Metabolism Clinical and Experimental 100 (2019) 153959

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Clinical Science

Placental proteases PAPP-A and PAPP-A2, the binding proteins they cleave (IGFBP-4 and -5), and IGF-I and IGF-II: Levels in umbilical cord blood and associations with birth weight and length

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ARTICLE INFO

Article history:
Received 15 May 2019
Accepted 6 August 2019

Keywords:
Cord blood
Fetal growth
IGF axis
IGF binding proteins
Placental proteases

ABSTRACT

Background: A newborn's birth weight for gestational age provides important insights into his or her fetal growth and well-being. While the underlying mechanisms regulating fetal growth remain to be fully elucidated, the IGF axis plays an important role. Some components of this axis have been well-characterized in umbilical cord blood, but others have not yet been studied. We measured the proteases PAPP-A and PAPP-A2, the binding proteins they cleave (IGFBP-4 and -5), and the established molecules IGF-I and –II in umbilical cord blood to better characterize the IGF axis in relation to birth weight and length.

Methods: We performed a case-control study of 180 neonates born at a tertiary teaching hospital in Boston. To maximize power, infants were recruited in a 1:3:1 ratio with 37 SGA, 111 AGA, and 37 LGA infants matched by gestational age, sex, and delivery mode. IGF-I, IGF-II, IGFBP-4, IGFBP-5, PAPP-A, and PAPP-A2 were measured in umbilical cord blood by ELISA. Associations between birth weight and birth length *Z*-scores and the *Z*-scores of the above molecules were analyzed using linear regression models and analysis of covariance.

Results: Birth weight and length *Z*-scores were positively associated with *Z*-scores of IGF-I, IGF-II, total IGFBP-4, and IGFBP-5, with IGF-I having the strongest association. Birth weight and length *Z*-scores were negatively associated with *Z*-scores of intact IGFBP-4, PAPP-A, and PAPP-A2 levels.

Conclusions: We confirm previous findings of significant associations between the IGFs in cord blood and newborn size and for the first time show positive associations between cord blood total IGFBP-4 and -5 and birth weight and a negative association between intact IGFBP-4 and birth weight. We also show for the first time a reciprocal relationship between cord blood levels of PAPP-A and PAPP-A2 and newborn size. The implications of these findings need to be further examined in large longitudinal studies and likely have diagnostic and therapeutic potential.

1. Introduction

A newborn's birth weight for gestational age provides important information about his or her fetal growth and well-being, which has considerable implications for postnatal health. There is an established association between birth weight for gestational age and adverse outcomes, where infants born both small for gestational age (SGA) and large for gestational age (LGA) are at risk for significant morbidity and mortality, both in the short- and long-term [1,2].

In addition to higher rates of neonatal death, SGA infants are more likely to have respiratory distress syndrome, hypoglycemia, hypothermia, hyperbilirubinemia, intraventricular hemorrhage,

Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; IGF, insulin-like growth factor; IGFBP-4, insulin-like growth factor binding protein-4; IGFBP-5, insulin-like growth factor binding protein-5; PAPP-A, pregnancy-associated plasma protein-A; PAPP-A2, pregnancy-associated plasma protein-A2; ELISA, enzyme-linked immunosorbent assay.

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https://doi.org/10.1016/j.metabol.2019.153959
0026-0495/

necrotizing enterocolitis, seizures, and sepsis in the neonatal period [3–10]. They also have worse neurocognitive outcomes in childhood, and, as adults, they are more likely to develop obesity, type 2 diabetes, and cardiovascular disease [11–15]. Similarly, LGA infants have increased perinatal risks, including shoulder dystocia, hypoglycemia, and increased NICU admissions, as well as long-term risks of obesity, type 2 diabetes, metabolic syndrome, and cardiovascular disease [13,16–18].

The associations between birth weight for gestational age and later health outcomes were first described in the Barker Hypothesis in the 1980s, which proposed that the origins of many adult diseases can be traced back to the fetal environment [19]. This idea of fetal programming suggests that a fetus's adaptations to its environment can result in permanent changes that affect his or her growth and metabolism throughout life [20]. Fetal adaptations to undernutrition include increased peripheral glucose and insulin sensitivity, decreased beta cell mass and insulin secretion, diminished skeletal muscle cell number and capacity for protein synthesis, and increased glucose production [21].

Despite these well-described associations, the underlying mechanisms regulating birth weight and length remain to be fully elucidated. In light of its known importance in regulating fetal growth, the insulin-like growth factor (IGF) axis likely plays a key role [22,23]. The IGF axis consists of insulin-like growth factors I and II (IGF-I, IGF-II), 6 regulatory IGF binding proteins (IGFBP-1-6), and two proteases (pregnancy associated plasma protein A and A2, PAPP-A, PAPP-A2). IGF-I and IGF-II are important factors in fetal growth and have been well-studied in umbilical cord blood. There is a strong positive association between cord blood IGF-I and growth indices and a weaker positive association between IGF-II and growth indices [24–33].

The IGFBPs are also influential in fetal growth, as they bind 99% of IGFs in fetal serum and regulate the amount of free IGF available to bind its receptor [34]. Multiple previous studies have shown that cord blood IGFBP-3 has a strong positive association with birth weight, whereas both IGFBP-1 and -2 are negatively associated with birth weight [25,26,28,31–33,35,36]. Much less is known about more novel, additional IGF binding proteins (IGFBP-4, -5, and -6) and the proteases that cleave them (PAPP-A and PAPP-A2). One previous study found an inverse relationship between cord blood PAPP-A levels and birth weight, which was hypothesized to be due to a compensatory regulation mechanism of IGF [37]. However, beyond that study, these proteases and binding proteins have not yet been measured in cord blood in relation to fetal growth.

Our study aims to measure these additional molecules and to assess them, in addition to important clinical variables, to better characterize the IGF axis in umbilical cord blood in newborns. This is an important step in more fully understanding the mechanisms by which fetal growth is regulated. Identifying the factors that predict birth weight and length may also provide key information about the link between abnormal fetal growth and long-term health outcomes, which will allow us to achieve the ultimate goal of intervening in the fetal or newborn period to prevent the life-long complications caused by fetal growth dysregulation. We hypothesize that the placental proteases, which regulate the availability of free IGF-I and –II through cleavage of the IGFBPs, will be inversely associated with birth weight *Z*-score, possibly through compensatory regulation. We hypothesize that the concentration of intact IGFBP-4 influences bioavailable IGF-I, and thus will also be inversely associated with birth weight *Z*-score.

2. Methods

2.1. Study population

This case-control study included 180 newborns born at Brigham and Women's Hospital between July 2010 and January 2018. Infants were recruited from a larger study, which included 980 umbilical cord blood samples that were indiscriminately and consecutively collected when staff was available. Fig. 1 details the recruitment of cases and controls for this study. Newborns with major congenital anomalies and those <34 weeks gestational age were excluded. Twins were not excluded, and there were 17 pairs of twins in the study. Infants were categorized as SGA (birth weight < 10th percentile), AGA (birth weight between 10th and 90th percentile), or LGA (birth weight > 90th percentile) based on the INTERGROWTH-21st growth curves [38]. SGA and LGA infants were selected consecutively from the larger study, and then matched for sex, gestational age and delivery mode in a 1:3:1 ratio to maximize power with 37 SGA, 111 AGA, and 37 LGA infants. Demographic and anthropometric data were collected from maternal and infant medical records for all study participants. The study was reviewed and approved by the institutional review board of Brigham and Women's Hospital.

2.2. Cord blood collection and hormone measurements

Cord blood samples were collected at time of delivery and stored at 4 °C for <24 h. They were then centrifuged, and serum was aliquoted and stored in Eppendorf tubes at −80 °C until assayed. Concentrations of insulin-like growth factor-I (IGF1, catalog number: AL-121), insulin-like growth factor-II (IGF-II, catalog number: AL-131), total and intact insulin-like growth factor binding protein-4 (IGFBP4, catalog number: AL-126 & AL-128), insulin-like growth factor binding protein 5 (IGFBP5, catalog number: AL-127), pregnancy-associated plasma protein A (PAPP-A pico, catalog number: AL-101), and pregnancy-associated plasma protein A2 (PAPP-A2, catalog number: AL-109) were all measured by ELISA with commercially available kits (Ansh Labs, Webster, TX, USA), according to the manufacturer's instructions. Assay characteristics are described in supplemental material (Supplementary Tables A1–A3) [39].

2.3. Statistical analysis

Data were analyzed using SPSS 19.0 (Chicago, IL, USA). Data were inspected for normality using the Shapiro-Wilk test, and variables not normally distributed were log transformed. Chi-squared test was used for comparison of baseline categorical variables, and one-way analysis of variance (ANOVA) and covariance (ANCOVA) were used for comparison of continuous variables among the three different birth weight categories (SGA, AGA, and LGA). Tukey HSD was then performed for post-hoc pairwise analyses between the groups. Pearson *r* correlation and Spearman rank correlation were both used to assess for associations between all variables in the study. Sequential linear regression models, controlling for various neonatal and maternal characteristics, were then performed to analyze the associations between the analytes as standardized values and birth weight *Z*-score and birth length *Z*-score separately.

3. Results

Comparisons of baseline maternal and neonatal characteristics are shown in Table 1. Demographics, including sex, gestational age, and delivery mode, were matched among the SGA, AGA, and LGA groups, per study design. Maternal age was slightly higher in the LGA group ($p = .001$). Gestational age was slightly different among the three groups, despite matching for this, with the lowest gestational age in the SGA group and highest in the LGA group ($p < .001$). The SGA group also had the most late-preterm infants, and the LGA group had the fewest, which is not unexpected, as many growth-restricted infants are delivered early. To control for the differences between the groups, these characteristics were entered as covariates in later analyses.

Table 2 shows the associations among variables for all study subjects using both Pearson *r* correlation and Spearman rank correlation. There were significant associations between several of the analytes measured

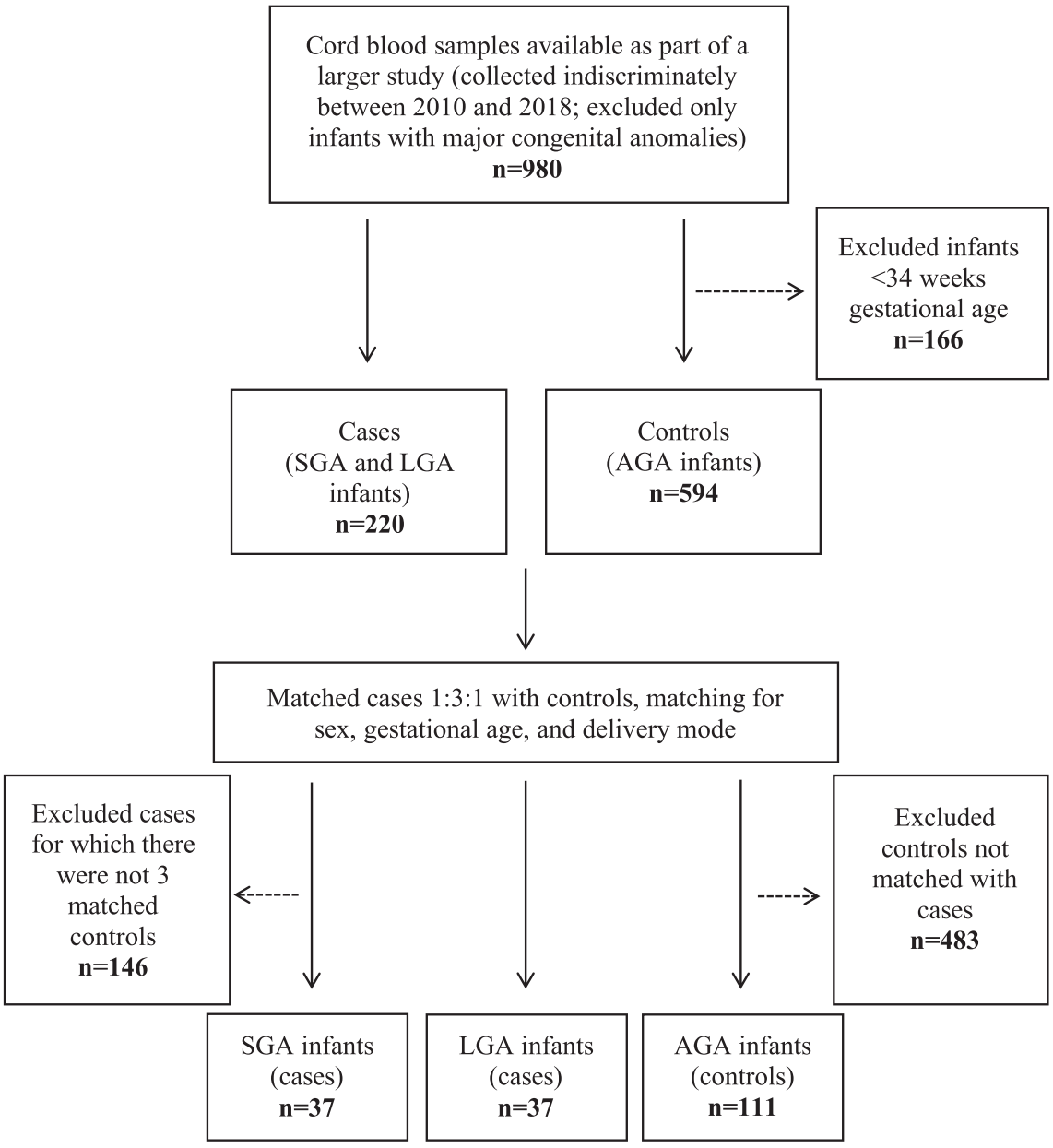

Fig. 1. Study population.

and clinical characteristics. IGF-I was positively associated with birth weight *Z*-score ($p < .001$), birth length *Z*-score ($p < .001$), gestational age ($p = .001$), maternal age ($p = .032$), and 1-minute Apgar score ($p = .006$). IGF-II was positively associated with birth weight *Z*-score ($p = .001$), length *Z*-score ($p < .001$), gestational age ($p < .001$), and sex ($p = .016$). Total IGFBP-4 was negatively associated with gestational age ($p = .008$) and 5-minute Apgar score ($p = .012$) only, whereas intact IGFBP-4 was negatively associated with birth weight *Z*-score ($p = .001$), length *Z*-score ($p = .004$), and gestational age ($p = .035$). IGFBP-5, in contrast, was positively associated with birth weight *Z*-score ($p = .002$), length *Z*-score ($p = .023$), and gestational age ($p < .001$). PAPP-A and PAPP-A2 were both negatively associated with birth weight *Z*-score ($p = .002$, $p < .001$), length *Z*-score ($p = .031$, $p < .001$), and 5-minute Apgar score ($p = .032$, $p = .004$). PAPP-A2 was additionally negatively associated with gestational age ($p = .009$). Gestational age was also positively associated with several clinical characteristics, including birth weight *Z*-score ($p < .001$), length *Z*-score ($p < .001$), 1-minute Apgar score ($p = .006$), and 5-minute Apgar score ($p = .002$), and was negatively associated with maternal age ($p = .035$). All of these characteristics were adjusted for in the linear regression analyses.

Sequential linear regression analyses were performed to examine the associations between each of the analytes measured with both birth weight and birth length for gestational age using birth weight *Z*-score (Table 3a) and birth length *Z*-score (Table 3b). Associations are expressed per standard deviation change in analytes. There were positive associations between birth weight and birth length *Z*-scores and IGF-I, IGF-II, total IGFBP-4 and IGFBP-5, and negative associations between birth weight and birth length *Z*-scores and intact IGFBP-4, PAPP-A, and PAPP-A2 ($p < .05$). After adjusting for gestational age, sex, race, delivery mode, maternal age, and Apgar scores, birth weight *Z*-score remained positively associated with IGF-I, IGF-II, and total IGFBP-4 and -5, and negatively associated with PAPP-A, and PAPP-A2 ($p < .05$). Notably, when IGF-I and IGF-II were added to the regression (Table 3a), these associations were almost all attenuated, with the exception of total IGFBP-4. Adjusting for the same characteristics, length

Table 1
Baseline maternal and neonatal characteristics.

	All ($N = 185$)	SGA ($N = 37$)	AGA ($N = 111$)	LGA ($N = 37$)	p-value
Maternal age (years)	32.6 ± 0.4	32.8 ± 0.9	31.6 ± 0.5[c]	35.3 ± 0.6[b]	0.001*
Delivery mode					0.285
Vaginal	41 (22.2%)	5 (13.5%)	29 (26.1%)	7 (18.9)	
C-section	144 (77.8%)	32 (86.5%)	82 (73.9%)	30 (81.1%)	
Marital status					0.183
Married	134 (72.8%)	27 (75%)	76 (68.5%)	31 (83.8%)	
Unmarried	50 (27.2%)	9 (25%)	35 (31.5%)	6 (16.2%)	
Race					0.161
White	102 (55.4%)	20 (55.6%)	55 (49.5%)	27 (73%)	
Black	27 (14.6%)	4 (11.1%)	20 (18.0%)	3 (8.1%)	
Hispanic	33 (17.9%)	5 (13.9%)	23 (20.7%)	5 (13.5%)	
Asian	22 (12.0%)	7 (19.4%)	13 (11.7%)	2 (5.4%)	
Gestational age (weeks)	37.9 ± 0.1	37.1 ± 0.3[b,c]	37.9 ± 0.2[a,c]	38.8 ± 0.2[a,b]	<0.001*
Full-term	135 (73%)	20 (54.1%)	80 (72.1%)	35 (94.6%)	
Late pre-term	50 (27%)	17 (45.9%)	31 (27.9%)	2 (5.4%)	
Sex					0.265
Female	93 (50.3%)	23 (62.2%)	52 (46.8%)	18 (48.6%)	
Male	92 (49.7%)	14 (37.8%)	59 (53.2%)	19 (51.4%)	
Birth weight (grams)	3105 ± 52	2270 ± 53[b,c]	3059 ± 42[a,c]	4081 ± 63[a,b]	<0.001*
Birth weight Z-score	0.08 ± 0.09	−1.7 ± 0.05[b,c]	0.03 ± 0.1[a,c]	2.0 ± 0.1[a,b]	<0.001*
Length (centimeters)	48 ± 0.25	44.6 ± 0.4[b,c]	48.0 ± 0.3[a,c]	51.5 ± 0.4[a,b]	<0.001*
Length Z-score	−0.2 ± 0.1	−1.7 ± 0.1[b,c]	−0.2 ± 0.1[a,c]	1.5 ± 0.2[a,b]	<0.001*
1 min Apgar	8.0 ± 0.07	7.8 ± 0.1	8 ± 0.1	8.2 ± 0.1	0.070
5 min Apgar	8.8 ± 0.03	8.7 ± 0.1[b]	8.9 ± 0.04[a]	8.9 ± 0.1	0.055

Values reported as n (%) or as mean ± standard error of the mean. ANOVA used to analyze continuous variables and chi-square test for categorical valuables. Asterisk (*) highlights p-value <.05. a: different from SGA ($p < .05$), b: different from AGA ($p < .05$), c: different from LGA ($p < .05$).
Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; PAPP-A, pregnancy-associated plasma protein A.

Z-score only remained significantly positively associated with IGF-I and IGF-II, and significantly negatively associated with PAPP-A2 ($p < .05$).

Adjusted R^2 values were derived from univariate and multivariate regression models to better understand how the various variables in our study contribute to birth weight and birth length Z-score variability. Clinical characteristics alone can explain approximately 17% of variability in birth weight for gestational age ($p < .001$), and cord blood concentrations of IGF-I and IGF-II each considered alone can explain 35% and 8% of the variability, respectively ($p < .001$; data not shown). Multivariate regression reveals that 46% of variability in birth weight for gestational age can be explained by combining clinical characteristics with the cord blood analytes measured ($p < .001$). A smaller percent of birth length for gestational age can be explained by these factors, with baseline clinical characteristics explaining 11% of the variance ($p < .001$), cord blood IGF-I alone explaining 20% ($p < .001$), cord blood IGF-II alone explaining 10% ($p < .001$), and the combination of clinical characteristics and the cord blood analytes explaining 27% ($p < .001$) of variability when analyzed in a multivariate regression.

Paternal anthropometric data was not available for our study participants, but we did have maternal height and pre-pregnancy weight for a small subset of subjects ($n = 37$, 20%). An exploratory analysis was performed in this subset, revealing that maternal height was associated with intact IGFBP-4 ($r = -0.263$, $p = .001$), but not with any of the other cord blood analytes measured, and maternal pre-pregnancy BMI was not associated with any of the cord blood analytes measured. When maternal height and pre-pregnancy BMI were added as covariates to the linear regression analyses in this subset, these variables did not modify the significance of the relationships between IGF-I, IGF-II, intact IGFBP-4, or IGFBP5 and birth weight Z-score, though the associations between total IGFBP-4, PAPP-A, and PAPP-A2 and birth weight Z-score did lose significance. Notably, neither maternal height nor pre-pregnancy BMI contributed significantly to the variability seen in birth weight and birth length Z-score, as the overall adjusted R^2 values did not increase when they were included in the model.

In addition to assessing linear associations with birth weight and birth length on a continuous scale, ANOVA and ANCOVA were also performed to assess for differences in the analytes among SGA, AGA, and LGA infants (Table 4). Cord blood IGF-I and IGF-II were both highest in the LGA group and lowest in the SGA ($p < .05$). In contrast, cord blood PAPP-A and PAPP-A2 were both highest in the SGA group and lowest in the LGA group ($p < .05$). After adjusting for gestational age, sex, race, delivery mode, maternal age, and Apgar scores, these differences remained significant across all groups for IGF-I and IGF-II ($p < .05$), and remained significant between the SGA group and the other two groups for both PAPP-A and PAPP-A2 ($p < .05$). There was no difference in total IGFBP-4 among the groups, but there was a trend towards intact IGFBP-4 being higher in the SGA group and lower in the LGA group, which was not significant once adjusted for other factors. There was also a trend towards IGFBP-5 being lower in the SGA group and higher in the LGA group, but this was not significant when adjusted for other factors.

4. Discussion

Identifying which factors determine a newborn's birth weight and birth length for gestational age is crucial to understanding the link between abnormal fetal growth and its long-term consequences. Maternal and neonatal characteristics alone, including maternal age, gestational age, sex, and race, explain a small percentage of the variability seen in birth weight and birth length for gestational age. We sought to identify and quantify the contributions of other factors to this variability, measuring several components of the IGF axis in cord blood in relation to growth indices for the first time.

Prior studies have established IGF-I and IGF-II as important factors in fetal growth with increasing levels of IGFs in both the maternal and fetal circulations throughout gestation [40,41]. Our study confirmed the established positive associations between both birth weight and birth length for gestational age and cord blood concentrations of IGF-I and

Table 2
Correlations between variables among all study participants.

		BW Z-score	Length Z-score	GA (weeks)	Sex	Race	Delivery Mode	Maternal Age (years)	Apgar 1 min.	Apgar 5 min.	IGF-I (ng/mL)	IGF-II (ng/mL)	T-IGFBP-4 (ng/mL)	I-IGFBP-4 (ng/mL)	IGFBP-5 (ng/mL)	PAPP –A (ng/mL)	PAPP -A2 (ng/mL)
BW	*r* or *ρ*		**.758**	**.377**	.109	-.125	.024	.142	**.169**	.140	**.588**	**.274**	.022	**-.251**	**.243**	**-.228**	**-.265**
Z-score	p		**.000**	**.000**	.141	.091	.746	.053	**.024**	.060	**.000**	**.001**	.772	**.001**	**.002**	**.002**	**.000**
Length	*r* or *ρ*	**.698**		**.371**	.015	-.095	-.029	.018	.041	.111	**.449**	**.313**	.003	**-.230**	**.182**	**-.170**	**-.286**
Z-score	p	**.000**		**.000**	.850	.218	.702	.813	.597	.149	**.000**	**.000**	.970	**.004**	**.023**	**.031**	**.000**
GA	*r* or *ρ*	**.340**	**.392**		.087	-.013	-.125	**-.155**	**.203**	**.231**	**.244**	**.300**	**-.199**	**-.163**	**.355**	-.038	**-.198**
(weeks)	p	**.000**	**.000**		.237	.863	.089	**.035**	**.006**	**.002**	**.001**	**.000**	**.008**	**.035**	**.000**	.616	**.009**
Sex	*r* or *ρ*	.085	.001	.106		.065	.036	.098	-.019	-.070	.019	**.196**	.039	.050	-.044	.059	.093
	p	.247	.991	.149		.383	.625	.183	.806	.352	.802	**.016**	.606	.523	.572	.438	.222
Race	*r* or *ρ*	-.129	-.085	-.006	.067		-.079	**-.342**	.045	.091	-.115	-.030	.080	.030	.085	.048	.113
	p	.082	.271	.933	.367		.289	**.000**	.549	.223	.132	.716	.290	.701	.277	.531	.138
Delivery	*r* or *ρ*	-.041	-.029	-.134	.036	-.108		**-.325**	-.027	-.029	.072	-.052	-.129	-.139	-.146	-.122	-.059
Mode	p	.578	.706	.070	.625	.145		**.000**	.716	.695	.343	.525	.086	.073	.059	.108	.441
Maternal Age	*r* or *ρ*	.140	.001	-.133	.106	**-.413**	**-.291**		-.057	.039	**.163**	-.131	-.114	-.143	-.092	-.140	-.129
(years)	p	.057	.989	.071	.151	**.000**	**.000**		.447	.606	**.032**	.108	.129	.066	.238	.065	.090
Apgar 1 min,	*r* or *ρ*	**.151**	.017	**.247**	-.022	.051	-.057	-.086		**.595**	**.214**	.044	-.142	-.131	.095	-.007	-.017
	p	**.044**	.829	**.001**	.767	.497	.452	.252		**.000**	**.005**	.594	.062	.099	.234	.928	.822
Apgar 5 min.	*r* or *ρ*	.131	.097	**.265**	-.117	.086	-.065	-.013	**.461**		.112	-.063	**-.189**	-.050	.048	**-.164**	**-.219**
	p	.080	.206	**.000**	.119	.251	.389	.862	**.000**		.146	.443	**.012**	.525	.548	**.032**	**.004**
IGF-I	*r* or *ρ*	**.556**	**.454**	**.202**	.009	-.117	.092	**-.184**	**.212**	.115		.141	-.131	**-.388**	**.169**	**-.273**	-.111
(ng/mL)	p	**.000**	**.000**	**.008**	.910	.125	.228	**.015**	**.006**	.135		.092	.089	**.000**	**.032**	**.000**	.154
IGF-II	*r* or *ρ*	**.343**	**.282**	**.278**	**.221**	-.022	-.044	-.114	.056	-.058	.116		**-.172**	-.051	**.243**	-.041	-.162
(ng/mL)	p	**.000**	**.001**	**.001**	**.006**	.790	.594	.160	.499	.484	.168		**.037**	.554	**.003**	.624	.052
T-IGFBP-4	*r* or *ρ*	-.009	.013	**-.182**	.032	.066	-.113	**-.112**	-.135	**-.218**	-.111	**-.170**		.086	.119	.019	**.189**
(ng/mL)	p	.905	.868	**.015**	.675	.383	.134	**.035**	.077	**.004**	.151	**.040**		.279	.131	.802	**.014**
I-IGFBP-4	*r* or *ρ*	**-.210**	**-.198**	-.135	.022	.053	-.129	**-.215**	-.019	-.066	**-.346**	.005	.068		**-.163**	.148	-.113
(ng/mL)	p	**.006**	**.013**	.081	.778	.495	.098	**.005**	.808	.402	**.000**	.958	.387		**.046**	.063	.158
IGFBP-5	*r* or *ρ*	**.225**	**.189**	**.324**	-.046	.086	-.148	-.104	**.157**	.101	**.158**	**.259**	.090	**-.175**		.076	.016
(ng/mL)	p	**.003**	**.018**	**.000**	.555	.268	.056	.179	**.048**	.202	**.046**	**.002**	.258	**.032**		.342	.844
PAPP-A	*r* or *ρ*	**-.216**	-.130	.087	.001	.136	**-.157**	-.144	.103	-.063	**-.265**	.031	-.023	.138	**.179**		**.155**
(ng/mL)	p	**.004**	.099	.255	.991	.075	**.038**	.056	.185	.417	**.001**	.711	.766	.084	**.023**		**.044**
PAPP-A2	*r* or *ρ*	**-.269**	**-.250**	**-.203**	**.150**	**.162**	-.052	-.105	-.011	-.142	-.062	**-.200**	**.207**	-.107	.010	.114	
(ng/mL)	p	**.000**	**.001**	**.007**	**.047**	**.032**	.496	.165	.891	.064	.430	**.016**	**.007**	.183	.905	.142	

Values displayed above grey line are Pearson *r* correlations (*r*), and values displayed below grey line are Spearman rank correlations (*ρ*).
Significant associations (*p*-value <.05) are highlighted in bold.
Abbreviations: BW, birth weight; GA, gestational age; IGF, insulin-like growth factor; T-IGFBP-4, total insulin-like growth factor binding protein-4; I-IGFBP-4, intact insulin-like growth factor binding protein-4; IGFBP-5, insulin-like growth factor binding protein-5; PAPP-A, pregnancy-associated plasma protein A; PAPP-A2, pregnancy-associated plasma protein A2.

IGF-II [22–31]. These associations remained significant after controlling for multiple maternal and neonatal characteristics, and regression analyses showed that cord blood IGF-I level explains a significant portion of the variability in both birth weight and birth length for gestational age. IGF-II, on the other hand, while positively associated with birth weight and birth length, explains much less of the variability.

In addition to IGF-I and IGF-II, we also measured two IGF binding proteins, IGFBP-4 and IGFBP-5. IGFBP-4 has only been measured in umbilical cord blood in one prior study and was found to be decreased in infants born to obese mothers, and IGFBP-5 has never before been measured in umbilical cord blood [42]. In our study total IGFBP-4 and IGFBP-5 were both positively associated with birth weight *Z*-score, even after adjusting for maternal and neonatal characteristics, similarly to what has been seen with IGFBP-3 [25–31]. However, perhaps more importantly, we were able to measure for the first time cord blood levels of intact IGFBP-4, which is the binding protein that has not been cleaved. Intact IGFBP-4 levels in cord blood were negatively associated with birth weight *Z*-score, which was an expected finding, as decreased levels of intact IGFBP-4 likely reflect higher availability of free IGF-I. Once adjusting for cord blood levels of IGF-I and IGF-II, this relationship lost significance. This is the first study to demonstrate the associations between these binding proteins and birth weight for gestational age. It is important for future prospective studies to measure not only total but also intact binding protein, to more fully understand the interactions among the IGF axis and their associations with fetal growth.

Finally, we measured the placental proteases that cleave these binding proteins. PAPP-A is an IGF-dependent protease that cleaves both IGFBP-4 and IGFBP-5, while PAPP-A2 only cleaves IGFBP-5 [43,44]. PAPP-A has been measured in umbilical cord blood in two prior studies, one of which found a trend towards an inverse relationship between cord blood PAPP-A and birth weight, and PAPP-A2 has never before been measured in umbilical cord blood [37,45,46]. Our study found significant inverse associations between both PAPP-A and PAPP-A2 levels in cord blood and birth weight *Z*-score, both of which remained significant after controlling for maternal and neonatal characteristics. We also found inverse relationships between cord blood PAPP-A and PAPP-A2 and birth length *Z*-score, though this association only remained significant for PAPP-A2 after adjusting for maternal and neonatal

Table 3a
Linear regression models using birth weight *Z*-score as dependent variable.

		Unadjusted	Adjusted for GA, race and sex	Adjusted for GA, race, sex, delivery mode, maternal age, and Apgar scores	Adjusted for GA, race, sex, delivery mode, maternal age, and Apgar scores, IGF-I and IGF-II
IGF-I (ng/mL)	B (95% CI)	0.753 (0.591, 0.915)	0.667 (0.511, 0.822)	0.651 (0.483, 0.818)	
	p-value	<0.001*	<0.001*	<0.001*	
IGF-II (ng/mL)	B (95% CI)	0.343 (0.149, 0.538)	0.197 (−0.002, 0.396)	0.222 (0.020, 0.425)	
	p-value	0.001*	0.052	0.031*	
Total IGFBP-4[a] (ng/mL)	B (95% CI)	0.040 (−0.148, 0.227)	0.132 (−0.048, 0.312)	0.204 (0.021, 0.387)	0.316 (0.139, 0.492)
	p-value	0.678	0.149	0.029*	0.001*
Intact IGFBP-4[a] (ng/mL)	B (95% CI)	−0.279 (−0.469, −0.089)	−0.202 (−0.387, −0.017)	−0.142 (−0.332, 0.048)	0.074 (−0.127, 0.276)
	p-value	0.004*	0.032*	0.143	0.466
IGFBP-5[a] (ng/mL)	B (95% CI)	0.328 (0.139, 0.516)	0.209 (0.019, 0.399)	0.226 (0.034, 0.417)	0.155 (−0.035, 0.344)
	p-value	0.001*	0.031*	0.021*	0.109
PAPP-A[a] (ng/mL)	B (95% CI)	−0.297 (−0.481, −0.112)	−0.294 (−0.465, −0.123)	−0.303 (−0.479, −0.127)	−0.194 (−0.412, 0.023)
	p-value	0.002*	0.001*	0.001*	0.079
PAPP-A2a (ng/mL)	B (95% CI)	−0.327 (−0.511, −0.143)	−0.214 (−0.394, −0.034)	−0.201 (−0.383, −0.018)	−0.171 (−0.35, 0.0080)
	p-value	0.001*	0.02*	0.032*	0.061

Relationships are expressed per standard deviation change in each analyte.
Abbreviations: GA, gestational age; IGF—I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGFBP-4, insulin-like growth factor binding protein-4; IFGBP-5, insulin-like growth factor binding protein-5; PAPP-A, pregnancy-associated plasma protein A; PAPP-A2, pregnancy-associated plasma protein A2.
[a] Denotes log transformation of variable.
* Highlights *p*-value <.05.

characteristics. We hypothesize that these inverse associations are due to IGF-related feedback mechanisms, with upregulation of the proteases in the setting of low levels of IGF in growth-restricted fetuses and down-regulation in the setting of high levels of IGF in well-grown fetuses. This hypothesis is supported by our finding that both associations lose significance when IGF-I and IGF-II are controlled for in the regression. Similarly to what was seen with IGFBP-4 and -5, the relative contribution of PAPP-A and PAPP-A2 to variation in birth weight and birth length are much less than that of IGF-I, and the significant upregulation of these proteases in growth-restricted fetuses is unable to compensate and restore normal growth.

It is unclear from our study whether maternal pre-pregnancy weight and/or gestational weight gain influence cord blood levels of the binding proteins and proteases. For the subset of patients for whom we did have maternal pre-pregnancy BMI, we found that this variable did not affect the relationships between the cord blood analytes and growth indices. It is difficult to draw any firm conclusions based on such a small number of patients ($n = 37$), but it highlights the importance of

Table 3b
Linear regression models using length *Z*-score as dependent variable.

		Unadjusted	Adjusted for GA, race and sex	Adjusted for GA, race, sex, delivery mode, maternal age, and Apgar scores
IGF-I (ng/mL)	B (95% CI)	0.641 (0.442, 0.840)	0.518 (0.321, 0.715)	0.612 (0.402, 0.822)
	p-value	<0.001*	<0.001*	<0.001*
IGF-II (ng/mL)	B (95% CI)	0.451 (0.227, 0.676)	0.290 (0.061, 0.520)	0.298 (0.046, 0.531)
	p-value	<0.001*	0.014*	0.02*
Total IGFBP-4[a] (ng/mL)	B (95% CI)	0.005 (−0.214, 0.223)	0.098 (−0.113, 0.309)	0.130 (−0.092, 0.352)
	p-value	0.967	0.361	0.25
Intact IGFBP-4[a] (ng/mL)	B (95% CI)	−0.292 (−0.514, −0.070)	−0.208 (−0.424, 0.008)	−0.207 (−0.439, 0.024)
	p-value	0.01*	0.059	0.079
IGFBP-5[a] (ng/mL)	B (95% CI)	0.271 (0.044, 0.498)	0.134 (−0.098, 0.367)	0.134 (−0.105, 0.374)
	p-value	0.02*	0.254	0.269
PAPP-A[a] (ng/mL)	B (95% CI)	−0.224 (−0.446, −0.003)	−0.207 (−0.412, −0.001)	−0.205 (−0.424, 0.014)
	p-value	0.047*	0.049*	0.067
PAPP-A2[a] (ng/mL)	B (95% CI)	−0.444 (−0.661, −0.227)	−0.296 (−0.515, −0.077)	−0.279 (−0.507, −0.051)
	p-value	<0.001*	0.008*	0.017*

Relationships are expressed per standard deviation change in each analyte.
Abbreviations: GA, gestational age; IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGFBP-4, insulin-like growth factor binding protein-4; IFGBP-5, insulin-like growth factor binding protein-5; PAPP-A, pregnancy-associated plasma protein A; PAPP-A2, pregnancy-associated plasma protein A2.
[a] denotes log transformation of variable.
* highlights p-value <.05.

Table 4
Comparison of cord blood analytes among SGA, AGA, and LGA infants.

	All ($N = 185$)	SGA ($N = 37$)	AGA ($N = 111$)	LGA ($N = 37$)	*p*-value (ANOVA)	*p*-value (ANCOVA)
IGF-I (ng/mL)	102.9 ± 3.6	51.1 ± 6.9[b,c]	108.5 ± 3.7[a,c]	138.3 ± 6.0[a,b]	<0.001*	<0.001*
IGF-II (ng/mL)	178.9 ± 3.2	161.3 ± 5.7[b,c]	177.4 ± 4.4[a,c]	205.2 ± 6.6[a,b]	<0.001*	0.003*
Total IGFBP-4 (ng/mL)	196.8 ± 3.1	195.7 ± 6.4	197.8 ± 4.1	195.0 ± 6.5	0.921	0.316
Intact IGFBP-4 (ng/mL)	91.9 ± 3.4	108.7 ± 11.2[c]	91.9 ± 3.8	75.4 ± 5.6[a]	0.009*	0.064
IGFBP-5 (ng/mL)	425 ± 6.9	388.9 ± 16.1[c]	428.5 ± 8.7	449.3 ± 14.5[a]	0.018*	0.173
PAPP-A (ng/mL)	1.2 ± 0.05	1.5 ± 0.2[b,c]	1.2 ± 0.1[a]	1.1 ± 0.1[a]	0.018*	0.013*
PAPP-A2 (ng/mL)	2.2 ± 0.08	2.8 ± 0.3[b,c]	2.2 ± 0.1[a]	1.8 ± 0.2[a]	<0.001*	0.01*

Values reported as mean ± standard error of the mean. *P*-values reported for both ANOVA and ANCOVA. ANCOVA analysis included the following covariates: gestational age, sex, race, delivery mode, maternal age, and Apgar scores. Asterisk (*) highlights *p*-value <.05; a: different from SGA ($p < .05$), b: different from AGA ($p < .05$), c: different from LGA ($p < .05$).
Abbreviations: GA, gestational age; IGF—I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGFBP-4, insulin-like growth factor binding protein-4; IFGBP-5, insulin-like growth factor binding protein-5; PAPP-A, pregnancy-associated plasma protein A; PAPP-A2, pregnancy-associated plasma protein A2.

including detailed maternal information in future studies to more comprehensively understand their influence on fetal growth regulation. Twin studies have suggested that genetics play an important role with much stronger correlations between cord blood IGF-I levels in monozygotic twins than dizygotic twins [47]. We were unable to address this question in our study, but in the small subset of patients for whom we had maternal height, we did find that maternal height and cord blood IGF-I levels were not associated with one another. This emphasizes the need for further exploration of the influences of genetics, epigenetics, and the environment on fetal IGF-I.

Strengths of the study are its adequate size and the inclusion of some molecules that have never been studied in cord blood in the past, specifically IGFBP-5 and PAPP-A2. Laboratory analyses were performed by blinded personnel not aware of the hypotheses, which increases the validity of the data reported herein. Bias was addressed by measuring several potential confounders and adjusting for them, whereas chance was addressed by performing appropriate bivariate and multivariate statistical analyses. A major limitation of this case-control study is the lack of paternal and the limited availability of maternal anthropometric data, which are important factors in analyzing fetal growth and may also influence levels of cord blood analytes. While we were able to do subset analyses with maternal height and BMI for 20% of our subjects, we did not have any information on gestational weight gain. Another limitation is the difference in gestational age among the three groups with a significantly greater number of late-preterm infants in the SGA group and fewer in the LGA group. While we included gestational age as a covariate in our analysis to control for the difference, future larger studies could better evaluate this topic. Finally, length measurements at birth are often inaccurate, but, notably, such errors are random; random misclassification may have affected some of the associations analyzed by suppressing effect estimates and increasing the relevant *p*-values but could not have resulted in statistically significant results such as those shown herein.

5. Conclusion

This is the first study to measure IGFBP-4, IGFBP-5, and the proteases that cleave these binding proteins (PAPP-A and PAPPA-2) in cord blood in relation to birth weight and birth length for gestational age, providing novel information about the relationship between the IGF axis and fetal growth regulation. We found positive associations between both total IGFBP4- and IGFBP-5 and birth weight and length *Z*-scores but negative associations between intact IGFBP-4 and birth weight and length *Z*-score, highlighting the importance of measuring both total and intact binding protein in future studies. We found negative associations between PAPP-A and PAPP-A2 and birth weight and length *Z*-scores, which we hypothesize is due to compensatory IGF-related regulation of these proteases. The study also reinforces that IGF-I is the major contributing factor to variation in birth weight and birth length for gestational age and emphasizes the need for further studies to understand what determines differences in IGF-I levels, the contributions of free IGF-I vis-a-vis total IGF-I and its binding proteins, and whether such differences during pregnancy, in cord blood and/or in the circulation early in life can be linked to appropriate fetal and neonatal growth as well as short and/or long-term fetal and neonatal growth dysregulation. These data need to be confirmed in larger prospective studies, and, if confirmed and extended, may provide novel diagnostic and therapeutic tools in the not so distant future.

Acknowledgements

We acknowledge Nikolaos Perakakis, MD, PhD and Natia Peradze, PhD for oversight and assistance with ELISAs. We also thank Eleni Pilitsi, MD and Konstantinos Stefanakis for help with sample organization, and we thank Kyoung Joung, MD for her support with statistical analysis.

Funding

The collection of study samples was funded in part by the Gerber Foundation and assays used herein were provided in part by Ansh Labs.

Declaration of competing interest

Authors A.K., B.K., and G.V.S. are all employees of Ansh Labs, which is the manufacturer of the ELISA kits used in this study. CSM is a consultant to Ansh Labs.

Author contributions

B.D. performed experiments, analyzed the data, and wrote the manuscript. A.K., B.K., S.V.G, and A.E.P all performed experiments. Z.M. collected umbilical cord blood samples and clinical data on study participants. O.M.F made major contributions to statistical analysis and edited the manuscript. H.C. helped with study design, oversaw collection of umbilical cord blood samples and edited manuscript. C.S.M. designed the study, directed data analysis and interpretation, and edited the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.153959.

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