REGULAR ARTICLE

Neonatal IGF-1/IGFBP-1 axis and retinopathy of prematurity are associated with increased blood pressure in preterm children

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ABSTRACT

Aim: Preterm children are at risk of developing increased blood pressure (BP). We evaluated possible associations between BP, early insulin-like growth factor-1 (IGF-1) and IGF-binding protein-1 (IGFBP-1) levels and retinopathy of prematurity (ROP) in preterm children.

Methods: The study included 32 infants: median gestational age 28.1 weeks (range 24.6–31.3) and birthweight standard deviation scores (SDS) ($\pm$SD) 1.0 $\pm$ 2.7. IGF-1 and IGFBP-1 at postnatal weeks 32.6–34.6 and ROP stages were established after birth. BP was measured at the age of 4 years. The ratio (IGF-1)^2/IGFBP-1 was created to investigate the influence of both IGF-1 and IGFBP-1 to later BP.

Results: Diastolic BP correlated with IGFBP-1, inversely correlated with IGF-1 and IGF-1^2/IGFBP-1 (r = −0.71, p < 0.0001) and positively correlated with catch-up growth velocity from lowest weight SDS to age 36.5 weeks (r = 0.48, p < 0.01), independent of gestational age. Children with moderate-to-severe ROP as neonates had higher mean arterial BP [78 ($\pm$95%CI 74–83) vs 71 ($\pm$95%CI 68–75) mm Hg, p < 0.05] adjusted for gestational age and birthweight SDS compared to children diagnosed with no to mild ROP.

Conclusion: Low neonatal IGF-1^2/IGFBP-1 and severe ROP were associated with higher BP in 4-year-old children born very preterm and may thus predict future cardiovascular morbidity.

INTRODUCTION

Preterm birth has been associated with an increased risk of high blood pressure (BP) in childhood (1) and this relationship seems to persist into adulthood (2,3). Early catch-up growth (4), very premature birth (5) and preterm infants born small for gestational age (2) are factors that appear to strengthen the association.

In 10-year-old control children, increased retinal arteriolar tortuosity has been found to correlate to elevated systolic and diastolic blood pressure (6). Increased retinal vessel tortuosity has been described in preterm children (7) as well as in adult ex-preterm subjects, (8,9), where it was associated with increased systolic blood pressure (3).

Retinopathy of prematurity (ROP) is a blinding disease that may develop during the early weeks after preterm birth. ROP is characterised by an arrest in normal retinal vascular development. The disease is initiated by a decrease in

vascular growth. The severity of neovascularisation and the stage of ROP are dependent on the amount of avascular retinal tissue present in the infant. Insulin-like growth factor-1 (IGF-1) has been shown to be crucial for retinal vessel development (10) and the development of ROP has been associated with decreased IGF-1 levels (10).

IGF-1 levels in the circulatory system are mainly derived from the liver. Its production, in children and adults, is stimulated by growth hormone. Malnutrition, especially protein deficiency, leads to low levels of IGF-1 (11). The regulators of IGF-1 and IGF-binding protein-1 (IGFBP-1)

Abbreviations
BP, Blood pressure; IGF-1, Insulin-like growth factor; IGFBP-1, Insulin-like growth factor binding protein-1; PMA, Postmenstrual age; ROP, Retinopathy of prematurity; SDS, Standard deviation scores; VEGF, Vascular endothelium growth factor.

Key notes
- Preterm infants are at risk of developing increased blood pressure.
- Our study of 32 preterm infants found an association between low postnatal IGF-1 and high IGFBP-1 serum levels and increased diastolic blood pressure at the age of 4 years.
- We also found that infants diagnosed with more severe retinopathy of prematurity in neonatal life had increased mean arterial blood pressure at follow-up.

during fetal life are largely unknown. In contrast, it is known that malnutrition gives rise to high levels of IGFBP-1, which inhibit the activity of IGF-1 in target tissues (12).

Whether more severe retinal vessel abnormalities during early life in preterm infants may correlate to functional vessel impairment changes later in life has not to our knowledge been evaluated previously. Our aim was to investigate a possible association between early IGF-1/ IGFBP-1 and ROP and blood pressure outcome in a group of preterm children at the age of approximately 4 years. Growth parameters during early infancy were carefully recorded for these children.

MATERIALS AND METHODS
Subjects
From an initial cohort of 74 infants, 65 subjects were followed from expected birth to five-years-of-age. The initial study included infants of postmenstrual age (PMA) < 32 weeks who were born at the Queen Silvia's Children's Hospital, Gothenburg, Sweden between February 2001 and April 2002. Fifty-nine infants in the follow-up cohort had postnatal IGF-1 levels measured weekly from birth to approximately 40 weeks PMA. At the age of 4 years, 33 children took part in this follow-up study with blood pressure measurements. Thirty-two subjects from the follow-up study cohort had either moved or refused blood pressure measurement. One infant, born in week 24 PMA, was subjected to severe extrauterine growth retardation due to necrotising enterocolitis. IGFBP-1 was missing and the subject was excluded. In total, 32 children were included in this study.

All infants were hospitalised in a neonatal intensive care unit and nourished according to the routines for premature infants (13).

Morbidity examination: ROP evaluation
Retinopathy of prematurity was classified according to International classification (14). Subjects were divided into stage 1 (demarcation line), stage 2 (ridge), stage 3 (ridge with extraretinal fibrovascular proliferations) or stage 4 (subtotal retinal detachment). In all gestational weeks, each infant was classified according to the most advanced ROP stage observed. The eyes of each infant were examined according to a routine protocol and dilated eye fundus examinations were performed once or twice weekly from the chronological age of 5–6 weeks until the eye was fully vascularised, or until the condition was considered stable. The procedure has been described in detail elsewhere (15). The study was retrospective and the highest ROP stage attained by an infant constituted the basis for the categorisation: for example, if an eye was stage 2 for 1 week and then went back to stage 1, this subject was categorised as ROP 2. In cases of asymmetric disease, the highest ROP stage was used for classification.

In this study, subjects were divided into two groups according to ROP staging: no-to-mild ROP (≤stage 1) (n = 18), or moderate-to-severe ROP (≥stage 2) (n = 14)

(Tables 1 and 3). The rationale for this categorisation was that it would be difficult to differentiate between ROP stage 0 and stage 1. At ROP stage 2, transparent retinal vessel changes occur. Two of four subjects with ROP stage 3 underwent laser treatment.

Maternal characteristics and neonatal illness
Table 1 contains details of some of the maternal characteristics. Although information about the use of antenatal corticosteroids was missing in some cases, there were no statistical differences between the no-to-mild and moderate-to-severe ROP groups with regard to antenatal corticosteroid administration (p = 0.31), preeclampsia (p = 0.73) or target height (p = 0.39) (Table 1). Three mothers in each ROP group had had preeclampsia during pregnancy. One

Table 1 Maternal and birth characteristics

	Preterm children n = 32	Missing values
Maternal Characteristics		
Antenatal corticosteriod, n	20	7
Maternal preeclampsia, n	6	
Target height SDS	−0.10 (−1.5–1.5)	
Birth Characteristics		
Sex, girls/boys, n	16/16	
Gestational age at birth, weeks	28.1 (24.6–31.3)	
Birthweight, grams	980 (530–2015)	
Birthweight SDS	−0.96 (−5.02–1.76)	
BPD, n	5	
Sepsis, n	6	
PDA, n	8	
ROP stages 0/1/2/3, n	15/3/10/4	
Time in days to lowest weight SDS	21 (5–34)	
Lowest weight SDS	−2.95 (−6.25–(−0.77))	
Time in days from lowest weight SDS to postmenstrual week 36.5	43 (23–63)	
Catch-up weight SDS from lowest weight SDS to postmenstrual week 36.5	1.16 (0.02–1.72)	
Catch-up weight SDS/day from lowest weight SDS to postmenstrual week 36.5	0.027 (0.0–0.064)	
Catch-up in gram/day from lowest weight SDS to postmenstrual week 36.5	28 (19–35)	
Mean IGF-1, µg/L, at postmenstrual week 32.6–34.6	37 (32–42)	3
Mean IGFBP-1, µg/L, at postmenstrual week 32.6–34.6	36 (27–48)	3
IGF-1^2/IGFBP-1 ratio	38 (23–61)	3

Maternal characteristics and birth characteristics at postnatal weeks 32.6–34.6. Values represent percentages or numbers (maternal and infant characteristics) or medians (ranges), except for IGF-1 and IGFBP-1 serum values and the IGF-1^2/IGFBP-1 ratio, which are presented as geometrical mean (±95% confidence interval).
BPD = Bronchopulmonary dysplasia; PDA = Patent ductus arteriosus; IGF-1 = Insulin-like growth factor 1; IGFBP-1 = Insulin-like growth factor binding protein 1; SDS = Standard deviation scores.

mother had type 1 diabetes. None of the mothers developed essential hypertension or gestational diabetes during pregnancy.

There were no differences in Apgar scores at 1, 5 or 10 min after birth between the two ROP groups (data not shown). There was a tendency towards a higher incidence of bronchopulmonary dysplasia in the moderate-to-severe ROP group compared to the no-to-mild ROP group (p = 0.07, Pearson's χ^2 test). A diagnosis of sepsis was based on clinical judgement, including evaluation of white blood count, C-reactive protein and blood culture. The incidences of sepsis and patent ductus arteriosus were not significantly different between the two groups (p = 0.20 and p = 0.66 respectively).

Data collection

GA for the infants was determined by ultrasound during early pregnancy. Standard deviation scores (SDS) are based on the Swedish reference curve at birth (16) and at the time of the study (17) (Tables 1 and 2).

Neonatal and anthropometric characteristics from birth to GW 40 were recorded at the neonatal unit and served as the basis for our early anthropometrical data. Maternal medical history was obtained at the time of the initial study. Maternal/paternal anthropometric characteristics were obtained by written/oral questions asked prior to or at the time of the initial study or at hospital visits during the follow-up study. Catch-up growth velocity in weight SDS/day was calculated according to the difference from lowest weight SDS (nadir) to weight SDS at approximately week 36.5 divided by the number of days between the two estimations (Tables 1 and 3 and Fig. 1). Week 36.5 rather than week 40 was applied because some infants were discharged at approximately 36.5 weeks PMA and growth anthropometrics at 40 weeks were missing.

The same physician (JS) performed all of the physical examinations and blood pressure recordings at follow-up without knowing the ROP stage of the child during

neonatal life. At the time of the study, all children were in good health. The Ethics Committee of the Medical Faculties at Gothenburg University approved the study. The parents of the study also provided informed consent.

Blood pressure measurements

The blood pressure of each participant was recorded by one investigator, after the children had rested for 15 minutes in a horizontal position, using the same automatic blood pressure recorder (Welch Allyn, Skaneateles Falls, NY, USA). Blood pressure was calculated as the mean of three recordings (n = 16) or as a single measurement (n = 16) due to a change in the protocol. The ratio of single to several recordings was 9:9 in the no-to-mild ROP group and 7:7 in the moderate-to-severe ROP group. Pulse pressure (Table 2) was derived from the difference between each individual's mean SP and DP. Mean arterial blood pressure (MAP) was calculated according to the following formula: DP + 1/3(SP−DP) (Table 2 and Fig. 2B).

Blood sampling

The laboratory methods for determining IGF-1 and IGFBP-1 levels have been described previously (13). The IGF-1 and IGFBP-1 levels presented in Table 1 represent either the mean of several (for 21 infants) or single (for nine infants) blood sample measurements at GW 32.6–34.6, corresponding to gestational days 228–242. This range was chosen to enable as many subjects as possible to be included. In two subjects, blood samplings from early neonatal weeks were missing, and in one subject, measurements were only performed twice during neonatal life and not within the range of the blood-sampling period.

Statistics

Anthropometric data for the individuals are presented as the median (range), and blood pressure measurements as the mean ±SD. Because IGF-1 and IGFBP-1 values were not normally distributed in the subjects, values were log-transformed and are presented as geometrical mean ± 95% confidence interval (CI). The algorithm (IGF-1)^2/IGFBP-1 was created because in multiple regression analysis with diastolic BP as the dependent variable and IGF-1 and IGFBP-1, the coefficient was 2-fold higher for IGF-1 than for IGFBP-1 (diastolic BP = −104 997 (logIGF-1) + 48 636 (logIGFBP-1) + 804 013). This equation was rounded to the proposed ratio: (log IGF-1 multiplied by 2)/(log IGFBP-1) which equals IGF-1^2/IGFBP-1 if values are not log-transformed (Tables 1 and 3 and Fig. 3).

The comparison between two independent groups was assessed by analysis of variance *t*-test. Pearson's χ^2-test was used to compare numerical values relating to maternal and birth characteristics in subjects. Covariance analyses to adjust for gestational age and birthweight SDS were performed for blood pressure measurements, with no-to-mild ROP and moderate-to-severe ROP as categorical variables. To investigate the impact of different exposures in relation to blood pressure, stepwise multiple analyses were performed with systolic BP, diastolic BP, mean arterial

Table 2 Age, height and body mass index of the children at the time of the study. Values represent medians (ranges) except for blood pressure measurements at follow-up, which are presented as geometrical mean (±95% CI)

Characteristics at follow-up	
Age, years	4.7 (4.0–5.5)
Weight, kg	17.3 (14.3–24.9)
Weight SDS	−0.62 (−2.23–2.25)
Height, cm	108 (99–117)
Height SDS	−0.22 (−1.55–1.73)
BMI, kg/m^2	15.0 (13.0–21.4)
Blood pressure, mmHg	
Mean systolic	103 (100–106)
Mean diastolic	60 (57–63)
Mean arterial pressure	74 (72–77)
Pulse pressure	44 (39–46)

BMI = Body mass index.

Table 3 Catch-up growth during early growth phases, mean serum IGF1 and IGFBP1 and the IGF1^2/IGFBP1 ratio at postnatal week 32.6–34.6 for the preterm children divided into no-to-mild ROP (n = 18) or moderate-to-severe ROP (n = 14) during neonatal life as well as blood pressure data at the time of the study. Values represent geometrical means (± 95% CI) except for gender and catch-up growth values that are presented as percentage and median (range) respectively. Blood pressure data for the investigated children were adjusted for GA and birthweight SDS (see Methods)

| | Groups | | | | |
	No-to-mild ROP	Missing values	Moderate-to-severe ROP	Missing values	p value
Characteristics after birth					
Sex, girls/boys, n	8/10		6/8		0.48†
Catch-up weight SDS from lowest weight SDS to postmenstrual week 36.5	1.12 (0.63–1.65)		1.22 (0.73–1.72)		0.49
Catch-up weight SDS/day from lowest weight SDS to postmenstrual week 36.5	0.025 (0.0–0.054)		0.033 (0.013–0.065)		0.11
Mean IGF-1, µg/L at postmenstrual week 32.6–34.6	42 (36–47)	1	30 (23–40)	2	*0.019*
Mean IGFBP-1, µg/L at postmenstrual week 32.6–34.6	33 (25–45)	1	49 (33–74)	2	0.099
IGF-1^2/IGFBP-1 ratio	52 (34–79)	1	18 (8–43)	2	*0.016*
Blood pressure, mmHg at follow-up					
Systolic	100 (96–104)		106 (101–112)		0.088‡
Diastolic	57 (53–61)		64 (59–69)		0.064‡
Mean arterial	72 (68–75)		78 (74–82)		*0.027‡*

IGF-1 = Insulin-like growth factor 1; IGFBP-1 = Insulin-like growth factor binding protein 1; ROP = Retinopathy of prematurity; SDS = Standard deviation scores. According to analysis of variance (ANOVA; t-test): †according to χ^2-test or ‡according to analysis of co-variances (ANCOVA) for the indicated group versus the other group. Significant p-values <0.05 are in italics.
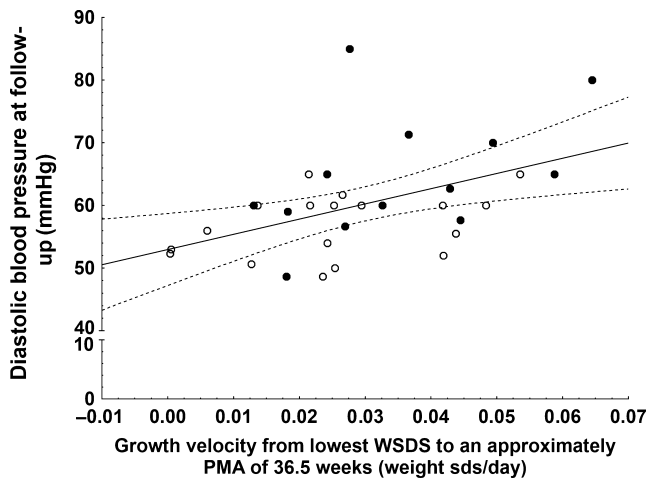

Figure 1 Catch-up growth velocity in weight SDS/day from lowest weight SDS to PMA of 36.5 weeks in relation to diastolic blood pressure at follow-up. The symbols indicate preterms with no-to-mild ROP (○) and preterms with moderate-to-severe ROP (●). The regression line (—) ± 95% confidence interval (- - -) indicates a significant positive correlation in the whole group (r = 0.48, p = 0.007, n = 31).

BP and pulse pressure as dependent variables. Gestational age, growth velocity from lowest weight SDS to PMA of approximately 36.5 weeks, the algorithm IGF-1^2/IGFBP-1, and ROP category were included as independent variables p < 0.05 was considered significant, and a tendency towards significance was used for the p range 0.1 to > 0.05. Statistical analyses were performed using Statistical Stat Soft, version 10 (Tulsa, OK, USA).

RESULTS
Anthropometric data
Basal characteristics at birth and follow-up
Basal characteristics at birth and at follow-up of the 32 children are shown in Table 1. Subjects diagnosed with a moderate-to-severe ROP after birth had lower median gestational age (range) [26.2 (24.7–30.9) vs 28.3 (25.7–31.1) weeks, p = 0.001] and lower median birthweight SDS (range) [−1.77 (−4.41–0.38) vs −0.86 (−3.41–1.76), p = 0.022] at birth and a longer time to lowest median weight SDS (range) [26 (7–34) vs 14 (5–29) days, p = 0.002]. Subjects with moderate-to-severe ROP did not differ from the no-to-mild ROP subjects in weight velocity in weight SDS/day from lowest weight SDS to a PMA of approximately 36.5 weeks (p = 0.11) (Table 1). At follow-up, subjects regarded as moderate-to-severe ROP postnatally had a tendency to lower corrected median age (range) [4.63 (3.95–4.80) vs 4.82 (4.26–5.49) years, p = 0.07] (Table 2), but no differences in weight SDS, height SDS or body mass index were seen between subjects classified as
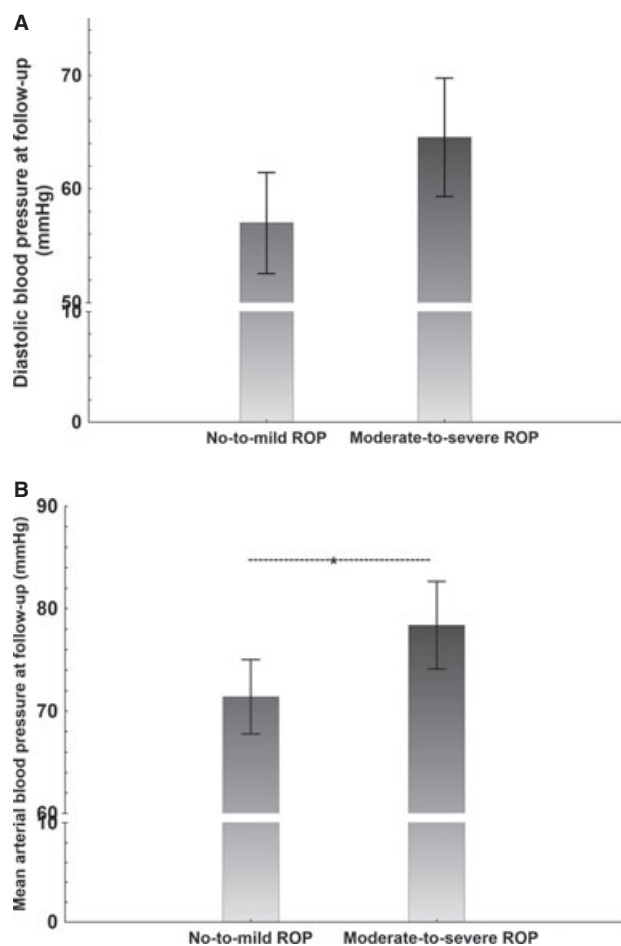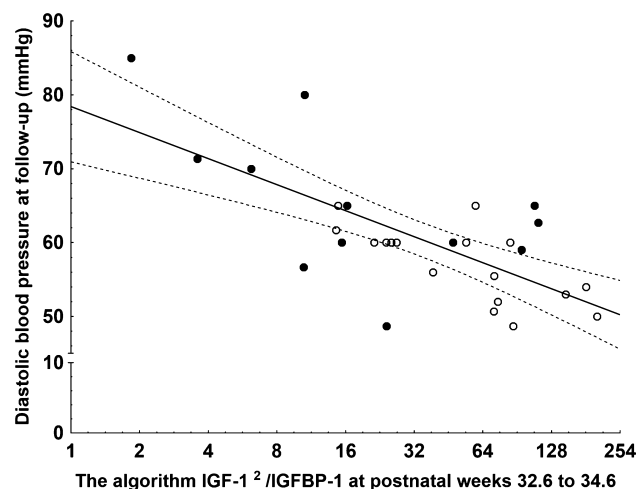
Figure 3 The algorithm/ratio IGF-1²/IGFBP-1 in relation to diastolic blood pressure at follow-up. Symbols as Figure 1. The IGF-1 and IGFBP-1 values correspond to mean levels at a mean PMA of 33.5 weeks (range 32.6–34.6). The regression line (—) ± 95% confidence interval (- - -) indicates a significant negative correlation in the whole group (r = −0.71, p < 0.0001, n = 29).

Figure 2 Diastolic and mean arterial blood pressure adjusted for age at birth and birthweight SDS. Vertical bars indicate the mean and relative blood pressure values ± 95% confidence interval in the no-to-mild ROP group versus the moderate-to-severe ROP group. There was a tendency in diastolic blood pressure (p = 0.064) (A) and a significant difference in mean arterial blood pressure between the groups (*p = 0.027) (B).

no-to-mild ROP or moderate-to-severe ROP after birth (p = 0.33, p = 0.70 and p = 0.13 respectively) (Table 2).

IGF-1 and IGFBP-1 levels
Mean levels of IGF-1 and IGFBP-1 at PMA 32.6–34.6 weeks are shown in Table 1. Mean serum levels of IGF-1 were significantly lower in infants with moderate-to-severe ROP at PMA 32.6–34.6 weeks compared to infants with no-to-mild ROP (p = 0.019, Table 3). In contrast, mean serum levels of IGFBP-1 tended to be higher in infants with moderate-to-severe ROP at a PMA of 32.6–34.6 weeks (p = 0.099, Table 3). The proposed ratio (see Materials and Methods) IGF-1²/IGFBP-1 was significantly lower in the moderate-to-severe ROP group compared to the no-to-mild ROP group (p = 0.016, Table 3).

Blood pressure measurement
Blood pressure recordings are shown in Tables 1 and 3. Infants with moderate-to-severe ROP had a tendency towards higher systolic BP (p = 0.09) and diastolic BP (p = 0.06) and a significantly higher mean arterial BP (p = 0.027) when gestational age and birthweight SDS were adjusted for in covariance analysis (ANCOVA test) (Table 3, Fig. 2A and B). Diastolic BP at follow-up correlated positively with catch-up velocity in weight SDS/day from lowest weight SDS to PMA 36.5 weeks (r = 0.48, p = 0.007, n = 31) (Fig. 1), independent of gestational age (p = 0.15). Postnatal levels of IGF-1 and IGFBP-1 were both correlated with diastolic BP at follow-up (r = −0.63, p < 0.001 and r = 0.57, p = 0.001, respectively). Diastolic BP at follow-up was inversely correlated with the algorithm IGF-1²/IGFBP-1 (r = −0.71, p < 0.0001, n = 29) (Fig. 3). In multiple regression analysis with diastolic BP as the dependent variable and with the algorithm IGF-1²/IGFBP-1 and gestational age as independent variables, gestational age had no influence on diastolic BP (p = 0.97) (adjusted r^2 = 0.49). Systolic BP at follow-up did not correlate with catch-up velocity in weight SDS/day from lowest weight SDS to PMA 36.5 weeks (r = 0.18, p = 0.33) or with the algorithm IGF-1²/IGFBP-1 (r = −0.22, p = 0.26). Gestational age had a more positive influence on the ratio IGF-1²/IGFBP-1 for infants in the no-to-mild ROP group (r = 0.38, p = 0.14) compared to infants in the moderate-to-severe ROP group (r = −0.01, p = 0.98). The algorithm IGF-1²/IGFBP-1 was positively correlated with pulse pressure (r = 0.44, p = 0.01).

Multiple regression analysis with SP variability as dependent variable
In forward stepwise multiple regression analysis with gestational age, growth velocity (weight SDS/day from lowest weight SDS to PMA 36.5 weeks) and ROP category, only ROP category was included as a dependent variable in

the analysis ($\beta = 0.43$, p = 0.01). ROP category explained (adjusted R^2) 16% of the systolic BP variability in the whole group (n = 31, p = 0.01). The ratio of early IGF-1^2/IGFBP-1 did not contribute to explaining the systolic BP variability within the whole group.

Multiple regression analysis with DP variability as dependent variable

Stepwise multiple regression analysis was conducted with gestational age, growth velocity (weight SDS/day from lowest weight SDS to PMA 36.5 weeks) and the ratio of early IGF-1^2/IGFBP-1. The IGF-1^2/IGFBP-1 ratio ($\beta = -0.63$, p < 0.0001) and growth velocity weight SDS/day ($\beta = 0.33$, p < 0.05) explained (adjusted R^2) 57% of the diastolic BP variability in the whole group (n = 29, p < 0.0003). In this analysis, gestational age did not contribute to explaining the diastolic BP variability within the whole group.

Multiple regression analysis with MAP variability as dependent variable

Multiple regression analysis was conducted with gestational age, the ratio of early IGF-1^2/IGFBP-1 and ROP category. The ratio IGF-1^2/IGFBP-1 ($\beta = -0.50$, p < 0.01) and ROP category ($\beta = 0.37$, p < 0.05) explained (adjusted R^2) 44% of the mean arterial BP variability in the whole group (n = 29, p < 0.0005). Gestational age did not add any further information (p = 0.64).

Multiple regression analysis with pulse pressure variability as dependent variable

In multiple regression analysis with pulse pressure as the dependent variable and the algorithm IGF-1^2/IGFBP-1 and gestational age, the ratio IGF-1^2/IGFBP-1 was included as a dependent variable ($\beta = 0.49$, p = 0.01), adjusted $R^2 = 0.15$, but not gestational age (p = 0.43). Adding ROP category and growth velocity (weight SDS/day) increased adjusted the R^2 to 0.18. Neither of these two variables was included as significant dependent variables (p = 0.23 and p = 0.24 respectively).

DISCUSSION

We found that preterm children at 4-years-of-age diagnosed with more severe ROP grades early in life had increased mean arterial blood pressure, adjusted for age at birth and birthweight SDS, compared to children with no or mild ROP in early life. At a postmenstrual age of 32.6–34.6 weeks, infants diagnosed with moderate-to-severe ROP exhibited lower IGF-1 levels and a tendency towards higher IGFBP-1 compared to subjects diagnosed with no-to-mild ROP. The diastolic blood pressure at 4-years-of-age was inversely correlated with IGF-1 levels and positively correlated with IGFBP-1 levels at a PMA of 32.6–34.6 weeks. To combine the influence of both of these variables on later diastolic BP, the ratio IGF-1^2/IGFBP-1 was created. The combination of the ratio IGF-1^2/IGFBP-1 at PMA 32.6–34.6 weeks and catch-up growth velocity

from lowest weight SDS to PMA 36.5 weeks explained 50% of the diastolic blood pressure variability at follow-up.

IGF-1 has been shown to be a permissive factor for vascular endothelial growth factor (VEGF) (18), which is a vessel recruiter in the retina (19) as well as in other organs, such as the lung and the brain. In the retina, during early life when IGF-1 is low, especially in more-severe ROP (15), VEGF signaling is depressed, but becomes upregulated despite low IGF-1 to recruit vessels in the avascular retina. When serum IGF-1 increases and rises over a certain threshold, this may lead to relative VEGF abundance and induce pathological retinal neovascularisation, a proposed scenario behind ROP development. The finding of increased blood pressure at age 4 years indicates that ROP changes in neonatal life are related to vessel disease. In another study, severe ROP correlated independently with poor outcome (20) and was a predictor of death or major disability at 11 years of age in children born preterm before 26 gestational weeks.

In one study, (21) the birthweight of infants was positively associated with maternal IGF-1 levels and inversely related to maternal IGFBP-1 levels during the last trimester. Furthermore, lower IGFBP-1 levels were associated with catch-up growth in preterm infants; (22) the IGF-1/IGFBP-1 ratio has been applied in growth-rate studies (11). We are not aware of any studies in which our ratio, IGF-1^2/IGFBP-1, was used to predict diastolic blood pressure. The present finding of a correlation between this ratio and blood pressure later in life raises the question of whether the IGF-1/IGFBP-1 system in preterm infants may influence future diastolic blood pressure. In our group of former preterm infants, we calculated the mean levels of IGF-1 and IGFBP-1 at several postnatal time intervals: at postmenstrual age 27.5–29.9, 30–32.5, 32.6–34.6 and 34.7–37.2 weeks. IGF-1 and IG-FBP-1 levels at PMA 32.6–34.6 weeks showed by far the strongest correlation with later diastolic blood pressure. IGF-1 levels during this postnatal period in preterm infants seem to be important for attaining a normal diastolic blood pressure later in life. Our results indicate an association between fetal IGF-1 levels during this time and later endothelial cell function and diastolic blood pressure. Very low IGF-1 levels at this period appear to be associated with elevated diastolic blood pressure later in life. That this association can already be detected in this group of former preterm infants may be crucial to these childrens' risk for adult hypertension.

Increased diastolic blood pressure has been attributed to increased systemic vascular resistance and endothelial dysfunction is thought to contribute a critical part in its development (23). Preterm children were shown to have an increased arterial stiffness compared with near term infants, both at their theoretical term (24) and in adolescence (25). IGF-1 has a wide spectrum of physiological effects such as regulation of cardiac contractility, tissue remodelling, glucose metabolism and vasomotor activity (26). IGF-1 has anti-apoptotic features and is present in cardiac tissue in children (27). In young adulthood, former preterm subjects

have been shown to have the highest relative mortality rates from cardiovascular-related disorders (28).

The algorithm IGF-1^2/IGFBP-1 during PMA 32.6–34.6 weeks may explain nearly 50% of the variation in diastolic blood pressure at 4 years of age in preterm children. This raises the question of whether this algorithm could be a useful surrogate marker to predict future outcome in preterm children. With the serum tests that are currently available, measuring IGF-1 and IGFBP-1 levels during this postnatal period could be one way to identify those infants most at risk for later increased diastolic blood pressure.

In this study, the small number of study subjects complicates the use of logistic analysis to predict elevated blood pressure with continuous variables. In addition, we do not know the causes behind the low algorithm levels in our small group of preterm children with ROP. Low levels of IGF-1 and elevated IGFBP-1 levels are generally found in children and adults with malnutrition (29), especially protein deficiency. High IGFBP-1 levels are also seen in anoxia (30). High levels of IGFBP-1 may serve as protection for those infants most subject to postnatal starvation by preventing a high metabolic rate; (12) immediate postnatal treatment with IGF-1 could potentially be used to prevent IGF-1 levels from decreasing when growth hormone has not yet stimulated IGF-1 expression. Such a therapy could result in a better prognosis for preterm children.

Recently, more gain in weight than length in early life (up to 3 months corrected age) was shown to be adverse for cardiovascular outcome (4) in adults born preterm, and there is an ongoing debate about how to feed preterm infants to attain an optimal outcome. The catch-up growth velocity in the study group correlated significantly with diastolic BP at follow-up. Our results thus support the idea of negative effects on future blood pressure outcome in preterm infants with an increased relative weight gain during the first months of life. This appears to be in line with previous results showing that infants who are severely malnourished immediately after birth (and thereafter show catch-up growth) are likely to develop IGF-1 deficiency and more severe ROP, thus predicting later blood pressure elevation.

Whether the differences in blood pressure between the no-to-mild ROP group and the moderate-to-severe ROP group are due to morphological causes such as an increased vessel tortuosity and reduced vessel branching or related to differences in vessel cell proliferation and differentiation, metabolic factors or a combination of these components remains to be established. Other influences such as differences in genetic factors or current hormonal status and weight could also contribute as well as an impact of a more preterm birth although we have tried to adjust for this factor in the calculations.

One weakness of this study is that in subjects with moderate-to-severe ROP the group consisted of infants born both appropriate for gestational age and small for gestational age, and these infants in general had lower birth weights and lower birthweight SDS compared with subjects

with no-to-mild ROP. Small for gestational age infants born preterm exhibit the highest blood pressure at follow-up in adulthood (2). Our total study group is small, and larger studies are needed to confirm our findings.

Our results indicate that the abnormal retinal vessel morphology seen in children born preterm who were diagnosed with moderate-to-severe ROP may reflect a more generalised vascular phenomenon and could be a risk marker or predict later cardiovascular disease. IGF-1 may play a causal role in this development. Efforts to minimise neonatal retinal abnormal development, noted as various degrees of ROP, may be important to reduce possible future cardiovascular risk in these fragile preterm infants.

DECLARATION OF INTEREST

The application to prevent ROP by means of administering IGF-1 is covered by patents and patent applications owned by Children's Medical Center Corporation, Boston, USA, and Premacure AB, Uppsala, Sweden. Two of the authors (CL and AH) owned shares in a company controlling Premacure AB. The remaining authors have no conflicts of interest to declare.

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