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Postnatal Decrease in Circulating Insulin-Like Growth Factor-I and Low Brain Volumes in Very Preterm Infants

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Context: IGF-I and IGF binding protein-3 (IGFBP-3) are essential for growth and maturation of the developing brain.

Objective: The aim of this study was to evaluate the association between postnatal serum concentrations of IGF-I and IGFBP-3 and brain volumes at term in very preterm infants.

Design: Fifty-one infants with a mean (SD) gestational age (GA) of 26.4 (1.9) wk and birth weight (BW) of 888 (288) g were studied, with weekly blood sampling of IGF-I and IGFBP-3 from birth until 35 gestational weeks (GW) and daily calculation of protein and caloric intake. Magnetic resonance images obtained at 40 GW were segmented into total brain, cerebellar, cerebrospinal fluid, gray matter, and unmyelinated white matter volumes.

Main Outcome Measures: We evaluated brain growth by measuring brain volumes using magnetic resonance imaging.

Results: Mean IGF-I concentrations from birth to 35 GW correlated with total brain volume, unmyelinated white matter volume, gray matter volume, and cerebellar volume [$r = 0.55$ ($P < 0.001$); $r = 0.55$ ($P < 0.001$); $r = 0.44$ ($P = 0.002$); and $r = 0.58$ ($P < 0.001$), respectively]. Similar correlations were observed for IGFBP-3 concentrations. Correlations remained after adjustment for GA, mean protein and caloric intakes, gender, severe brain damage, and steroid treatment. Protein and caloric intakes were not related to brain volumes. Infants with BW small for GA had lower mean concentrations of IGF-I ($P = 0.006$) and smaller brain volumes ($P = 0.001–0.013$) than infants with BW appropriate for GA.

Conclusion: Postnatal IGF-I and IGFBP-3 concentrations are positively associated with brain volumes at 40 GW in very preterm infants. Normalization of the IGF-I axis, directly or indirectly, may support normal brain development in very preterm infants. **(*J Clin Endocrinol Metab* 96: 0000–0000, 2011)**

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Abbreviations: AGA, Appropriate for GA; BW, birth weight; CBV, cerebellar volume; CSF, cerebrospinal fluid; CSFV, CSF volume; GMV, gray matter volume; GW, gestational week(s); IGFBP-3, IGF binding protein 3; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; MWM, myelinated white matter; PDw, proton density-weighted; PMA, postmenstrual age; SGA, small for GA; TBV, total brain volume; TE, echo time; TR, repetition time; T1w, T1-weighted; T2w, T2-weighted; UWMV, unmyelinated white matter volume; WMD, white matter damage.

Many extremely preterm infants display neurodevelopmental and cognitive dysfunctions, even in the absence of major brain damage (1). These impairments have been associated with a concomitant reduction in gray and white matter volume accompanied by an increase in cerebrospinal fluid (CSF) volume, compared with full-term infants assessed at term (2, 3). Very preterm birth is followed by poor postnatal growth, which has been associated with retinopathy of prematurity, lower brain volumes, and neurocognitive impairments (4–6).

IGF-I is an essential fetal growth factor, and circulatory concentrations increase during gestation (7). In experimental models, IGF-I promotes proliferation and differentiation of neural tissues and has antiapoptotic effects after induced ischemic injury or inflammation (8–10). The major binding protein of IGF-I is IGF binding protein 3 (IGFBP-3). IGFBP-3 regulates tissue availability of IGF-I and also exerts exclusive intracellular actions independently of IGF-I, such as vascular regrowth (11).

After birth, a rapid decline in circulatory concentrations of IGF-I and IGFBP-3 occurs (12). In preterm infants, low levels of IGF-I and IGFBP-3 may persist for several weeks, depending on low endogenous production (13). Postnatal levels of IGF-I and IGFBP-3 have been associated with short-term growth velocity in preterm infants (14, 15). Our aim was to prospectively assess the relationship between postnatal serum concentrations of IGF-I and IGFBP-3 and cerebral volumes at 40 gestational weeks (GW) after very preterm birth, hypothesizing that low concentrations of these factors are associated with decreased postnatal cerebral growth.

Subjects and Methods

Study population

For this prospective cohort study, 64 preterm infants were enrolled at the Neonatal Unit of Lund University Hospital between January 2005 and May 2007.

Inclusion criteria were a gestational age (GA) less than 31 wk at birth, informed parental consent, and no major anomalies. Recruitment and parental information were initiated before delivery, and written informed consent was obtained from both parents before enrollment. The study was approved by the Regional Ethical Review Board, Lund, Sweden.

All pregnancies were dated by ultrasound at 17–18 GW. Birth weight (BW) that was small for GA (SGA) was defined as weight at birth more than 2 SD below the age-related mean of the population (16). Weight was measured weekly on the same day as sampling of IGF-I and IGFBP-3 until discharge.

The nutritional strategy was based on individualized enteral nutrition using maternal or donor breast milk (fortified if required) and additional parenteral nutrition. The latter was started within the first hour after birth, and minimal enteral feeding (1–2 ml/meal) was initiated within 3 h of age and thereafter administered every 2 to 3 h and gradually increased. Nutritional intake aimed at a protein intake of 3.5 g/kg · d and an energy intake of 120 kcal/kg · d within 5–7 d. Administered breast milk was analyzed weekly for protein (g/100 ml) and energy (kcal/100 ml) content in a 24-h sample. Daily enteral and parenteral intakes of protein (g/kg · d) and energy (kcal/kg · d) were calculated. Clinical routine care included iv insulin administration for persistent hyperglycemia, steroid treatment for resistant arterial hypotension or hypoglycemia (hydrocortisone), and betamethasone on pulmonary indication to facilitate weaning from ventilator. Routine cerebral ultrasound was performed at d 1, 3, and 7, at 3 and 6 wk of age, and at term age (40 GW). Severe intracranial hemorrhage was defined in the presence of intraventricular hemorrhage (IVH) grade III or periventricular hemorrhagic infarction and white matter damage (WMD) in the presence of periventricular echodensities persisting for more than 7 d or periventricular cysts. Severe brain damage was defined as severe intracranial hemorrhage and/or WMD.

IGF-I and IGFBP-3 measurements

IGF-I and IGFBP-3 concentrations were measured on d 3 and 7, and thereafter once a week at the same day as weight measurements until at least a GA of 35 wk.

The blood samples were drawn from an umbilical or peripheral arterial catheter and thereafter by venous puncture and were transferred within 30 min to the local chemical laboratory.

After centrifugation, serum samples were stored at −80 C until assayed. The IGF-I samples were diluted 1:50, and the IGF-I concentrations were analyzed using IGFBP-blocked RIA (Mediagnost GmbH, Tübingen, Germany). The intraassay coefficients of variation for IGF-I were 18, 11, and 7% at concentrations of 9, 33, and 179 μg/liter. All samples were analyzed within the same assay. The method has been described in detail previously (17). All values of IGF-I were omitted for further evaluation if the infant had received transfusion of fresh frozen plasma during the same day and before sampling of IGF-I was performed.

Magnetic resonance imaging (MRI)

Image acquisition

MRI was performed on a 3-Tesla Siemens Magnetom Allegra head scanner (Siemens AG, Medical Solutions, Erlangen, Germany) at term age [mean (SD), 40.1 (0.6) GW]. The infants were fed and swaddled into a vacuum fixation pillow and received a single dose of chloral hydrate (35 mg/kg) as sedation.

The protocol consisted of a three-dimensional T1-weighted (T1w) magnetization-prepared rapid gradient echo (MPRAGE) sequence [1-mm coronal slices; flip angle, 8°; repetition time (TR), 9.6 msec; echo time (TE), 4.38 msec; rectangular matrix, 160 × 256]; a T2-weighted (T2w) turbo spin echo sequence (1-mm coronal slices; flip angle, 120°; TR, 4190 msec; TE, 82 msec; rectangular matrix, 128 × 256), and a proton density-weighted (PDw) turbo spin echo sequence (1-mm coronal slices; flip angle, 120°; TR, 3050 msec; TE, 16 msec; rectangular matrix, 128 × 256). The voxel size for all sequences was 1 × 1 × 1 mm.

Image processing

A sequence of image processing algorithms was applied. As a consequence of a consistent signal in homogeneity in all acquisitions, a parametric bias field correction was performed on all images (MRI bias correction, EPFL, Lausanne, Switzerland).

The matrix of the T2w and PDw images was adapted to the 160 × 256 matrix for the T1w images. The T1w and PDw images

were linearly coregistered to the T2w images, using the Statistical Parametric Mapping 5-fMRI software (Wellcome Trust Center for Neuroimaging, London, UK, www.fil.ion.ucl.ac.uk/spm). Thus, a three-channel (T1w, T2w, PDw) dataset was created. A template modified, statistical classification algorithm (κ nearest neighbor) was used for image classification into myelinated white matter (MWM) and unmyelinated white matter, total gray matter, and CSF, based on signal intensities on these three channels (18).

The voxels of every tissue class were summed to calculate the tissue volumes. Total brain volume (TBV) was defined as the total volume of gray matter, MWM, and unmyelinated white matter, including the cerebellum.

The cerebellar volume (CBV), including the cerebellar peduncles, were measured by manual outlining on both T1w and T2w images, using an image analysis tool (www.slicer.org). The mean of the volume measurements on T1w and T2w images was taken as the CBV.

All segmentations were performed by the same person (H.H.), unaware of nutritional and IGF-I results. The intraobserver variation was tested, reperforming a complete segmentation on six randomly chosen infants, for κ nearest neighbor and cerebellum, respectively. Because the intraindividual correlation of repeated measurements for MWM was as low as 0.40, MWM was excluded from further analysis. For all other brain volumes, r values between 0.91 and 0.99 were obtained.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences software for Microsoft Windows v. 14.0 (SPSS Inc., Chicago, IL).

For analysis of intraobserver variation for repeated brain volume measurements (test-retest), paired-samples *t* test was used. Relationships between categorical variables and IGF-I, IGFBP-3, or brain volumes were assessed using the unpaired *t* test. Correlations between continuous variables were assessed using Pearson correlation. Adjustment for other variables was performed using linear regression analysis. The independent variables included in the multivariate analysis were GA at birth, gender, BW for GA, severe brain damage, mean intake of protein (g/kg · d), and energy (kcal/kg · d), accumulated dose of hydrocortisone (mg/kg), betamethasone (mg/kg), insulin (U/kg) from birth to 35 GW. *P* values < 0.05 were considered as significant.

Results

Study population

Of the 64 recruited infants, nine died during the study period, and four chose to leave the study before term. The characteristics of the 51 infants with a complete study protocol including MRI at 40 GW are presented in Table 1.

Clinical parameters and brain volumes

In all 51 infants, segmentations of the cerebellum could be performed, whereas five (including two infants with a posthemorrhagic shunt) had to be excluded for segmentation of the remaining cerebral volumes due to major artifacts. Thus, complete segmentations of TBV, unmyelinated white matter volume (UWMV), gray matter volume (GMV), and CBV were obtained in 46 infants.

TABLE 1. Neonatal characteristics of infants examined with MRI (n = 51)

GA at birth (wk)	26.4 (1.9)
BW (g)	888 (288)
Male gender, n (%)	25 (49)
BW < -2 SD (SGA), n (%)	12 (24)
GA at discharge (wk)	38.3 (2.0)
Mean protein intake birth to 35 GW (g/kg/d)	3.14 (0.29)
Mean energy intake birth to 35 GW (kcal/kg · d)	121.3 (10.2)
Severe brain damage[a] defined by cerebral ultrasound, n (%)	6 (12)
Posthemorrhagic shunt, n (%)	2 (4)
Insulin treatment, n (%)	8 (16)
Hydrocortisone treatment, n (%)	13 (26)
Betamethasone treatment, n (%)	16 (31)

Data are expressed as mean (SD) unless described otherwise.

[a] Severe brain damage; IVH grade III, and/or parenchymal hemorrhage, and/or WMD.

GA at birth correlated positively with TBV, UWMV, GMV, and CBV at term [r = 0.58 ($P < 0.001$), r = 0.60 ($P < 0.001$), r = 0.46 ($P = 0.001$), and r = 0.52 ($P < 0.001$), respectively]. Brain volumes did not differ according to gender and did not differ between infants with severe brain damage or any cerebral hemorrhage as defined by cerebral ultrasound compared with those without brain damage.

Taking GA into account, brain volumes did not correlate with accumulated dose (mg/kg) of hydrocortisone, betamethasone, or insulin from birth until 35 GW.

Infants with BW SGA (n = 12) had smaller mean (SD) brain volumes compared with infants with BW appropriate for GA (AGA); TBV, 356 (50) *vs.* 410 (45) ml, $P = 0.001$; UWMV, 174 (27) *vs.* 197 (24) ml, $P = 0.006$; GMV, 177 (30) *vs.* 206 (27) ml, $P = 0.002$; and CBV, 19.1 (3.8) *vs.* 22.0 (3.4) ml, $P = 0.013$. Mean (SD) concentrations of IGF-I from birth to 35 GW were lower in infants with BW SGA [19.2 (6.0) μg/liter], compared with those in infants with BW AGA [25.3 (7.0) μg/liter; $P = 0.006$]. Corresponding mean concentrations of IGFBP-3 in infants with BW SGA were 936 (231) μg/liter, compared with those in infants with BW AGA [1036 (191) μg/liter; $P = 0.122$]. Infants with BW SGA had the same mean GA (26.0 wk) at birth as infants with BW AGA.

IGF-I, nutrition, and brain volumes

To evaluate relationships between longitudinal changes in levels of IGF-I and nutritional intake, respectively, and MRI-determined brain growth at term, data on CBV were categorized using the 25th percentile as the cutoff limit. CBV was used for categorization because data were available in all 51 infants. The 13 infants with a CBV within the lowest quartile had a mean (SD) CBV of 17.0 (1.4) ml, and the remaining 38 infants above the 25th percentile a CBV of 22.6 (3.1) ml. Relationships between CBV category at term age and weekly

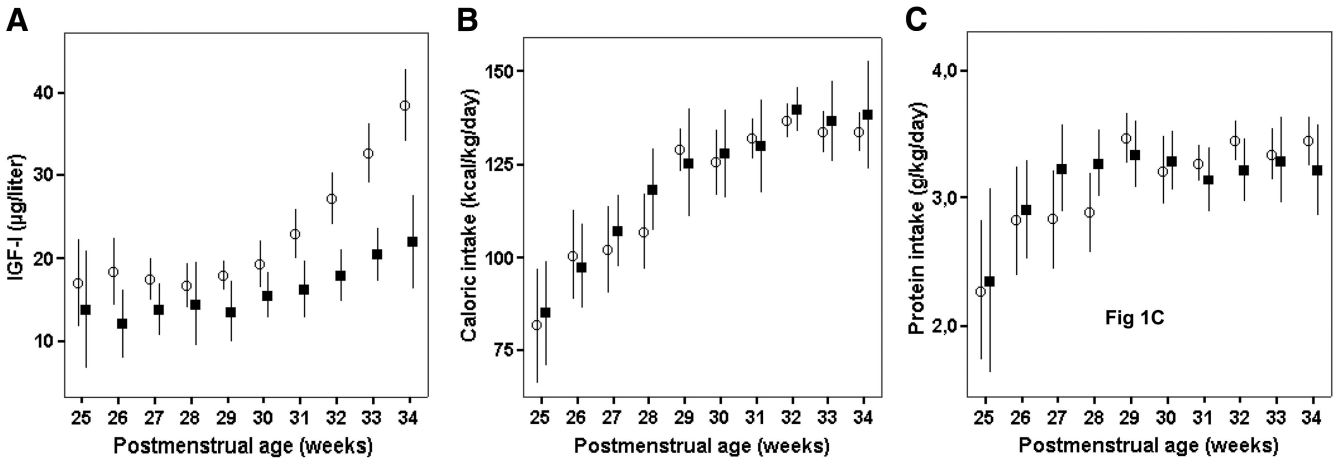

FIG. 1. A–C, Mean IGF-I (μg/liter), caloric intake (kcal/kg · d), and protein intake (g/kg · d) according to PMA (wk). *Filled squares* denote infants with CBV below the 25th percentile (n = 13); *open circles* denote infants with CBV above the 25th percentile (n = 38). *Error bars* denote ±95% confidence interval.

levels of IGF-I and nutritional intake, respectively, are illustrated in Fig. 1. Neither protein intake nor caloric intake differed according to CBV category during the observed period of postmenstrual weeks. In contrast, weekly IGF-I concentrations from a postmenstrual age (PMA) of 30 wk and onward were lower in infants with CBV below the 25th percentile, compared with those with CBV above the 25th percentile (Fig. 1A).

Mean IGF-I concentration and mean protein/caloric intakes were calculated for each infant based on weekly and daily data, respectively, from birth to 35 GW. Mean protein (g/kg · d) or energy intake (kcal/kg · d) did not correlate with any cerebral volume. Mean IGF-I concentration correlated positively with TBV, UWMV, GMV, and CBV [r = 0.55 ($P < 0.001$), r = 0.55 ($P < 0.001$), r = 0.44 ($P = 0.002$), and r = 0.58 ($P < 0.001$), respectively], whereas no correlation was observed for CSF volume (CSFV; r = 0.19; $P = 0.20$]. Correlations between mean IGF-I concentrations and TBV, UWMV, and CBV remained significant ($P = 0.009$, $P = 0.009$, and $P = 0.001$, respectively) after adjustment for GA at birth, gender, severe IVH and/or WMD, mean intake of protein and energy, and accumulated dose of insulin, hydrocortisone, and betamethasone. After inclusion of SGA/AGA as an additional independent variable, only CBV remained significantly associated with mean IGF-I ($P = 0.007$). Individual brain volumes according to BW for GA in relationship to mean IGF-I are given in Fig. 2.

Mean concentration of IGFBP-3 correlated positively with TBV, UWMV, and CBV [r = 0.29 ($P = 0.05$), r = 0.28 ($P = 0.055$), and r = 0.36 ($P = 0.009$), respectively], but not with GMV or CSFV. After adjustment for GA at birth, gender, severe IVH and/or WMD, mean intake of protein and energy, and accumulated dose of insulin, hydrocortisone, and betamethasone, mean IGFBP-3 concentrations were significantly associated with TBV, UWMV, GMV, and CBV ($P = 0.001$, $P = 0.002$, $P = 0.02$, and $P = 0.001$, respectively). After including SGA/AGA as an additional independent variable, TBV, UWMV, and CBV remained significantly associated with IGFBP-3 ($P = 0.026$, $P = 0.023$, and $P = 0.007$, respectively).

Concentrations of IGF-I and IGFBP-3 at 40 GW did not correlate with brain volumes.

Discussion

Our study is the first to show that decreased postnatal IGF-I and IGFBP-3 concentrations are associated with lower brain volumes at term age in very preterm infants. This association was evident from a PMA of 30 wk and onward. Protein and caloric intake exhibited no clear independent association with brain volumes. The subgroup of infants growth restricted at birth had low postnatal IGF-I and IGFBP-3 concentrations concomitant with reduced brain volumes.

The results of the present study infer that improved protein and caloric intake alone may not suffice in altering the well-recognized postnatal growth restriction associated with reduced brain growth, occurring in very preterm infants. Growth of the healthy fetus remaining *in utero* is a consequence of a complex interplay between maternal, placental, and fetal factors regulating nutrient utilization (19). The IGF system plays crucial roles for both pre- and postnatal brain growth and development. A null mutation for IGF-I in mice results in reduced brain size, hypomyelination, and a reduction in neuronal populations (20). In humans, inability to synthesize IGF-I results in severe intrauterine growth restriction (IUGR) and mental retardation (21). IGF-I, by acting through the IGF-I receptor, influences all of the mechanisms involved in normal brain development apart from migration,

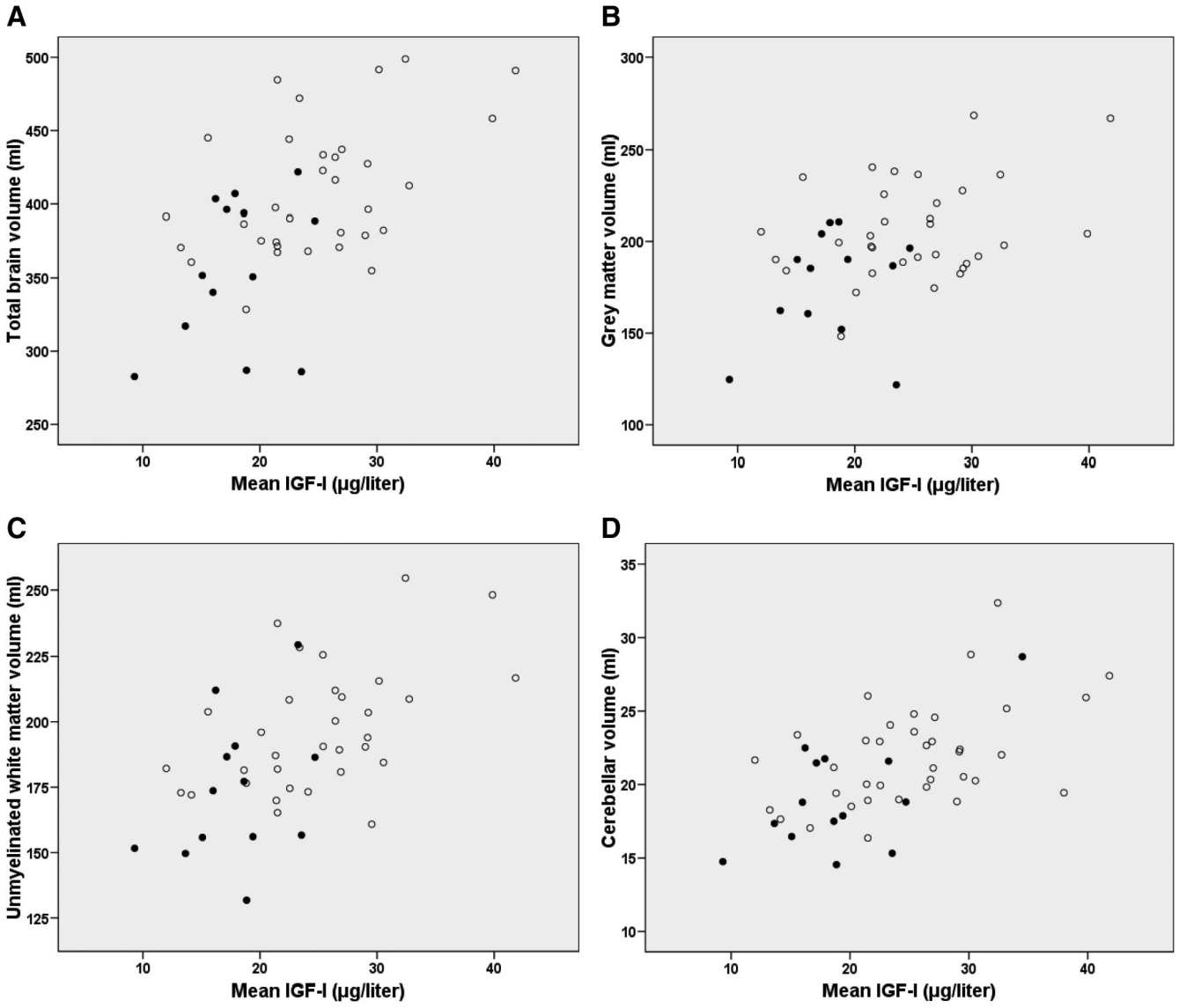

FIG. 2. A–D, Individual values of TBV, GMV, UWMV (ml) (n = 46, respectively), and CBV (n = 51) at term in relation to mean IGF-I concentrations from birth until 35 GW. *Filled circles* denote infants with BW SGA; *open circles* denote infants with BW AGA.

i.e. proliferation, differentiation, maturation, and apoptosis (22–24). Furthermore, IGF-I protects the brain after postnatal undernutritional insults in mice (25). IGFBP-3 both inhibits and promotes IGF-I actions, but also has independent effects such as preventing loss and stimulating regrowth of retinal vessels (11).

Very preterm birth and the interruption of the fetoplacental unit are followed by an inevitable fall in circulating levels of IGF-I and its major circulatory binding protein, IGFBP-3 (12). Some infants fail to increase their postnatal circulating levels of IGF-I/IGFBP-3, which has been related to poor weight gain and development of severe retinopathy of prematurity (13, 26). We show that decreased circulating levels of IGF-I/IGFBP-3 are associated with lower brain volumes at term age. In the immature brain, transfer from the blood system occurs predominantly via CSF (27). IGF-I enters the brain using a specific transport mechanism, predominantly located in the choroid plexus (28, 29). It is, as yet, unknown to which degree circulatory IGF-I/IGFBP-3 concentrations pass the blood-brain barrier in extremely preterm infants.

Circulating levels of IGF-I/IGFBP-3 were associated with brain volumes representing both gray and white matter, which is consistent with the known effects of IGF-I on neuronal and oligodendrocyte populations (8, 24). CBV displayed the strongest association with IGF-I and IGBP-3. Preterm infants examined at term and in adolescence have lower CBV compared with term controls, which has partly been ascribed as a secondary effect to WMD (30–32). Cerebellar growth in the last trimester of gestation is fast and exceeds that of the cerebral hemispheres, indicating that the cerebellum may be more vulnerable to environmental changes than other parts of the brain (31). IGF-I promotes development and survival of cerebellar neurons (33, 34).

Using CBV at the 25th percentile as a cutoff limit, we show that the relationship between low levels of circulating IGF-I and decreased brain volume was most evident at a PMA of 30 GW and onward (Fig. 1A). During this time period, the majority of infants display catch-up growth, as expressed by SD scores, and this coincides with a weekly increase in circulating levels of IGF-I (our unpublished data). This increase in IGF-I was clearly blunted in infants with CBV below the 25th percentile. We have previously observed a positive association between nutrient intake and circulating IGF-I during the period of catch-up growth, as opposed to no clear association during the initial period of postnatal growth restriction (our unpublished data). Protein and caloric intakes did not differ between CBV categories and were not associated with any brain volume at term age. This may reflect an insufficient effect of nutrient intake on hepatic synthesis of IGF-I in infants developing smaller brain volumes. All four evaluated brain volumes were lower in infants with BW SGA than in those born AGA with a similar GA at birth. Furthermore, the group of infants with BW SGA had lower mean values of IGF-I, and this difference was most pronounced at a PMA beyond 30 wk (data not shown). The persisting low values of IGF-I during the period of catch-up growth and the hypothesized insufficient effect of nutrient intake in SGA infants may be a consequence of IUGR.

Preterm infants with IUGR already exhibit reduced TBV, cortical GMV, and hippocampal volume 2 wk after birth, which may indicate that a restriction in cerebral growth is already established before birth (35, 36). These findings are supported by experimental studies where induced IUGR results in a reduced number of neurons in the cerebellum and hippocampus (37). It is unknown whether retarded brain growth in growth-restricted infants may be ameliorated by improved postnatal nutritional intake. However, our data indicate that the current clinical regime aiming at a similar nutrient intake per kilogram in infants with BW SGA as in infants with BW AGA is insufficient in halting the adverse effects of IUGR on brain growth.

Decreased cerebellar and cortical volumes have been observed in infants with cerebral hemorrhage or WMD, but reduced cerebral volumes also occur without major brain damage (38, 39). In our study, severe brain damage, as detected by ultrasound, was not associated with brain volumes in univariate analysis. This may be explained by the low number of infants with ultrasound-defined severe brain damage (n = 6), of which two infants were further excluded from volumetric analysis of TBV, UWMV, and GMV due to the presence of shunt artifacts.

Exogenous IGF-I has proven neuroprotective effects after hypoxic-ischemic insult to the developing brain in experimental studies (8, 40). Acquired brain damage in premature infants is accompanied by increased CSFV, which indicates brain atrophy (2). We did not observe any correlation between IGF-I/IGFBP-3 concentrations and CSFV. The positive association between circulating IGF-I/IGFBP-3 and brain volumes in the absence of a negative association with CSFV suggests a relationship with brain growth rather than protection from atrophy secondary to brain damage.

Conclusion

Circulating levels of IGF-I/IGFBP-3 during postnatal life were positively associated with MRI-determined brain volumes at term age in very preterm infants. Of note, variability in nutrient intake did not display any clear relationship to brain growth. Normalization of the IGF-I axis, directly or indirectly, may support normal brain development in very preterm infants. This may be particularly relevant in very preterm infants with BW SGA.

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Disclosure Summary: The application to prevent retinopathy of prematurity by means of administering IGF-I is covered by patents and patent applications owned by the Children's Medical Center Corporation (Boston, MA) and Premacure AB (Uppsala, Sweden). Four of the authors (I.H.-P., C.L., A.H., and D.L.) own shares in a company controlling Premacure AB. The remaining authors have nothing to declare.

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