AORTIC AND CAROTID WALL THICKNESS IN TERM SMALL-FOR-GESTATIONAL AGE NEWBORNS AND RELATIONSHIP WITH PRENATAL SIGNS OF SEVERITY

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KEY WORDS

aortic intima-media thickness; blood pressure; carotid intima-media thickness; late onset intrauterine growth restriction; small-for-gestational age

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ABSTRACT

Objective: To assess carotid and aortic intima-media thickness (IMT) in term growth restricted newborns with and without prenatal signs of severity.

Methods: This was a prospective study comprising 67 cases diagnosed prenatally and 134 normally grown newborns. Cases were sub-classified into small-for-gestational age (SGA) with no signs of severity and those with signs of severity, defined as a birth weight below 3rd percentile, or abnormal uterine artery Doppler or cerebro-placental ratio. Blood pressure and vascular IMT were evaluated.

Results: SGA newborns showed a trend for higher values of blood pressure. IMT values were significantly increased in SGA, with and without signs of severity, as compared with controls. The magnitude of the increase was higher in SGA with signs of severity.

Conclusions: Vascular IMT was increased in SGA irrespective of the presence or absence of prenatal signs of severity. The results challenge the notion of "constitutionally small" SGA, and support that the majority of SGA represent forms of true growth restriction and suffer fetal cardiovascular programming.

INTRODUCTION

Fetal growth restriction (FGR) is one of the most common conditions affecting ongoing pregnancies¹. A substantial body of evidence has reported a wide spectrum of unfavorable perinatal and lifelong effects associated with FGR²⁻⁶. The pathophysiology linking growth restriction to the adverse short and long term outcomes could be possibly attributed to the profound changes in fetuses' metabolism⁷, cardiovascular system^{8,9} and brain structure¹⁰. The fetal cardiovascular adaptation to hypoxia and undernutrition is thought to represent a central adaptive mechanism and induces both cardiac^{9,11} and vascular remodeling^{12,13}. Vascular intima-media thickness (IMT) is a standard diagnostic procedure in assessing cardiovascular risk in asymptomatic adults¹⁴. Recently, an inverse relation between aortic IMT^{12,13}, arterial stiffness^{15,16} and low birth weight is reported.

There is a current tendency to distinguish between early and late FGR if it occurs before or after 32 weeks gestation¹⁷. The importance of gestational age at diagnosis is reflected on the severity of placental dysfunction and the prognosis. Early FGR is mainly characterized by severe placental insufficiency expressed by abnormal umbilical artery^{17,18}. On the other hand, late FGR is associated with a milder placental dysfunction, illustrated merely by brain redistribution and abnormal uterine arteries^{19,20}. Recent evidence has demonstrated that late FGR fetuses, often referred as term small for gestational age (SGA) due to normal umbilical artery, are characterized by subtle cardiovascular remodeling^{8,21}. These cases have worse cardiovascular²² and neurodevelopmental²³ outcomes in postnatal life than initially anticipated. Over recent years several prognostic factors, including abnormal cerebroplacental ratio²⁴ and uterine artery Doppler¹⁹, and birth weight below the 3rd centile²⁵, have been identified as predictors of poor neonatal outcome, and

consequently as surrogate markers of true growth restriction among near term SGA fetuses. Although these factors are associated with poorer neonatal outcome, it is unknown whether they also classify well the risk of abnormal vascular outcome.

We hypothesized that vascular structure and function in SGA neonates could be similarly anticipated by the aforementioned prognostic factors of placental insufficiency. We explored this hypothesis by conducting a prospective cohort study to assess blood pressure and vascular IMT in late-onset growth restricted newborns classified by surrogate markers in different stages of severity.

METHODS

Study population

This was a prospective cohort study including 201 term newborns prenatally diagnosed as small or appropriate for gestational age and delivered after 37 weeks, subdivided into: A) SGA with signs of severity defined by estimated fetal weight (EFW) and confirmed birth weight below 3rd percentile or abnormal uterine artery mean pulsatility index (PI) (above 95th percentile) or cerebro-placental ratio (below 5th percentile); B) SGA without signs of severity defined by EFW and birth weight between 3rd and 10th percentile together with normal uterine artery mean PI and cerebro-placental ratio; and C) controls defined by EFW and confirmed birth weight above 10th percentile with no pregnancy complications. Prenatal ultrasonographic examination was performed using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) with a 6-MHz curvilinear transducer. Feto-placental Doppler examination before delivery was considered and included uterine arteries, umbilical artery and middle cerebral artery. Cerebro-placental ratio was calculated as a simple ratio of the middle cerebral artery PI divided by the umbilical artery PI²⁶.

Information on maternal demographic characteristics, reproductive history and pregnancy outcome was obtained in the interview at the day of the scan and a posteriori through medical history notes. Gestational age at delivery, mode of delivery, birth weight, birth weight centile, Apgar score and umbilical pH were also recorded. Cases and controls were identified in fetal life among pregnancies attended in the Department of Maternal-Fetal Medicine in Hospital Clinic (Barcelona) and evaluated in the neonatal period during their first week of life. Controls were matched 2 to 1 with cases by gender and gestational age at delivery (± 1 week). Exclusion criteria were chromosomal or

genetic disorders, monochorionic twin pregnancy and evidence of infection. In all pregnancies gestational age was calculated based on the crown-rump length at first trimester ultrasound²⁷. Fetal and neonatal weight centile were calculated according to local reference curves²⁸. The study received ethical approval by the local Research Ethics Committee and written informed consent form was obtained from parents.

Neonatal vascular assessment

A controlled environment contributed to the achievement of all neonatal measurements. Blood pressure was obtained from brachial artery using a validated ambulatory automated Omron 5 Series device. An appropriate cuff size covering the 40 percent of the arm circumference was used to ensure accurate measurement²⁹. Each newborn's blood pressure was evaluated twice during quiescence and the average was determined. Due to the lack of recently published blood pressure normal values for our population of healthy newborns, systolic and diastolic blood pressure percentiles were calculated using local standards (supplemental data).

Carotid and aorta ultrasound assessment was performed by skilled sonographers using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer. Longitudinal clips of the far wall of the proximal abdominal aorta were obtained in the upper abdomen by a 10-MHz linear probe. Carotid and aorta IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package), as depicted in Figure 1. To obtain IMT, three end-diastolic frames were selected across a length of 10 mm and analyzed for mean and maximum IMT, and the average reading from these three frames was calculated. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements in end-diastole.

Vascular wall thickness reproducibility

Intra-observer variability was determined for each parameter by comparing data from three measurements of the same sonographer and inter-observer variability by comparing data from two sonographers at one occasion.

Statistical analysis

The intra-observer reproducibility was assessed by calculating the intra class correlation coefficients and coefficients of variation (CV). The inter-observer reproducibility was assessed by calculating the CV for each parameter³⁰.

Sample size was calculated taking into account comparison between control and SGA neonates both with and without signs of severity. We used balanced ANOVA type of analysis for 2 factors and 3 groups, considering standard deviation (SD) of 0.08 mm¹² and detectable contrast of 0.07 for both factors (cIMT and aIMT). An

estimated sample size of 32 women per study group was achieved for a power of more than 90% and 5% type I error level³¹. Results are presented as median (interquartile range) or as percentage after checking parametric assumptions by Kolmogorov-Smirnov test. One way analysis of variance based on log transformed data adjusted with Bonferroni post-hoc test and Pearson chi square were used to compare quantitative and qualitative data respectively. Doppler ultrasound measurements were converted into Z-scores^{26,32,33}. Models for vascular results were adjusted by multiple linear regression by gender, gestational age at birth, age at evaluation. Additionally, polynomial orthogonal contrasts were constructed in order to test for linear trends of parameters across severity groups. All statistical tests were performed at the 2-sided 5% level of significance. The analyses were carried out using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS

Study populations

Clinical and perinatal data are presented in Table 1. Maternal characteristics were similar among the study groups with the exception of a higher prevalence of nulliparity and smokers in the SGA group with signs of severity as compared to controls. Median birthweight of control neonates was 3350g (minimum 2530g and maximum 4400g). By definition, both SGA groups had a lower birth weight; median birthweight for SGA without signs of severity was 2603g (minimum 2190g and maximum 2920g), while median birthweight for SGA with signs of severity was 2200g (minimum 1630g and maximum 2650g). SGA cases with signs of severity had worse prenatal Doppler as compared to controls. Most of SGA with signs of severity were characterized by EFW <p3 and only 30% of them presented abnormal uterine

artery Doppler or cerebroplacental ratio. As expected, SGA cases with signs of severity had a higher prevalence of cesarean section and admission to the neonatal intensive care unit.

Neonatal vascular assessment

Results on intra- and inter-observer reproducibility are presented in tables 2 and 3.

Results on vascular parameters are shown in table 4 and figure 2.

Blood pressure values were similar among groups with a non-significant tendency for higher values in SGA without and with signs of severity.

Mean and maximum carotid artery were significantly increased in both SGA cases as compared to controls (mean cIMT: 0.017 mm [SE 0.008], $P=0.041$ and maximum cIMT: 0.035 mm [SE 0.011], $P=0.002$ per SGA without signs of severity; mean cIMT: 0.023 mm [SE 0.010], $P=0.028$ and maximum cIMT: 0.026 mm [SE 0.013], $P=0.050$ per SGA with signs of severity). These differences remained significant after normalization of IMT values by neonatal weight and vessel diameter. There was a significant linear tendency for higher values across groups ($P<0.001$, $P=0.001$ for mean and maximum IMT, respectively). Mean aortic IMT showed a non significant trend for higher values between controls and SGA cases (mean aIMT: 0.023 mm [SE 0.017], $P=0.194$ per SGA without signs of severity; mean aIMT: 0.036 mm [SE 0.018], $P=0.055$ per SGA with signs of severity), that became significantly different after adjusting by neonatal weight and vessel diameter. Furthermore, maximum aorta IMT was significantly increased in both SGA cases as compared to controls (maximum aIMT: 0.055 mm [SE 0.018], $P=0.003$ per SGA without signs of severity and maximum aIMT: 0.057 mm [SE 0.022], $P=0.010$ per SGA with signs of severity). Similarly to previous parameters, the association remained significant after normalization of IMT values by neonatal weight and vessel diameter.

DISCUSSION

This study demonstrates that term SGA cases with and without signs of severity show increased vascular IMT, irrespective of the absence of signs of severity.

No significant differences for both systolic and diastolic blood pressure within groups were demonstrated in our study. However, a tendency for higher values in SGA newborns with signs of severity was shown as compared to SGA without signs of severity. No prior studies aimed at analyzing blood pressure of neonates with late-onset growth restriction. Yet, it is well documented that cases with growth restriction have higher blood pressure values than controls in childhood^{11,34,35}. Nevertheless, our results are concordant with previous data showing no differences between preterm controls and early-onset growth restricted neonates¹⁵. The non significant tendency to higher blood pressure values in our neonates could be explained by our limited sample size or selection of a different postnatal age at examination. The lack of differences in the neonatal period could also be explained by an intrinsic limitation of measuring blood pressure in the neonatal period.

Vascular IMT was increased in growth restricted neonates irrespective whether they presented signs of severity or not. The differences in both SGA cases were statistically significant after adjusting by neonatal weight and vessel diameter. These results are in agreement with previous data demonstrating associations between low birth weight and aortic IMT adaptations in newborns and young children¹¹⁻¹³. We showed a significant linear trend for higher values of IMT in relation to growth restriction severity. Although there was a tendency to worse values in SGA cases with signs of severity, SGA neonates without signs of severity also presented clear changes suggesting vascular remodeling, without though significant differences among the two groups. This association challenges the notion of "constitutionally

small" SGA, and suggests that SGA contain a substantial proportion of cases representing forms of true fetal growth restriction. The observed changes in SGA neonates without signs of severity suggest that severity markers, which are useful to predict adverse perinatal outcome, such as uterine artery pulsatility index, cerebroplacental ratio, estimated fetal weight less than the 3rd percentile^{19,24,25}, fail to predict a normal postnatal cardiovascular outcome. These results are in line with previous studies evaluating postnatal long term cardiac function in SGA²². Thus, the need to develop biomarkers allowing the detection of long term cardiovascular outcome in term low birth weight subjects could not be overemphasized. Whether vascular IMT could be used as a prognostic imaging biomarker, to improve stratification of risk among term growth restricted newborns, needs to be further investigated in large prospective studies.

In order to complete vascular assessment with further information, additional vascular parameters were included in our study (provided in the supplemental data). Arterial tensile stress, a major determinant of arterial hemodynamic forces³⁶, and arterial distensibility showed comparable values between cases and controls. The interpretation of these results is challenging. Theoretically, these findings were not anticipated when considering the previously reported increased afterload and arterial stiffness in fetuses and preterm growth restricted neonates when evaluated shortly after delivery^{11,15,37}. However, as previously mentioned the neonatal period is a transitional pathophysiological state for cardiovascular regulation. The vascular behavior in our cohort would support the hypothesis that high placental resistance and subsequently increased afterload and raised blood pressure occur merely during fetal life^{11,15,37}. In the immediate postnatal period however, the multiple stimuli that are responsible for the aforementioned changes would cease to exert their influence.

Consequently, tensile stress would show a behavior similar to blood pressure during this period. This assumption could be in line with our previously published results in 3-6 years old children with late-onset growth restriction without signs of severity. These children showed a non significant trend for higher values of normalized mean cIMT values and significant linear tendency for this parameter across stages of severity²². It seems therefore that despite normalization of hemodynamic conditions in the early neonatal period, mild changes still persist in childhood.

This study has strengths and limitations. To our knowledge, it was the first time to focus on structural and functional parameters of carotid and aorta as distinct components of the vascular tree³⁸⁻⁴⁰. Furthermore, the thorough classification of neonates according to their prenatal data in stages of severity has allowed observing vascular remodeling in all growth restricted neonates. The adjustment of results by both neonatal weight and lumen diameter further confirmed this finding. On the other side, our relatively limited sample size could account for the non significant trend for higher values in blood pressure. We also acknowledge that the different prevalence of maternal smoking among groups could have partly affected our results. Finally, long term pediatric follow up of our cohort is warranted in order to better understand the pathophysiology of cardiovascular remodeling in growth restriction and subsequently implement preventive strategies.

In conclusion, our study suggests that SGA neonates, both with and without signs of severity, demonstrate similar changes in vascular structure and function. These results challenge the notion of "constitutionally small" SGA, and support that the majority of SGA represent forms of true growth restriction and suffer fetal cardiovascular programming. Identifying these neonates at risk of vascular remodeling could make a significant contribution to screening public health

programs, given that growth restriction occurs in 10% of general maternity population.

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Table1. Baseline characteristics and perinatal outcomes of the study groups.

Characteristics	Controls	SGA without signs of severity	SGA with signs of severity	P value [†]
<i>N</i>	134	32	35	
<i>Maternal characteristics</i>				
Maternal age (years)	32.0 (8.3)	32.5 (11)	31.0 (9)	0.820
Body mass index (kg/m ²)	22.8 (3.8)	23.0 (5.1)	21.4 (4.5)	0.190
Nulliparity (%)	78 (58.0)	20 (62.5)	27 (77.1)	0.132
White ethnicity (%)	74 (55.2)	18 (56.6)	22 (62.8)	0.906
Smoking (%)	22 (16.4)	8 (25.0)	13 (37.1)	0.028
<i>Prenatal ultrasound</i>				
Gestational age at prenatal scan (weeks)	34.1 (4.0)	37.7 (2.0) [*]	37.6 (1.3) [*]	<0.001
Uterine artery mean PI (z-scores)	-0.93 (2.56)	0.36 (1.29)	-0.72 (1.22)	0.050
Umbilical artery PI (z-scores)	-0.04 (0.42)	0.06 (0.67)	0.19 (1.29) [*]	0.040
Middle cerebral artery PI (z-scores)	1.06 (1.10)	0.48 (1.18)	-0.39 (1.94)	0.345
Cerebro-placental ratio (z-	-0.29 (1.48)	-0.31	-0.71	0.353

scores)		(1.10)	(2.18)	
<i>Delivery data</i>				
Gestational age at delivery (weeks)	39.5 (1.8)	38.7 (1.5)	38.5 (1.2) [*]	0.012
Caesarian section (%)	22 (16.0)	6 (18.7)	15 (42.5) [*]	0.006
Caesarian section for non reassuring fetal status (%)	4 (2.9)	2 (6.2)	5 (14.2) [*]	0.033
Male gender (%)	78 (58)	19 (59)	20 (57)	0.983
Birth weight (g)	3350 (545)	2603 (273) [*]	2200 (290) [*]	<0.001
Birth weight percentile	55 (50)	4 (3)	1 (1)	<0.001
5-min Apgar score	10 (0)	10 (0)	10 (0)	0.464
Umbilical artery pH	7.24 (0.10)	7.26 (0.09)	7.25 (0.08)	0.346
Days in NICU	0 (0)	0 (0)	4 (3)	<0.001
<p>Data are presented as median (interquartile range) or percentage (N).</p> <p>[*]P<0.05 as compared to controls.</p> <p>[†]P value calculated by one-way ANOVA or Pearson's chi-square test.</p> <p>All Doppler parameters are presented as Z-scores.</p> <p>SGA, small-for-gestational age; <i>PI</i>, pulsatility index;</p> <p><i>NICU</i>, neonatal intensive care unit.</p>				

Table 2. Intra-observer reproducibility of neonatal carotid and aortic arteries intima-media thickness evaluation.

	Correlation coefficient	Coefficient of variation
	ρ	CV, %
Mean right cIMT	0.827 (0.790-0.859)	2.7%
Mean left cIMT	0.870 (0.842-0.895)	2.6%
Maximum right cIMT	0.908 (0.887-0.926)	2.8%
Maximum left cIMT	0.921 (0.903-0.937)	2.6%
Mean aIMT	0.861 (0.830-0.887)	2.6%
Maximum aIMT	0.934 (0.919-0.947)	2.1%

ρ indicates intraclass correlation coefficient (Lower bound-higher bound); CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Table 3. Inter-observer reproducibility of neonatal aortic and carotid arteries intima-media thickness evaluation.

	Coefficient of variation
	CV, %
Mean right cIMT	1.3%
Mean left cIMT	1.5%
Maximum right cIMT	1.2%
Maximum left cIMT	1.0%
Mean aIMT	7.6%
Maximum aIMT	4.5%

CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Table 4. Results on neonatal vascular assessment in the study groups.

Characteristic	Controls	SGA without signs of severity	SGA with signs of severity	Linear tendency P-value ²
<i>N</i>	134	32	35	
<i>Blood pressure</i>				
Systolic BP (mmHg)	82 (17)	85 (20)	86 (13)	0.143
Systolic BP percentile	57 (50)	57 (48)	62 (48)	0.420
Diastolic BP (mmHg)	46 (15)	48 (15)	52 (16)	0.107
Diastolic BP percentile	44 (53)	53 (50)	65 (51)	0.324
<i>Carotid arteries</i>				
Mean cIMT(mm)	0.226 (0.06)	0.247 (0.05) [*]	0.268 (0.08) [*]	<0.001
Mean cIMT / neonatal weight (mm/kg)	0.067 (0.02)	0.090 (0.02) [*]	0.090 (0.03) [*]	<0.001
Mean cIMT / diameter	0.094 (0.04)	0.117 (0.04)	0.112 (0.03)	<0.001
Maximum cIMT(mm)	0.303 (0.08)	0.336 (0.08) [*]	0.341 (0.10) [*]	0.001
Maximum cIMT / neonatal weight (mm/kg)	0.090 (0.02)	0.122 (0.02) [*]	0.120 (0.04) [*]	<0.001
Max cIMT / diameter	0.126 (0.04)	0.148 (0.05) [*]	0.157 (0.04) [*]	<0.001
<i>Aorta</i>				

Mean aIMT(mm)	0.419 (0.10)	0.493 (0.20)	0.446 (0.12)	<i><0.001</i>
Mean aIMT / neonatal weight (mm/kg)	0.127 (0.03)	0.183 (0.08) [*]	0.210 (0.06) [†]	<i><0.001</i>
Mean aIMT / diameter	0.085 (0.03)	0.104 (0.05) [*]	0.113 (0.04) [*]	<i><0.001</i>
Maximum aIMT(mm)	0.547 (0.11)	0.622 (0.20) [*]	0.604 (0.15) [*]	<i>0.002</i>
Maximum aIMT / neonatal weight (mm/kg)	0.165 (0.05)	0.222 (0.08) [*]	0.284 (0.09) [†]	<i><0.001</i>
Maximum aIMT /aortic diameter	0.111 (0.02)	0.128 (0.07) [*]	0.141 (0.05) [*]	<i><0.001</i>
<p>Data are presented as median (interquartile range).</p> <p>[*]<i>P</i><i><0.05</i> as compared to controls calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group</p> <p>[†]<i>P</i><i><0.05</i> as compared to SGA without signs of severity calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group</p> <p>[‡]<i>P</i><i><0.05</i> calculated by linear trends across severity groups</p> <p>SGA, small-for-gestational age; BP, blood pressure;</p> <p>c/MT, carotid intima-media thickness; a/MT, aortic intima-media thickness.</p>				

Figure 1. Illustration of the mean carotid and aortic intima-media thickness (IMT) assessment by ultrasound in control, small-for-gestational age neonates without signs of severity and small-for-gestational age neonates with signs of severity.

Figure 2. Vascular intima-media thickness (IMT) in controls, late-onset growth restricted neonates without signs of severity and late-onset growth restricted neonates with signs of severity. Data are presented as mean (95% CI). * $P<0.05$ as compared to controls adjusted by gender, gestational age at birth, age at evaluation and group.