third trimester among the smoke-exposed fetuses (Fig. 5a). Overall, this resulted in an altered relationship between kidney volume and fetal weight with smoke exposure compared to the unexposed fetuses across gestation (Fig 2).

Cardiac growth

Cardiac dimensions of the unexposed fetuses were close to those previously published in second-trimester reference samples

Fig. 3. Kidney growth. Best-fit trends for (a) kidney volume (cm^3), (b) thickness, (c) length, and (d) width (mm). All are three-parameter logistic functions with the exception of smoke-exposed kidney length, a second-degree polynomial. Smoke-exposed subjects shown in broken lines.

TABLE 4. Best fit growth curve trend functions for kidney dimensions and estimated volume[a]

Kidney	Bestfit curve	R^2	H_o of equality
Volume			
No smoke	$20/(1 + \exp(-0.03 \times (\text{age}) - 240))$	0.94	0.003
Smoke	$14.5/(1 + \exp(-0.03 \times (\text{age}) - 215))$	0.95	
Thickness			
No smoke	$30.4/(1 + \exp(-0.016 \times (\text{age}) - 190))$	0.98	0.003
Smoke	$23/(1 + \exp(-0.019 \times (\text{age}) - 148))$	0.98	
Length			
No smoke	$-32\ \text{age}^2 + 0.48\ \text{age} - 0.0007$	0.81	0.004
Smoke	$-40\ \text{age}^2 + 0.57\ \text{age} - 0.001$	0.76	
Width			
No smoke	$26/(1 + \exp(0.02) \times (\text{age}) - 164)$	0.99	
Smoke	$-12.6\ \text{age}^2 + 0.21\ \text{age} - 0.0003$	0.73	

[a]Best fit determined by a comparison of R^2 values and nonsignificant runs' test of the residuals. Smoke exposure growth functions were compared to unexposed under the null hypothesis that one curve was an equally good fit of both growth data. Significance identifies failure of the null hypothesis by *F*-ratio and Akaike and Bayes information criteria.

TABLE 5. Effects of smoke exposure on the slope of linear trends suggested by best-fit growth models, controlling for gestational age

	Kidney dimensions with smoke exposure				
	Age (days)	β	*P*	R^2	Model *P*
Volume	<210	0.86	0.000	0.71	0.000
Thickness	<210	1.2	0.01	0.59	0.0000
	>220	−1.1	0.05[a]	0.44	0.0000
Width	<210	2.07	0.002	0.39	0.0000
Length	—	1.2	0.03	0.77	0.0000

Note: Results identify significant time-specific smoke-exposure effects on kidney growth (*xtreg* mixed-model STATA 8).
[a]Controlling for kidney width and length to assess overall morphology by exposure.

for gestational age and biparietal diameter (Gembruch, 2000).

Among the equations tested as estimations of the growth trends, a variable-slope sigmoidal function was the best descriptor for all cardiac dimensions (Fig. 4, Table 6). A comparison of three-parameter logistic models identified that the growth trajectory of cardiac length was not significantly different with exposure. By contrast, the three- and four-parameter logistic functions fit the exposed fetuses' cardiac volume better than the unexposed, who had significant runs' test results ($P = 0.03$), suggesting that an alternative untested function might better describe their growth trajectory. Unexposed fetuses began the second trimester with larger volumes but had a lower upper asymptote ($P < 0.001$), indicating a smaller size late in gestation. Thus, the null hypothesis that an identical curve fit both samples' cardiac volume growth pattern was rejected ($P < 0.01$).

These analyses identified a smaller cardiac volume with smoke exposure in the second trimester, followed by a surge in cardiac volume growth, surpassing the unexposed fetuses early in the third trimester and resulting in a greater cardiac volume subsequently. The mixed-effects model confirmed the magnitude of smoke exposure with a nearly 3-fold greater slope (more rapid growth) after 210 days of age (~30 weeks) for cardiac volume. Cardiac morphology was also altered at this time: smoke-exposed fetal cardiac width growth was characterized by a 1.5-fold greater slope (both analyses controlled for fetal weight, Table 7). The significant morphological results can be visualized graphically as the smoke-exposed hearts became relatively wide for their length between the second and third trimester (Fig. 5b).

DISCUSSION

Maternal smoking effects

In this sample, the hypothesis that maternal smoking alters the in utero growth patterns of the key organs of the postnatal cardiovascular system, the heart and kidneys,

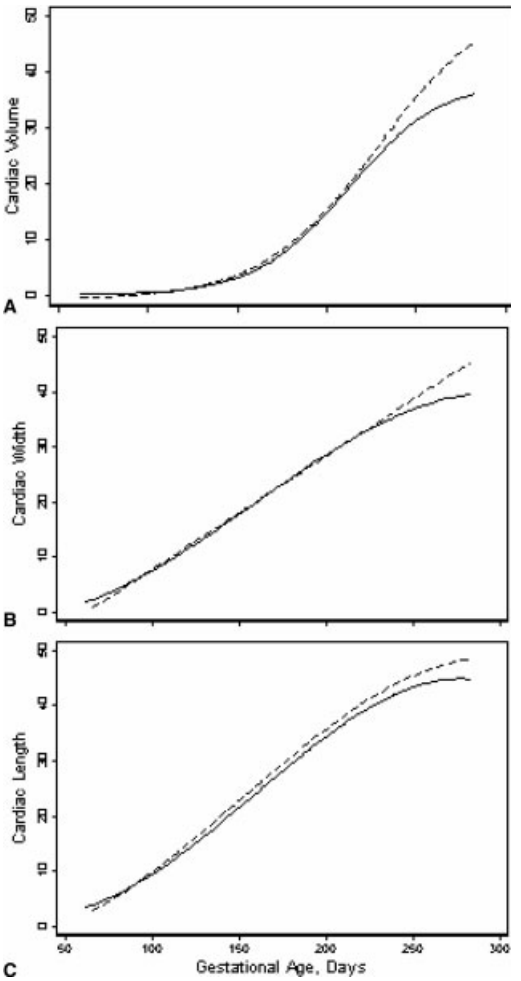


Fig. 4. Heart growth. Best-fit trends for (a) cardiac volume (cm^3), (b) width, and (c) length (mm). All are three-parameter logistic functions. The null hypothesis that both exposed and unexposed fetal cardiac growth are well described by one curve is rejected for cardiac volume and width but not for cardiac length. Smoke-exposed subjects are shown in broken lines.

was confirmed. Both specific timing patterns and differences in morphology were identified. This was a sample level analysis and may not apply to all individuals; the patterns of differences described here are hypothesis-generating and remain to be investigated with larger samples.

As a group, smoke-exposed fetuses were characterized by an early kidney growth spurt during the second trimester, followed by decreasing renal growth rates relative to unexposed fetuses with a transition from relatively thick to relatively thinner kidneys during the third trimester. By contrast, smoke exposure was associated with a smaller cardiac volume during the second trimester, followed by a growth surge resulting in smoke-exposed fetuses surpassing nonsmokers during the third trimester with a heart that was wide for its length, and for fetal weight. Sex-specific risks could not be identified with the available sample.

Normal versus compensatory growth

The smoke-exposed organ growth patterns found here deviated from normal in both developmental timing and morphology in line with previous reports of compensatory organ growth. Taken together, these observations suggest a "hand off" of compensatory strategies from the kidney to the heart during midgestation in the fetus exposed to maternal smoking, reflected in the relative size of the kidney to heart across gestation (Fig. 6).

For example, in this study, smoke exposure resulted in early renal expansion, followed by reduced growth and thin, relatively longer kidneys, both absolutely and normalized to biparietal diameter across the third trimester ($P = 0.01$). This type of pattern has been previously described as indicative of compensatory renal growth in both humans (Glazebrook et al., 1983; Mandell et al., 1993; Hill et al., 2000) and animal models, where it was associated with early renal growth rate acceleration and hyperplasia, but no concurrent nephrogenic development (Peters et al., 1993). The specific insults associated with maternal smoking that may have caused the fetal kidneys to appear anatomically similar to previous reports of compensatory renal growth remain to be elucidated. It is worth considering that increased kidney size may occur without expanded functional units (the nephrons) and form an anatomical basis for subsequent cardiovascular risk.

From the viewpoint of maternal smoking-related hypoxia, the proximal mechanisms underlying fetal growth responses to hypoxia are poorly detailed to date. Fetal hypoxia has been reported to influence insulin growth factors 1 and 2 (IGF-1, IGF-2) and IGF binding protein (IGFBP) levels with tissue and developmental specificity (Green et al., 2000; Verhaeghe et al., 2003). The pattern of kidney growth and decline described here is in line with early hypoxia-induced over-

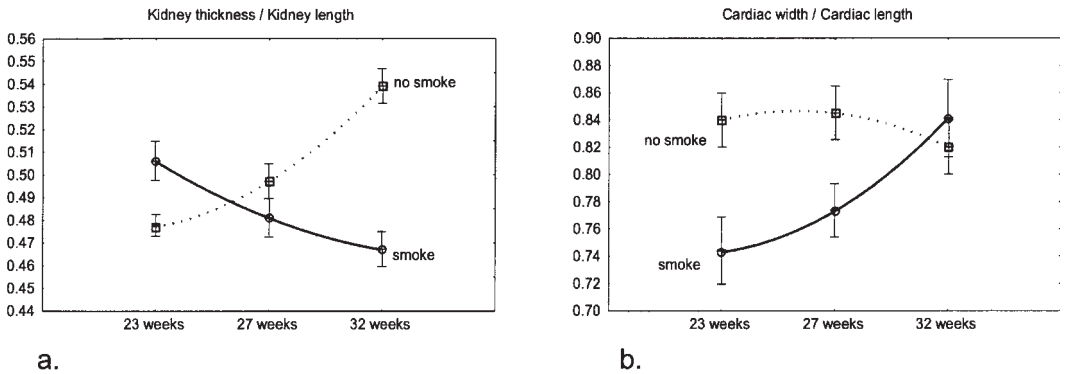


Fig. 5. Significant smoke exposure effects on the morphology of the kidney (a) and heart (b). (a) Ratio of kidney thickness to kidney length at 23, 27, and 32 gestational weeks of age (mean ± SEM). The smoke-exposed kidney has grown more rapidly prior to 23 weeks and is relatively thicker than the non-exposed. Subsequently, the non-exposed kidney grows three times more than the exposed. The growth in relative thickness of the smoke-exposed kidney declines significantly between 23 and 32 weeks of age, both absolutely ($P = 0.04$) and relative to fetal weight ($P = 0.01$). (b) Ratio of cardiac width to length at 23, 27, and 32 gestational weeks of age (mean ± SEM). Between 23 and 32 weeks, the non-smoke-exposed fetal heart does not change significantly in shape with increasing growth. By contrast, the smoke-exposed fetuses have a heart that is significantly narrower at 23 weeks, and subsequent growth includes a surge in longitudinal diameter by 27 weeks, accompanied by an overall greater growth rate in transverse diameter, both absolutely ($P = 0.01$) and relative to fetal weight ($P = 0.02$) over the entire interval.

TABLE 6. Best fit growth curve trend functions for measured heart dimensions and estimated volume

Heart	Best-fit curve	R^2	H_o of equality
Volume			
No smoke	$39/(1 + \exp(-0.04 \times (\text{age} - 212)))$	0.96	0.0001
Smoke	$52/(1 + \exp(-0.03 \times (\text{age} - 226)))$	0.97	
Width			
No smoke	$43/(1 + \exp(-0.02 \times (\text{age} - 167)))$	0.99	0.004
Smoke	$49/(1 + \exp(-0.02 \times (\text{age} - 183)))$	0.99	
Length			
No smoke	$49/(1 + \exp(-0.02 \times (\text{age} - 161)))$	0.99	nsd
Smoke	$51/(1 + \exp(-0.02 \times (\text{age} - 161)))$	0.99	

Note: Best fit determined by a comparison of R^2 values and nonsignificant runs' test of the residuals. Smoke-exposure growth functions were compared to unexposed under the null hypothesis that one curve was an equally good fit of both longitudinal growth data. Significance identifies failure of the null hypothesis by *F*-ratio and Akaike and Bayes information criteria.

TABLE 7. Smoke exposure effects on linear aspects of best fit growth models identifying significant time-specific effects on cardiac growth, controlling for gestational age and fetal weight (mixed-effects model, xtreg, STATA 8)

	Cardiac dimensions with smoke exposure				
	Age (days)	β	*P*	R^2	Model *P*
Volume	>210	2.8	0.03	0.61	0.0000
Width	>210	1.5	0.04	0.55	0.0000

expression of IGF-2-mediated hyperplastic growth (Rosendahl et al., 1992; Bae et al., 1999) followed by IGFBP-1 over-expression. IGFBP-1 has been documented to increase with decreasing fetal blood oxygen levels as well as maternal smoking specifically among a sample of 25- to 36-week-old preterm human fetuses (Verhaeghe et al., 2003). As IGF-1 has been shown to stimulate kidney growth (Tarantal et al., 1997; Marsh et al., 2001), increasing IGFBP-1 levels have the potential to down-regulate IGF-1 availability, leading to reduced growth (Doublier et al., 2001; Lee et al., 2001).

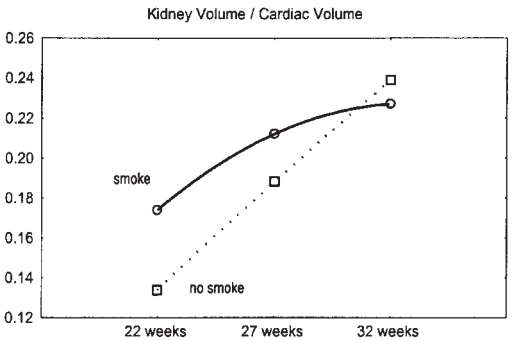


Fig. 6. Kidney volume/cardiac volume. The ratio of kidney volume to cardiac volume was 30% greater among smoke-exposed fetuses at 23 weeks, followed by a decline across gestation relative to unexposed fetuses. Controlling for gestational age at birth and day of ultrasound, the kidney volume to cardiac volume ratio at 32 weeks of age predicted 23% of the variance in ponderal index at birth ($P = 0.04$). Thus, a smaller kidney volume to cardiac volume ratio at 32 weeks of age predicted a lower ponderal index at birth.

The declining renal growth rates after ~30 weeks in the present study parallel the timing of reduced growth in biparietal diameter and tibia among this sample (Lampl et al., 2003), suggesting increasing hypoxia. As this is also the time of greatest nephrogenic development (Gasser et al., 1993), the third trimester may be a critical period of risk for the smoke-exposed fetal kidneys and may be the anatomical basis for long-term sequelae in blood pressure regulation as previously proposed (Brenner et al., 1988). A pattern of early renal overgrowth followed by a subsequent decline in nephrons has been associated with elevated postnatal blood pressure in animal models (Langley-Evans et al., 1996).

By mid third trimester, the smoke-exposed fetuses in the present study had kidneys that were long and thin. Relatively long, sausage-shaped kidneys have been previously reported among small-for-gestational age neonates, accompanied by high cord renin concentrations (Konje et al., 1997). Thin kidneys have been identified by ultrasound in children and adolescents born with low birth weight from a population in which systolic blood pressure has been inversely correlated with kidney volume (Spencer et al., 2001; Singh and Hoy, 2004). Thus, the developmental and morphological patterns found here among the smoke-exposed fetuses parallel previous studies that have correlated renal anatomy with signs of compensatory growth, hypoxia, and adult hypertension.

In the smoke-exposed fetuses, heart size in mid second trimester was reduced. The subsequent acceleration in cardiac volume and shape, preferencing width for length, has also been previously reported in small for gestation age infants and fetuses undergoing compensatory cardiac growth patterns (Veille et al., 1993; Tsyvian et al., 2002).

Similar to effects at high altitude, the natural analogue to fetal hypoxia, the early lag in cardiac volume may have been due to underperfusion of the cardiac circulation (Thompson, 2003). Animal research has identified that the fetal heart remodels its coronary vasculature as an adaptive response to hypoxemia (Thornburg and Reller, 1999), under the constraints of developmental timing (Dalshaug et al., 2002), and hypertrophy is an important stimulus to angiogenesis normally (Lewis et al., 1999). This may be emphasized with unremitting hypoxemia.

The challenge of fetal growth with smoke exposure

Contrary to common notions that compromised environments put an overall constraint on growth with the goal of tuning down energy conflicts, the smoke-exposed fetuses in this sample were growing in body size at pace with the unexposed by mid second trimester (Lampl et al., 2003). During the second trimester, overall body growth of the smoke-exposed fetuses was not reduced in this sample: no significant lags in the size of the head or limbs were evident. Only by mid third trimester did smoke exposure predict a decreasing slope in tibial length and altered biparietal diameter (Lampl et al., 2003). A reasonable hypothesis is that, in order to maintain these growth rates, fetal organs underwent facultative adjustments to support the body's metabolic needs, employing normal growth-signaling mechanisms to distribute resources.

For example, it has been reported previously that cardiac output increases to match body growth by increasing ventricular dimensions (Veille et al., 1990). Whether this is a tandem or a sequential process, and, if the latter, which comes first—heart expansion or body growth—is presently undocumented. One hypothesis is that imminent bone, organ, and tissue expansion signal local energy needs and cardiac output negotiates delivery. This

proposed pathway preferences hypoxia signals as important to the growth cascade (Lampl, 2005). The details of such a process remain to be elucidated, but the present data allude to such a putative biology. In this sample, individuals' incremental growth in cardiac volume across midgestation (between 23 and 32 weeks of age, a period of rapid organ growth), significantly predicted the slope of incremental growth in tibial length during this interval ($\beta = 0.44$, $P = 0.04$, $R^2 = 0.31$, model $P = 0.02$). This may have been a genetically based developmental pattern as both maternal and paternal height significantly predicted incremental growth of fetal cardiac volume and tibial length over the 23- to 32-week interval in the present sample (Table 8).

As it was the smoke-exposed fetuses who had the relatively tall parents (Table 2), one hypothesis is that their significantly increased cardiac volume growth may merely have reflected genetics. However, as smoke exposure was associated with third trimester decline in tibial length growth, it appears that the organ-level responses were ultimately unable to keep pace with lower limb growth potential. These observations support previous suggestions that it is the fetus with a genetic drive to grow more, restricted in utero, who is at greatest subsequent risk (Harding et al., 1992; Leon et al., 1996): the energetically challenged heart, in its effort to grow a larger body, may itself be structurally altered. Further research is needed to clarify this.

What mechanisms drive facultative organ growth? A hypothesis regarding kidney and heart collaboration

Any explanatory framework for the present observations must address the evidence of a sequence of rapid early kidney growth followed by cardiac growth among the smoke-exposed fetuses in the context of early overall growth maintenance and subsequent selective decline. Our working hypothesis is that the anatomical observations in this study reflect hypoxia-induced adaptive responses at the tissue, cell, and molecular levels resulting from the hypoxia-inducible factor (HIF) cascade's (Semenza, 2000) normal signaling systems that functioned to augment growth due (at the least) to maternal smoke-induced hypoxemia and nicotinic vasoconstriction.

The present observations suggest a pattern of collaborative organ growth involving the kidney and heart in driving and supporting fetal growth. We hypothesize that the kidneys were triggered to take the lead in an early response sequence. With maternal smoking, carboxyhemoglobin levels rise (Seidman et al., 1999), inducing relative fetal hypoxia and engaging the HIF cascade to up-regulate oxygen carrying capacity via erythropoiesis (Stroka et al., 2001), focused in the liver and kidney in the first two fetal trimesters (Zanjani, 1980), and a sequence of events that upregulate mechanisms for increasing oxygen-carrying capacity, blood volume, and delivery. Multiple HIF downstream effects, including early upregulation of vascular endothelial growth factor (VEGF) and IGF-1 (Flyvbjerg et al., 2002), are mechanistic candidates contributing to the enlarged kidney dimensions by mid-to-late second trimester. Increased erythrocytosis can contribute to renal volume expansion and increase cardiac preload, or the volume of blood that reaches the right atrium, which in turn promotes cardiac growth via cell stretch-mediated growth factor effects (Isgaard et al., 1994; Seko et al., 1999; Zheng et al., 2001). In this way, the kidneys are engaged in a cascade of responses and play a central role in increasing overall blood volume (Donnelly, 2001; Antunes-Rodrigues et al., 2004).

In a collaborative effort, the kidneys and heart effect augmented blood volume and distribution to keep pace with a growing fetus. In this process, the normally stiff fetal ventricles (Grant, 1999) must work to increase cardiac output against a back pressure of nicotinic-

TABLE 8. Regression results for model predicting cardiac volume and tibial length growth velocity between 23 and 32 gestational weeks of age from parental height

	Cardiac volume growth	Tibial length growth
Maternal height	$\beta = 2.3$, $P = 0.02$, $R^2 = 0.37$, model $P = 0.001$	$\beta = 0.06$, $P = 0.04$, $R^2 = 0.16$, model $P = 0.08$
Paternal height	$\beta = 2.0$, $P = 0.04$, $R^2 = 0.34$, model $P = 0.003$	$\beta = 0.07$, $P = 0.02$, $R^2 = 0.19$, model $P = 0.04$

[a]Individual growth increments were converted to mm/day to accommodate lack of identical time intervals between measurements.

induced vasoconstriction from the fetal side of the placenta with maternal smoking (Morrow et al., 1988). Thus, it is likely that the smoke-exposed fetal heart faces both chronic volume and pressure stresses that result in alterations not only in terms of overall morphology but also at the cellular level (Sidi and Rosa, 2004). Experimental studies have identified increased levels of both erythropoietin (EPO, prompting red blood cell differentiation) and vascular epidermal growth factor (VEGF, prompting increasing blood vessels and thereby increasing blood flow) associated with fetal renal and cardiac tissue undergoing hypoxia-driven hypertrophy and angiogenesis (Marti and Risau, 1998; Martin et al., 1998). Animal models of compensatory cardiac growth, including carbon monoxide exposure-associated cardiomegaly (Clubb et al., 1986), involve both cellular hyperplasia and hypertrophy (Calderone et al., 1995; Murotsuki et al., 1997; Barbera et al., 2000). Altered cellular growth patterns may underlie the risk for postnatal sequelae including arryhthmias (Sartiani et al., 2004).

Implications of facultative fetal growth negotiated by organ growth patterns

While the present observations derive from a modest sample, the results describe an anatomical pattern that parallels a number of reports from animal models (Lampl, 2005). The specific sequence of organomegaly found in the present sample of smoke-exposed fetuses has been previously reported among hypoxemic mice, where renal hyperplasia preceded cardiomegaly: compared to HIF-deficient mice, normal animals exposed to chronic hypoxia up-regulated EPO and VEGF in kidney and heart within hours. Elevated red blood cells appeared within days, and cardiac hypertrophy followed in weeks (Yu et al., 1999).

Observations in the spontaneously hypertensive rat (SHR) have identified the potential for postnatal sequelae from anatomical alterations such as those observed here. The increased heart weight-to-body weight ratios found in the SHR during gestation and cardiomegaly at birth continue, resulting in a hypertrophic adult heart and hypertension (Lewis et al., 1997). Investigations aimed at specifically delineating in utero nicotinic effects have also identified the importance of genetic background in physiological responsivity (Pausova et al., 2003). In the SHR, nicotine exposure was associated with a selective decrease in kidney weight to body weight ratio at birth, while not reducing the relatively large heart/body weight characteristic of the strain. This was accompanied by increased systolic blood pressure and serum cholesterol in contrast to control rats, who experienced no organ effects.

There is some question as to how effectively the HIF cascade may rescue the hypoxemia of fetal smoke exposure, as a recent animal study identified impaired HIF-1 alpha stabilization with cigarette smoke exposure in a postnatal mouse model of muscle ischemia (Michaud et al., 2003). However, it was also found that additional HIF reversed these effects. It is likely that in the developing fetus, redundant pathways contribute to HIF stability and signaling activation of the downstream effects of the HIF cascade (Lee et al., 2004). Growth factors themselves upregulate HIF-1 alpha in an autocrine, cell-specific manner (Feldser et al., 1999) with developmental timing patterns (Adachi et al., 2004).

Similar observations are not yet available from humans and more research is needed to characterize the underlying mechanisms of the anatomical observations found here; it is likely that the numerous chemical constituents of smoke exposure are contributory to alterations that are not measurable by anatomical description alone. To date, elevated levels of erythropoietin (EPO) have been identified in cord blood of in utero smoke-exposed fetuses (Varvarigou et al., 1994; Jazayeri et al., 1998).

Debate currently ensues regarding causality in the observations put forward under the notion of fetal programming (Marchand and Langley-Evans, 2001; Huxley et al., 2003). Collectively, data at present suggest that what has been called fetal programming with a view towards explaining negative adult health sequelae, is clinically outcome oriented when, in fact, adjusting size to metabolic signals is what fetuses do. The process of normal fetal growth is a facultative process by which growth occurs as cell level signals integrate mitotic activity and differentiation within available resources according to a genetically based and developmentally timed schema (Louey et al., 2003). Organ growth patterns reflect these negotiations.

While a number of epidemiological studies have reported that smoking is associated with a dose/response reduction in birth weight (Dougherty and Jones, 1982; Haug et al., 2000), smaller samples have not consistently identified these effects (Vio et al., 1995;

Bernstein et al., 2000). These differences may reflect not only the constellation of confounders associated with smoking in some populations (e.g., diet, SES, exercise) but also the importance of maternal size effects on birth outcome (Witter and Luke, 1991; Hediger et al., 1994; Goldenberg et al., 1997), a factor more likely to bias small samples.

Recent reports have focused attention on altered body proportionality as a significant outcome of maternal smoking during pregnancy. Among the infants from the Swedish Birth Register, a dose–response effect was found between smoke exposure and increased ponderal index, identifying a differential effect of smoke exposure on weight versus length growth (Zelzer et al., 1998; Lindley et al., 2000). Thus, some organ growth patterns may provide a mechanism for the fetus to preserve its birthweight, with selective tissue expansion and reduction (Bernstein et al., 2000). It is not surprising that birthweight itself is not an entirely robust predictor of smoke (or other prenatal) exposure effects. Genetically based differences likely influence the specific organ-level responses through metabolic responsivity, or cross-talk between organ cytokine cascades (Carmeliet et al., 1998; Wang et al., 2002), resulting in variable weight outcomes for similar environmental circumstances. Likewise, equal birth weights are not equal at the organ level: developmental timing effects are critical as shown in IUGR sheep where nephron endowment due to twinning was significantly reduced compared to late gestation-induced IUGR (Mitchell et al., 2004). Taken together, these observations suggest organ-level responses by which a variety of genetically based hypoxia-responsive mechanisms may be selected for, as they contribute to infant survival through birth weight preservation (Beall et al., 2004).

Whether the present observations are unique to this sample or more generally characterize organ-level smoke exposure effects remains to be tested on larger samples. This is a hypothesis-generating report. The evidence of altered cardiovascular organ growth documented here adds to previous reports of abnormal fetal coronary artery development (Lehtovirta et al., 1984) to establish an anatomical foundation for adult disease risk after prenatal smoke exposure. The present study provides the first evidence for direct effects of maternal smoking during pregnancy on the longitudinal growth patterns of organs of the human fetal cardiovascular system. These results suggest that the kidneys and heart are functional collaborators in the growth of the fetal body, with a relationship that is altered by smoke exposure.

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