

Aortic Wall Thickness and Amniotic Fluid Albuminuria in Growth-Restricted Twin Fetuses

Vincenzo Zanardo, Silvia Visentin, Martina Bertin, Martina Zaninotto, Daniele Trevisanuto, Francesco Cavallin, and Erich Cosmi

Department of Pediatrics, Department of Gynecological Science and Human Reproduction, Section of Maternal Fetal Medicine, and Department of Laboratory Medicine, University of Padua School of Medicine, Padua, Italy

Background and objective: Intrauterine growth restriction (IUGR) may be associated with significantly higher aortic intima–media thickening (aIMT) values. It is unknown if fetal aIMT is associated with glomerulosclerosis and amniotic albuminuria in utero. **Design, setting, participants, and measurements:** Fetal abdominal aIMT and amniotic albumin/creatinine ratio (ACR) were measured in 126 individual twin fetuses, recruited by the Obstetrics and Gynaecology Clinics of the University of Padua (Italy) Medical Center. The IUGR twin fetuses were classified into two groups: Group A were those fetuses whose estimated fetal weight (EFW) was <10th percentile with pulsatility index >2 SD and Group B were those fetuses whose EFW was <10th percentile and had no velocimetry abnormalities. **Results:** The median fetal aIMT was significantly different in the three groups (Group A = 0.9 mm; Group B = 0.7 mm; and appropriate for gestational age (AGA) = 0.5 mm; $p < .0001$). It was significantly higher in Group A than in the AGA group ($p < .0001$) and than in the Group B fetuses ($p = .003$), respectively. In addition, ACR was different in the three groups (Group A = 183,500 mg/g; Group B = 6,4720 mg/g; and AGA = 8,2750 mg/g; $p = .0002$). It was significantly higher in Group A than in the AGA group ($p = .03$) and than in Group B ($p = .02$), respectively. **Conclusions:** Growth-restricted twin fetuses with velocimetry abnormalities present are associated with aIMT and higher ACR levels in amniotic fluid, which could be possible markers in utero of preclinical atherosclerosis, and early glomerulosclerosis.

■ **Keywords:** IUGR, twinning, aortic intima–media thickness, amniotic fluid albuminuria

Intrauterine growth restriction (IUGR; ACOG, 2001) has recently been shown to be associated with higher rates of renal and cardiovascular diseases in adult life (Barker, 2006; Brenner & Chertow, 1993; Johansson et al., 2005). According to Barker's fetal origin hypothesis, these diseases, resulting in permanent changes in body structure and function, may be due to metabolic or endocrine adaptations that occur when a fetus is undernourished (Byrne & Phillips, 2000; Gluckman & Hanson, 2004).

Early endothelial dysfunction and aortic intima–media thickening (aIMT) occurring in utero may play an important role in premature in-utero stiffening of the aortic vessels (Skilton et al., 2005) and predispose these fetuses to hypertension, nephropathies, and the metabolic syndrome (Franco et al., 2006). Moreover, the developing kidneys appear to be extremely susceptible to IUGR (Manalich et al., 2000), and several studies in animals and humans have reported a reduced number of nephrons after IUGR (Hughson et al., 2006). A reduced number of nephrons result in a decreased glomerular filtration surface area, while renal

blood flow per glomerulus is increased in the attempt to maintain a normal overall glomerular filtration rate. According to Brenner's hyperfiltration hypothesis (Brenner et al., 1998), this could lead to glomerular hypertension and hypertrophy, which could ultimately result in glomerulosclerosis and albuminuria (Crews et al., 2011; Hostetter et al., 2003; Luyckx & Brenner, 2005).

It is unclear if glomerulosclerosis and albuminuria develop together with endothelial dysfunction and aIMT during intrauterine life, and as a result, a study on twin fetuses, considered a common, natural model of pregnancy nutrient restriction, was designed. Twins are thought to represent a

RECEIVED 13 April 2012; ACCEPTED 8 October 2012. First published online 25 March 2013.

ADDRESS FOR CORRESPONDENCE: Vincenzo Zanardo, Department of Pediatrics, Padua University, Via Giustiniani 3, 35128 Padua, Italy. E-mail: zanardo@pediatria.unipd.it

special category of stunted fetal growth (one of the twins is commonly below the 10th percentile; Blickstein, 2005; Muhlhausler et al., 2011), and umbilical artery Doppler exams are generally carried out for early detection of umbilical artery vasculopathy (Figueras & Giardosi, 2011). Twins, therefore, would be expected to have a significant risk for diseases associated with lower birth weight (Fox et al., 2011) and this is an important consideration in an epoch in which the use of assisted reproductive technology, together with multiple gestations, has increased dramatically (Blondel & Kaminski, 2002). Compared with singletons, the growth rate of twin fetuses is characterized by a higher incidence of IUGR, varying from 15% to 25% (Secher et al., 1985). The important role of non-genetic factors in the etiology of cardiovascular and renal diseases in adult twins supports the notion of pre- and early post-natal programming as key contributors to the development of various components of the metabolic syndrome in adult life (Neitzke et al., 2011; Phillips et al., 2001; Poulsen et al., 2009; Vågerö & Leon, 1994). We hypothesized that evaluating the intrauterine development of twins could provide a unique opportunity to mimic a scientific experiment to study fetal growth restriction, as well as in utero endothelial vascular damage, predictors of preclinical atherosclerosis and of early glomerulosclerosis risk.

The aim of this was then to assess: (1) abdominal aIMT in intrauterine growth-restricted twin fetuses with and without Doppler umbilical artery vasculopathy and in appropriate-for-gestational-age (AGA) twin fetuses, and (2) the relationship between fetal aortic thickening (a marker of preclinical atherosclerosis) and amniotic fluid albumin concentration (a marker of early glomerulosclerosis).

Materials and Methods

Subjects were recruited by the Obstetrics and Gynaecology Clinics of the University of Padua (Italy) Medical Center between January 2009 and December 2010 at the time patients underwent a first trimester ultrasonography examination to assess chorionicity or during the third trimester if a patient was referred from other centers. The study design was approved by the University Hospital Committee for Research on Human Subjects and written informed consent was obtained from all the patients who participated. Some of the subjects had also been recruited as part of a separate study (Cosmi et al., 2009). Data concerning the patients and their pregnancies were recorded according to routine practice of the Department of Obstetrics and Gynaecology. Inclusion criteria included multiple pregnancy, a gestational age determined on the basis of the patient's last known menstrual period and/or ultrasound dating before the 20th week, and that the patient lived in the Veneto region (Italy). We included dichorionic and monochorionic diamniotic twin pregnancies, chorionicity being diagnosed at 10–14 weeks of gestation in the presence or absence of T or λ sign.

Exclusion criteria were triplets, monochorionic monoamniotic twin pregnancies, major congenital anomalies, pregnancies complicated by maternal history of cardiovascular disease, or endocrine disorders such as diabetes, hypercholesterolemia, pre-eclampsia, thyroid or adrenal dysfunctions, clinical chorioamnionitis, PPRM, anhydramnios, and twin-to-twin transfusion syndrome. Patients who drank alcohol, smoked, or were taking medications such as ritodrine or corticosteroids (except for fetal lung maturation) were excluded.

To define birth weight percentiles for gestational age, we used standard tables for singleton pregnancies (Hadlock, 1990). Estimated fetal weight (EFW) and Doppler umbilical artery pulsatility index (PI) were calculated according to indications or guidelines recommending the use of Doppler ultrasonography in multiple pregnancies (Baschat 2004). The IUGR twin fetuses were thus classified in two groups: Group A if the EFW was <10th percentile and the PI umbilical artery >2 standard deviation (SD) above the mean and Group B if the EFW was <10th percentile in the absence of velocimetry test abnormalities. In the AGA twins, the EFW was between the 10th and 90th percentiles. As previously reported (Zanardo et al., 2011), fetal aIMT was measured in each IUGR and AGA twin subject at a median gestational age of 32 weeks (Interquartile Range [IQR] 30–34 weeks) by high-resolution ultrasound scan using an ultrasound machine (Antares, Siemens Medical Solutions, Mountain View, CA) equipped with a 3.5–5 MHz linear array transducer. Intima–media thickness was measured in a coronal or sagittal view of the fetus at the dorsal arterial wall of the most distal 15 mm of the abdominal aorta sampled below the renal arteries and above the iliac arteries, as previously described (Cosmi et al., 2009); gain settings were used to optimize image quality. Abdominal aIMT was defined as the distance between the leading edge of the blood–intima interface to the leading edge of the media–adventitia interface on the far wall of the vessel (Koklu et al., 2007). Three measurements were taken and the arithmetic mean aIMT was utilized in the calculations for this study. All images were taken at the end diastole in order to minimize variability during the cardiac cycle.

All the patients were examined once a month if the pregnancy was diamniotic dichorionic and every two weeks if the pregnancy was monochorionic diamniotic. Fetal growth, ultrasound and Doppler studies, and biophysical profile scores were utilized to evaluate fetal well-being. The referring obstetrician decided the delivery time on the basis of fetal growth and Doppler patterns, gestational age, Bishop score, and parity. All pregnancies were delivered via caesarean section following antenatal corticosteroids administration, if performed before the 34th week of gestation.

A sample of amniotic fluid for albumin, creatinine, and albumin/creatinine ratio (ACR) determinations was obtained from both sacs by amniocentesis after

TABLE 1
Clinical Measurements Among Twin Gestations

	IUGR		AGA	<i>p</i> value
	Group A	Group B	Group C	
Twins (<i>n</i>)	20	26	80	—
Mothers				
Maternal age (years)*	33 (31–34)	34 (32–37)	33 (32–37)	.17
Parity (%)	12 (62.5)	17 (66.7)	55 (69.2)	.93
Monochorionicity (%)	8 (40)	8 (30)	21 (26)	.40
Prenatal steroids (%)	16 (43)	8 (22)	13 (35)	.71

Note: IUGR = intrauterine growth restriction; AGA = appropriate for gestational age.

Data expressed as *n*, number (%) or *median (IQR, interquartile range).

hysterotomy. Amniotic fluid albumin was assayed using an immunonephelometric method (BNTMII, Siemens Medical Solutions). Amniotic fluid creatinine was measured using a colorimetric Jaffé method (Roche Diagnostics, Monza, Italy).

Statistical Analysis

Continuous data were expressed as median and interquartile range (IQR), whereas categorical data were expressed as a number (%). Continuous and categorical data from the three groups were compared using the Kruskal–Wallis test and Fischer's exact test. Comparisons between individual twin groups and twin-pair groups were also made for the aIMT, amniotic fluid albumin, and the ACR variables using the Mann–Whitney test and Bonferroni correction for multiple comparisons. A *p* value less than .05 was considered significant. Statistical analysis was performed using the R 2.12 software.

Results

Sixty-three twin pregnancies met our inclusion criteria for the study and data on 126 individual twins (*n* = 20 IUGR, Group A; *n* = 26 IUGR, Group B; and *n* = 80 AGA, Group C) were included in our final statistical analysis. The group-specific sample size twin-pairs include 28 AGA–AGA; 11 AGA–IUGR, Group A; 13 AGA–IUGR, Group B; 2 IUGR, Group A–IUGR, Group A; 5 IUGR, Group A–IUGR, Group B; 4 IUGR, Group B–IUGR, Group B, respectively. IUGR Doppler patterns were associated with five cases of gravidic hypertension, two of placental abnormalities, and one of pre-eclampsia. Anthropometric, sonographic, and clinical data regarding the IUGR and AGA groups are outlined in Tables 1 and 2.

All the patients were comparable with regard to median age, parity, chorionicity, and prenatal (single course) of steroid administration, and all underwent cesarean section. Nevertheless, absent umbilical artery, end-diastolic flow velocity and deterioration of the ductus venosus waveform were noted in six and eight cases respectively, making cesarian delivery necessary.

The median birth weight of the 20 Group A fetuses was 1,665 g (<5th percentile) and the median gestational age was 35 weeks. The median birth weight of the 26 Group B fetuses was 2,172 g (<5th percentile) and the median gestational age was 36 weeks. The 80 AGA fetuses had a median gestational age of 35 weeks and a median birth weight of 2,295 g (50th percentile). No gender disparity was found when IUGR and AGA fetuses were compared.

The median fetal aIMT was different in the three groups (Group A = 0.9 mm; Group B = 0.7 mm; and AGA = 0.5 mm; *p* < .0001; Table 2).

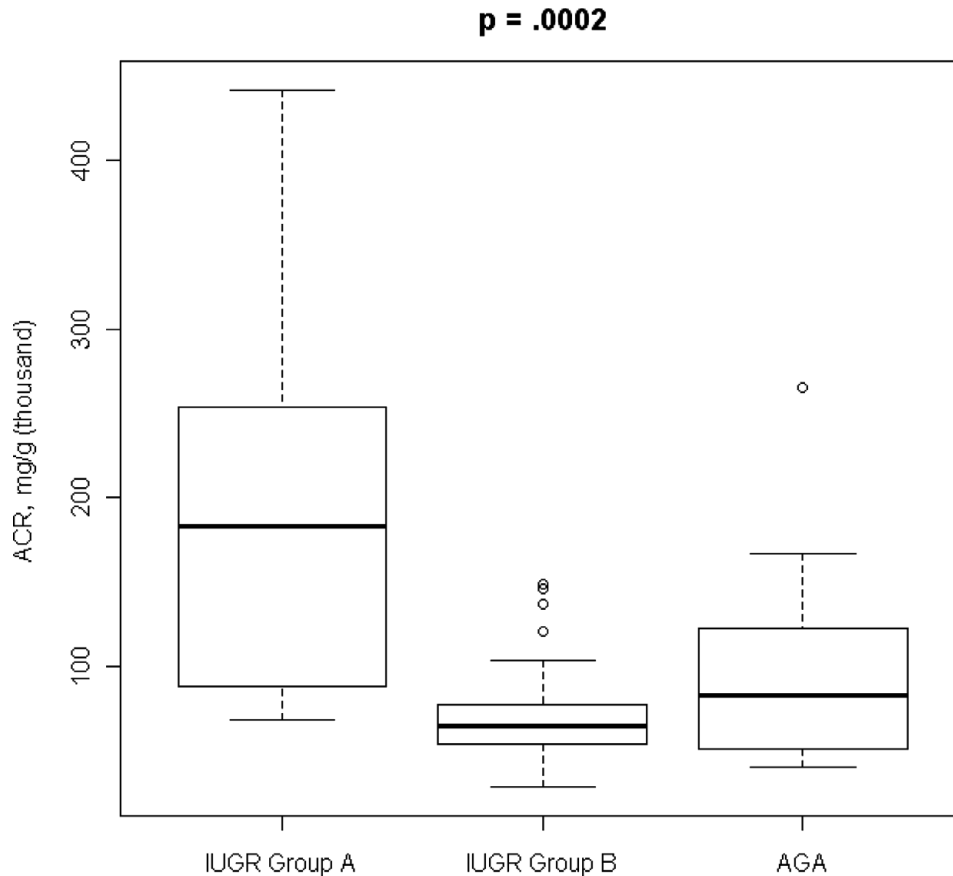
The aIMT was higher in Group A than in the AGA group (*p* < .0001, adjusted for multiple comparison), it was higher in Group B than in the AGA group (*p* = .003, adjusted for

TABLE 2
Anthropometric, Sonographic, and Clinical Measurements Among Twin Fetuses

	IUGR		AGA	<i>p</i> value
	Group A	Group B	Group C	
Twins (<i>n</i>)	20	26	80	—
Ultrasonography				
* aIMT (mm)	0.9 (0.8–1.2)	0.7 (0.6–0.8)	0.5 (0.5–0.6)	<.0001
Neonates				
Female: male	11:9	17:9	39:41	.32
Gestational age (w)*	35 (32–36)	36 (34–36)	35 (31–36)	.21
* Birth weight (g)	1,665 (1,181–1,846)	2,172 (1,680–2,333)	2,295 (1,810–2,550)	<.0001
Amniotic fluid				
* Creatinine (mg/L)	0.10 (0.08–0.12)	0.13 (0.12–0.16)	0.14 (0.06–0.16)	.02
* Albumin (g/L)	1.42 (1.19–2.33)	1.07 (0.82–1.40)	1.24 (1.04–1.56)	.01
* ACR (mg/g)	183,500 (89,370–246,600)	64,720 (53,990–76,220)	82,750 (51,070–119,200)	.0002

Note: IUGR = intrauterine growth restriction; AGA = appropriate for gestational age; aIMT = aortic intima–media thickness; ACR = albumin/creatinine ratio.

Data expressed as *n*, number (%) or *median (IQR, interquartile range).

**FIGURE 1**

Albumin/creatinine ratio (ACR, mg/g) among twin fetuses. Note that median albumin/creatinine ratio (ACR, mg/g) was significantly higher in the IUGR, Group A individual twins, 183,500 (89,370–246,600), compared with IUGR, Group B, 64,720 (53,990–76,220), and AGA, Group C individual twin fetuses, 183,500 (89,370–246,600); $p = .0002$ by the Kruskal–Wallis non-parametric test.

multiple comparison), and it was higher in Group A than in the Group B fetuses ($p = .003$, adjusted for multiple comparison).

The median amniotic fluid albumin concentration was different in the three groups (Group A = 1.42 g/L; Group B = 1.07 g/L; and AGA = 1.24 g/L; $p = .01$; Table 2).

Amniotic fluid albumin was significantly higher in Group A than in Group B ($p = .02$, adjusted for multiple comparison), while it was similar in Group A and the AGA group ($p = .11$, adjusted for multiple comparison), and similar in Group B and the AGA group ($p = .25$, adjusted for multiple comparison).

ACR was different in the three groups (Group A = 183,500 mg/g; Group B = 64,720 mg/g; and AGA = 82,750 mg/g; $p = .0002$; Table 2 and Figure 1).

ACR was higher in Group A than in the AGA group ($p = .03$, adjusted for multiple comparison), it was similar in Group B and AGA group ($p = .39$, adjusted for multiple comparison), and it was higher in Group A than in Group B ($p = .02$, adjusted for multiple comparison).

The comparison between twin-pair groups showed a significant difference between the AGA group and Group A and between the AGA group and Group B with regard

to aIMT (0.3 [0.1–0.6] mm, $p = .001$ and 0.2 [0.1–0.3] mm, $p = .0006$, respectively) and to ACR (2,777 [1,098–59,364], $p = .004$ and 3,457 [2,939–4,324], $p = .23$, respectively). No significant differences in the aIMT and the ACR were found; instead they were found between the AGA group and Group A and the AGA group and Group B pairs ($p = .11$ and $.76$, respectively) or in the other twin-pair groups.

Discussion

The natural model of IUGR represented by the twins pregnancies and adopted in this study, using EFW < 10th percentile and umbilical artery PI > 2 SD as diagnostic criteria, shows, for the first time, that IUGR individual twin and twin-pair fetuses with umbilical artery vasculopathy have a greater risk of preclinical atherosclerosis and in utero glomerulosclerosis (Koklu et al., 2007; Levine et al., 1994; Loos et al., 2001).

Early endothelial dysfunction and intima–media thickening could contribute significantly to premature stiffening of the arterial tree, which might ultimately predispose these individuals to systemic hypertension and increased renal

and cardiovascular risk (Järvisalo et al., 2001; Litwin & Niemirska, 2009; Zhang et al., 2001). It is now possible, thanks to external ultrasonography, to measure in vivo aortic wall thickness during fetal and early postnatal life in an accurate and reproducible way (Baschat, 2004). Ultrasound measurement of aIMT in IUGR fetuses has been found to be a sensitive marker of hypertension and atherosclerosis risk in adult life, supporting the epidemiological link between impaired fetal growth and cardiovascular disease later in life (Koklu et al., 2007). After Barker (2004) proposed his hypothesis concerning fetal programming of adult disease, Skilton et al. (2005) reported that newborns with growth restriction have significant aortic thickening. Crispi et al. (2010) also reported that IUGR resulted in remodeled and less efficient hearts in children, higher blood pressure and increased intima-media thickness, demonstrating a linear increase in relation to the severity of growth restriction. Unlike those studies focusing on older age groups, we measured abdominal aIMT during the second–third trimesters of intrauterine life and found that IUGR fetuses had significantly higher abdominal aIMT values compared with age-matched controls when assessed in utero and at 18 months of age. It would seem then that increased arterial thickness can be present even in intrauterine life and this could play a role in programming adult disease (Koklu et al., 2007; Skilton, 2008).

Developing kidneys appear to be extremely susceptible to IUGR and are often found to be small in proportion to body weight in these neonates (Luyckx & Brenner, 2005; Manalich et al., 2000). According to the hyperfiltration hypothesis proposed by Brenner et al. (1998), this could lead to glomerular hypertension and hypertrophy, which cause systemic hypertension and higher sodium reabsorption and glomerular damage resulting in albuminuria and glomeruloclerosis (Brenner et al., 1998; Hostetter et al., 2003; Luyckx & Brenner, 2005). Microalbuminuria is often observed during the early stages of kidney disease due to altered glomeruli permeability of the kidney to protein and predicts risk of cardiovascular and non-cardiovascular mortality (Crews et al., 2011; Keijzer-Veen et al., 2005). The presence of higher amniotic microalbuminuria in IUGR twin fetuses with umbilical artery vasculopathy may have implications in pediatric and adult life. Over the past decade, clinical data have become available regarding renal function impairment in IUGR infants. Singh and Hoy (2004) described an association between low birth weight, kidney size, and albuminuria in Aboriginal Australians between 4 and 72 years of age. Recently, the Keijzer-Veen et al. (2005) prospective follow-up study reported an association between the severity of IUGR and renal function in young adults who were born very prematurely. On average, their low birth weight was associated with lower glomerular filtration rates, higher serum creatinine concentrations, and higher microalbumin secretion at the age of 19. A meta-analysis published by Teeninga et al. (2008) showed that

low birth weight has an important influence on glomerular filtration rate and proteinuria and is associated with a higher risk of developing several complications, including hypertension. More recently, we described the ultrasound-based measurement of abdominal aIMT in utero in IUGR singletons, categorized by EFW < 10th percentile and PI > 2 SD, presenting with persistent aortic wall thickening and higher albuminuria at the 18-month follow-up (Zanardo et al., 2011).

Although the mechanisms that underlie these associations remain unclear, these data represent another step forward in understanding IUGR. Further studies should focus on additional factors that impact on early endothelial dysfunction, in addition to placenta-induced developmental disorders and Doppler umbilical artery vasculopathy. In particular, there is a growing interest in podocytes and endothelial cells, which are joined by a common embryological mesenchymal origin. Albuminuria is one of the first signs of renal disease, anticipating a decrease in glomerular filtration rate. If fetal kidneys are damaged, absorption of albumin is altered and some albumin will appear in the amniotic fluid. The main source of amniotic fluid production is the fetal kidney, together with the fetal lung, while the intramembranous circulation is a secondary source. Baschat (2004) states that amniotic fluid volume is determined by the concentration of oxygen and the renal vessels perfusion, which affect fetal urine production. A progressive deterioration of acid base and vascular status is accompanied by a progressive decline in amniotic fluid volume (Baschat, 2004). Nevertheless, despite these observations and the results of our data, it is still unclear if albumin alone or ACR is more reliable in detecting amniotic albuminuria in selected and/or non-selected AGA or IUGR twins. A spot urine sample seems to be a reliable screening method to detect albuminuria in infancy and adulthood (Crews et al., 2011).

There are some limitations inherent in this study. First, the progression of IUGR is determined by when and how it starts and by the degree of umbilical artery abnormality at onset, all data for the moment impossible to determine and thus limiting the possibility of making comparisons. Second, cross-sectional observations are unable to specify how to monitor individual singleton or twin fetuses not yet compromised. Third, we are unable to establish if renal function will worsen more rapidly with age compared with aortic wall thickening. It is also unknown whether screening for amniotic albuminuria and aIMT could help prevent renal function failure, atherosclerosis, and metabolic syndrome at an early stage.

In conclusion, the present study shows, for the first time, that in utero aortic wall thickness and amniotic albumin are significantly higher in IUGR twin fetuses with Doppler velocimetry anomalies than in IUGR fetuses without anomalies or AGA twin fetuses. Although the exact mechanisms underlying these associations remain unclear, these data,

indicating early glomerular damage, extend the findings of our previous study on the natural course of aIMT and glomerulosclerosis in IUGR fetuses and those in IUGR children, adolescents, and young adults at risk for premature cardiovascular and kidney diseases. Follow-up studies are of course warranted to confirm the prognostic role of these markers of atherosclerosis and renal disease development.

References

- American College of Obstetricians and Gynecologists (ACOG). (2001). Intrauterine growth restriction. Clinical management guidelines for obstetrician-gynecologists. American College of Obstetricians and Gynecologists. *International Journal of Gynecology & Obstetrics*, 72, 85–96.
- Barker, D. J. (2004). The developmental origins of adult disease. *Journal of the American College of Nutrition*, 23, 588S–595S.
- Barker, D. J. (2006). Adult consequences of fetal growth restriction. *Clinical Obstetrics and Gynecology*, 49, 270–283.
- Baschat, A. A. (2004). Fetal response to placental insufficiency: An update. *BJOG: An International Journal of Obstetrics and Gynaecology*, 111, 1031–1041.
- Blickstein, I. (2005). Growth aberration in multiple pregnancy. *Obstetrics and Gynecology Clinics of North America*, 32, 39–54.
- Blondel, B., & Kaminski, M. (2002). Trends in the occurrence, determinants, and consequences of multiple births. *Seminars in Perinatology*, 26, 239–249.
- Brenner, B. M., & Chertow, G. M. (1993). Congenital oligonephropathy: An inborn cause of adult hypertension and progressive renal injury. *Current Opinion in Nephrology and Hypertension*, 2, 691–695.
- Brenner, B. M., Lawler, E. V., & Mackenzie, H. S. (1998). The hyperfiltration theory: A paradigm shift in nephrology. *Kidney International*, 49, 1774–1777.
- Byrne, C. D., & Phillips, D. I. (2000). Fetal origins of adult disease: Epidemiology and mechanisms. *Journal of Clinical Pathology*, 53, 822–828.
- Cosmi, E., Visentin, S., Fanelli, T., Mautone, A. J., & Zanardo, V. (2009). Aortic intima media thickness in intrauterine growth restricted fetuses and infants: A longitudinal prospective study. *Obstetrics & Gynecology*, 114, 1109–1114.
- Crews, D. C., Boulware, L. E., Gansevoort, R. T., & Jaar, B. G. (2011). Albuminuria: Is it time to screen the general population? *Advances in Chronic Kidney Disease*, 18, 249–257.
- Crispi, F., Bijmens, B., Figueras, F., Le Noble, F., Ahmed, A., & Gratacós, E. (2010). Fetal growth restriction results in remodeled and less efficient hearts in children. *Circulation*, 121, 2427–2436.
- Figueras, F., & Giardosi, J. (2011). Intrauterine growth restriction: New concepts in antenatal surveillance, diagnosis, and management. *American Journal of Obstetrics and Gynecology*, 204, 288–300.
- Fox, N. S., Rebarber, A., Klauser, C. K., Roman, A. S., & Saltzman, D. H. (2011). Intrauterine growth restriction in twin pregnancies: Incidence and associated risk factors. *American Journal of Perinatology*, 28, 267–272.
- Franco, M. C. P., Christofalo, D. M. J., Sawaya, A. L., Ajzen, S. A., & Sesso, R. (2006). Effects of low birth weight in 8- to 13-year-old children: Implications in endothelial function and uric acid levels. *Hypertension*, 48, 45–50.
- Gluckman, P. D., & Hanson, M. A. (2004). Living the past: Evolution, development, and patterns of disease. *Science*, 305, 1733–1736.
- Hadlock, F. P. (1990). Sonographic estimation of fetal age and weight. *Radiologic Clinics of North America*, 28, 39–50.
- Hostetter, T. H. (2003). Hyperfiltration and glomerulosclerosis. *Seminars in Nephrology*, 23, 194–199.
- Hughson, M. D., Douglas-Denton, R., Bertram, J. F., & Hoy, W. E. (2006). Hypertension, glomerular number, and birth weight in African Americans and white subjects in the southeastern United States. *Kidney International*, 69, 671–678.
- Järvisalo, M. J., Jartti, L., Näntö-Salonen, K., Rönnemaa, T., Hartiala, J. J., Celermajer, D. S., & Raitakari, O. T. (2001). Increased aortic intima-media thickness: A marker of pre-clinical atherosclerosis in high-risk children. *Circulation*, 104, 2943–2947.
- Johansson, S., Iliadou, A., Bergvall, N., Tuvemo, T., Norman, M., & Cnattingius, S. (2005). Risk of high blood pressure among young men increases with the degree of immaturity at birth. *Circulation*, 112, 3430–3436.
- Keijzer-Veen, M. G., Schrevel, M., Finken, M. J., Dekker, F. W., Nauta, J., Hille, E. T., . . . Dutch POPS-19 Collaborative Study Group. (2005). Microalbuminuria and lower glomerular filtration rate at young adult age in subjects born very premature and after intrauterine growth retardation. *Journal of the American Society of Nephrology*, 16, 2762–2768.
- Koklu, E., Ozturk, M. A., Gunes, T., Akcakus, M., & Kurtoglu, S. (2007). Is increased intima-media thickness associated with preatherosclerotic changes in intrauterine growth restricted newborns? *Acta Paediatrica*, 96, 1858.
- Levine, R. S., Hennekens, C. H., & Jesse, M. J. (1994). Blood pressure in prospective population based cohort of newborn and infant twins. *BMJ*, 308, 298–302.
- Litwin, M., & Niemirska, A. (2009). Intima-media thickness measurements in children with cardiovascular risk factors. *Pediatric Nephrology*, 24, 707–719.
- Loos, R. J., Fagard, R., Beunen, G., Derom, C., & Lietinck, R. (2001). Birth weight and blood pressure in young adults: A prospective twin study. *Circulation*, 104, 1633–1638.
- Luyckx, V., & Brenner, B. (2005). Low birth weight, nephron number, and kidney disease. *Kidney International*, 68, 68–77.
- Manalich, R., Reyes, L., Herrera, M., Melendi, C., & Fundora, I. (2000). Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. *Kidney International*, 58, 770–773.
- Muhlhausler, B. S., Hancock, S. N., Bloomfield, F. H., & Harding, R. (2011). Are twins growth restricted? *Pediatric Research*, 70, 117–122.

- Neitzke, U., Harder, T., & Plagemann, A. (2011). Intrauterine growth restriction and developmental programming of the metabolic syndrome: A critical appraisal. *Microcirculation*, *18*, 304–311.
- Phillips, D. I., Davies, M. J., & Robinson, J. S. (2001). Fetal growth and the fetal origins hypothesis in twins—Problems and perspectives. *Twin Research*, *4*, 327–331.
- Poulsen, P., Grunnet, L. G., Pilgaard, K., Storgaard, H., Alibegovic, A., Sonne, M. P., . . . Vaag, A. (2009). Increased risk of type 2 diabetes in elderly twins. *Diabetes*, *58*, 1350–1355.
- Secher, N. J., Kaern, J., & Hansen, P. K. (1985). Intrauterine growth in twin pregnancies: Prediction of fetal growth retardation. *Obstetrics & Gynecology*, *66*, 63–68.
- Singh, G. R., & Hoy, W. E. (2004). Kidney volume, blood pressure, and albuminuria: Findings in an Australian aboriginal community. *American Journal of Kidney Diseases*, *43*, 254–259.
- Skilton, M. R. (2008). Intrauterine risk factors for precocious atherosclerosis. *Pediatrics*, *121*, 570–574.
- Skilton, M. R., Evans, N., Griffiths, K. A., Harmer, J. A., & Celermajer, D. S. (2005). Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet*, *365*, 1484–1486.
- Teeninga, N., Schreuder, M. F., Bökenkamp, A., Delemarre-van de Waal, H. A., & van Wijk, J. A. (2008). Influence of low birth weight on minimal change nephrotic syndrome in children, including a meta-analysis. *Nephrology Dialysis Transplantation*, *23*, 1615–1620.
- Vågerö, D., & Leon, D. (1994). Ischaemic heart disease and low birth weight: A test of the fetal-origins hypothesis from the Swedish Twin Registry. *Lancet*, *343*, 260–263.
- Zanardo, V., Fanelli, T., Weiner, G., Fanos, V., Zaninotto, M., Visentin, S., . . . Cosmi, E. (2011). Intrauterine growth restriction is associated with persistent aortic wall thickening and glomerular proteinuria during infancy. *Kidney International*, *80*, 119–123.
- Zhang, J., Brenner, R. A., & Klebanoff, M. A. (2001). Differences in birth weight and blood pressure at age 7 years among twins. *American Journal of Epidemiology*, *153*, 779–782.
-