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Clinical Research Article

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Dynamic Changes in Serum IGF-I and Growth During Infancy: Associations to Body Fat, Target Height, and *PAPPA2* Genotype

Emmie N. Upners,[1,2] Marie Lindhardt Ljubicic,[1,2] Alexander S. Busch,[1,2] Margit Bistrup Fischer,[1,2] Kristian Almstrup,[1,2] Jørgen H. Petersen,[1,2] Rikke Beck Jensen,[1,2] Casper P. Hagen,[1,2] and Anders Juul[1,2,3]

[1]Department of Growth and Reproduction, Copenhagen University Hospital - Rigshospitalet, 2100 Copenhagen, Denmark; [2]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Copenhagen University Hospital - Rigshospitalet, 2100 Copenhagen, Denmark; and [3]Department of Clinical Medicine, University of Copenhagen, 2100 Copenhagen, Denmark

ORCiD numbers: 0000-0002-7843-7590 (E. N. Upners); 0000-0002-7418-6878 (M. L. Ljubicic); 0000-0003-4417-569X (A. S. Busch); 0000-0002-7999-548X (M. B. Fischer); 0000-0002-1832-0307 (K. Almstrup); 0000-0003-3979-7443 (J. H. Petersen); 0000-0002-4522-672X (R. B. Jensen); 0000-0002-0250-8049 (C. P. Hagen); 0000-0002-0534-4350 (A. Juul).

Abbreviations: AGA, appropriate for gestational age; IGFBP-3, IGF binding protein-3; PAPP, pregnancy plasma protein; SDS, SD score; SNP, single nucleotide polymorphism.

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Abstract

Context: IGF-I is important for postnatal growth and may be of diagnostic value in infants suspected of pituitary disease; however, little is known about the impact of IGF-I and its determinants on infant growth. Importantly, detailed reference ranges for IGF-I and IGF binding protein-3 (IGFBP-3) concentrations during infancy are lacking.
Objective: To evaluate the rapid changes in weight and length as well as their determinants in healthy infants, and to establish age- and sex-specific reference curves for IGF-I and IGFBP-3 in children aged 0 to 1 years.
Design: Prospective longitudinal study.
Setting: Cohort study.
Participants: A total of 233 healthy children (114 girls) with repeated blood samples during the first year of life.
Main Outcome Measure(s): Serum concentrations of IGF-I and IGFBP-3, length velocity, weight velocity, and *PAPPA2* (rs1325598) genotype.
Results: Individual trajectories of length and weight velocities were sex specific. We provide detailed reference curves based on longitudinal data for IGF-I and IGFBP-3 during infancy. In both girls and boys, IGF-I decreased during infancy, whereas IGFBP-3

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remained stable. IGF-I and IGFBP-3, but not *PAPPA2* genotype, were positively associated with weight gain, but not with longitudinal growth. When stratified by sex, the association between weight gain and IGF-I only remained significant in girls.

Conclusions: Interestingly, we found a significant association between IGF-I and infant weight gain in girls, but not with longitudinal growth in the first year of life. Our findings highlight the role of IGF-I as an important anabolic hormone that is not limited to linear growth.

Key Words: IGF-I, IGFBP-3, infancy, growth, PAPP-A2, growth velocity

Growth velocity peaks in early life and the path of individual's growth trajectories is influenced by genetic, hormonal, and nutritional factors. Importantly, rapid weight gain during infancy is associated with higher risk of childhood overweight and obesity (1). Moreover, previous studies have reported that rapid changes in length during the first years of life appear to contribute to higher risk of overweight (2, 3). Although, nutrition is believed to be an important factor (4), the major determinants of growth in infancy are not fully elucidated.

IGF-I is an important regulator of growth in infancy and childhood promoting linear bone growth (5), muscle mass (6), and adipocyte maturation (7). Individual IGF-I trajectories follow a distinct pattern during childhood. In brief, IGF-I concentrations slowly increase during childhood and peak in midpuberty, presenting acromegalic levels 2 to 4 years after the pubertal growth spurt. Subsequently, IGF-I concentration decreases throughout adult life (8). The majority of circulating IGF-I are bound to IGF binding proteins (IGFBPs), which regulate the amount of free and bioactive IGF-I accessible to peripheral target tissues. Measurements of IGF-I and IGFBP-3 serum concentrations are valuable tools in the evaluation of children with postnatal growth failure. During childhood, IGF-I secretion from the liver is primarily stimulated by GH from the pituitary gland. However, during infancy, nutritional status and insulin are key regulators of IGF-I (9). Formula-fed infants have, in addition to being heavier and taller, higher IGF-I concentrations compared with breastfed infants (10, 11). Although substantial amounts of data exist on IGF-I in older children, the dynamics of IGF-I in infancy remain largely undescribed.

Early postnatal growth is largely GH independent (ie, patients with congenital GH deficiency present with only marginally decreased birth weight and birth length, as well as mild growth impairment during the first year of life) (12). In contrast, IGF-I stimulates fetal growth and rare mutations in the IGF-I gene cause severe intrauterine growth retardation and reduced postnatal growth (13, 14). There is a well-described positive association between cord blood concentrations of IGF-I and birth weight supporting that IGF-I in utero increases fetal growth (15, 16). Interestingly, a recent study found that cord blood concentrations of pregnancy plasma protein-A2 (PAPP-A2) and PAPP-A were negatively associated with birth weight as well as birth length (17). PAPP-A2 and PAPP-A are proteases that cleave IGF-I from its binding proteins and thereby increase the IGF-I bioactivity. Approximately 80% of the variation in adult height is genetically determined and in a previous genome wide association study, variants in *PAPPA2*, *PAPPA*, and *Stanniocalcin 2* (*STC2*), genes that code for regulators of IGF-I bioavailability, were strongly associated with adult height (18, 19). In a recent study by our group, we found an association between height and the same common variant of *PAPPA2* in healthy Danish children (20).

Therefore, the aims of this study were: (1) to evaluate the dynamic and rapid changes in length and weight as well as their determinants in healthy infants during infancy; (2) to establish detailed age- and sex-specific reference curves for IGF-I and IGFBP-3 serum concentrations in infants during first year of life; and (3) to evaluate the possible impact of IGF-I, target height, body fat, and *PAPPA2*, *PAPPA*, and *STC2* genotypes on infant length and weight velocity.

Subjects and Methods

Study Population

The COPENHAGEN Minipuberty Study (ClinicalTrials.gov ID NCT02784184) is a prospective, longitudinal, observational study of healthy infants born at term and followed throughout the first year of life. The study population, design, and methods have been published in detail previously (21). In brief, healthy pregnant women with singleton pregnancies were recruited between 2016 and 2018 to participate in the study at Department of Growth and Reproduction, Rigshospitalet, Denmark. The following inclusion criteria were applied after birth: (1) healthy newborn; (2) gestational age ≥ 37 + 0 and ≤ 42 + 0 weeks; (3) born appropriate for gestational age (AGA); birth weight SD score (SDS) ≥ -2 SD and ≤ 2 SD (22). A total of 233 infants (114 girls) were

included and scheduled to 6 visits during the first year of life. Each study visit included a clinical examination and blood sample. The visits were scheduled at age 0 and 12 months and at 1, 3, 5, and 7 months or 2, 4, 6, and 8 months. A total of 186 children completed all 6 visits.

Anthropometric Measurements

Length was measured in supine position to the nearest 0.5 cm with a baby length measuring mat (ADE Germany GmbH & Co, Hamburg, Germany). Weight was measured without clothing on an electronical calibrated scale (Babyscale, Solotop Oy, Finland) to the nearest 0.005 kg. The measurements were made in triplicate and mean values were used. As previously reported, interexaminer intraclass correlations for length were 0.954 (95% CI, 0.915-0.975) (21). Body fat percent was calculated by Slaughter's equation (23) using triceps and subscapular skinfolds. SDSs for body fat percent were calculated using reference data (24). Birth weight, birth length, and gestational age were obtained from medical records. Parental heights were recorded and target height was calculated. Target height SDSs were calculated using Danish reference data (25).

IGF-I and IGFBP-3

IGF-I and IGFBP-3 concentrations were quantified in serum from 197 of 233 infants (6 samples: n = 3; 5 samples: n = 17; 4 samples: n = 33; 3 samples: n = 50; 2 samples: n = 44; and 1 sample: n = 50) and in serum from cord blood from 142 infants. Blood samples were drawn from the antecubital vein, clotted, and centrifuged within 8 hours of collection and cord blood was drawn from the umbilical vein after birth. Samples were stored at -20°C for a maximum of 1 year before analyses of all samples within 4 batches. Serum concentration of IGF-I and IGFBP-3 were measured using IDS-iSYS IGF-I and IDS-iSYS IGFBP-3 assays (Immuno-diagnostic Systems LTD, Bolton, UK) based on chemiluminescence technology. Limits of detection were 10 ng/mL and 80 ng/mL and interassay coefficients of variation were < 7.1% and < 7.2% for IGF-I and IGFBP-3, respectively.

Genotyping

Genomic DNA was isolated from EDTA-preserved peripheral blood using LEV Blood DNA Kits with the Maxwell 16-MDx instrument (Promega, Madison, WI, USA) and the DNA was quantified by NanoDrop ND-1000 spectrophotometer (Saveen Werner, Limhamn, Sweden). All small nucleotide polymorphisms (SNPs) were analyzed at the laboratory of Department of Growth and Reproduction (Rigshospitalet, Copenhagen, Denmark) using KASP genotyping assays designed by LGC genomics (United Kingdom) toward the following sequences: rs1325598 (*PAPPA2*): CATAAATGAAKAAM[R]TAATTTTTCCAGC; rs751543 (*PAPPA*): GAGCAGACTC[Y]GGCTACTTCT; and rs889014 (*STC2*): ARYTATTAACT[Y]TCAAYTACTAGA. The SNPs were analyzed in all children with available DNA (n = 225). Because of poor DNA quality or nearby genetic variation, genotyping was unsuccessful in a few cases, resulting in *PAPPA2*, *PAPPA*, and *STC2* genotype being available in 224, 223, and 224 children, respectively.

Statistics and Calculations

Descriptive statistics are reported as medians (25th percentile; 75th percentile) and Mann-Whitney *U* tests were used for nonparametric comparisons between groups. Feeding groups were divided in (1) exclusively breastfed, (2), exclusively formula-fed, and (3) combination of breastfed and formula-fed based on their feeding pattern until 4 months of age.

The reference curves for growth and growth velocities (length and weight) were based on modelling of the longitudinal growth data: $Y_{ij} = A_i + f(t_{ij} - B_i) + C_i t_{ij} + \varepsilon_{ij}$

where Y_{ij} represents the *j*th growth measurement of the *i*th individual. The function f is modelled using natural cubic splines allowing a flexible and continuous modelling of the growth. A, B, and C are random individual-specific effects following a 3-dimensional Gaussian distribution. This allows individuals to have different growth profiles including different birth weights, growth rates, and individual-specific time translation allowing for some individuals to have a faster or slower growth rate the first few months. The model is an extension of the SITAR model (26), allowing for a parameterized individual linear growth rate at the end of the first year.

Reference charts for IGF-I and IGBP-3 were developed using the generalized additive models for location, scale, and shape (GAMLSS), based on the Box-Cox transformation, which transforms data to follow a Gaussian distribution at each age. In this model, data are summarized in 3 smooth age-dependent curves LMS where the L curve adjust for age-dependent skewness, the M curve corresponds to the age-dependent median, and S is the age-dependent coefficient of variation curve. Calculation of individual SDSs were based on the following equation:

$$SDS = \frac{(X/M)^L - 1}{L * S}, \text{if } L \neq 0$$

Because of the marked dynamic changes in IGF-I and IGFBP-3 during the first month of life, reference curves for IGF-I and IGFBP-3 were calculated separately for cord blood and postnatal blood samples using the same method.

Differences in IGF-I and IGFBP-3 between birth (cord blood) and day 10 (range, 4-14 days) divided by sex were analyzed by paired samples *t* test. Differences in IGF-I concentrations between girls and boys at 12 months of age were analyzed by Mann-Whitney *U* test. Correlations between IGF-I SDS and IGFBP-3 SDS were calculated as the average IGF-I and IGFBP-3 concentrations postnatally for each child and assessed using Pearson correlation. To describe the variation of intraindividual IGF-I SDSs, mean dispersions were calculated in Microsoft Excel, Office 2016, and are shown as medians (25th percentile; 75th percentile).

Univariate regression was performed to test for associations between mean IGF-I (SDS) and (1) mean length velocity (SDS) and (2) mean weight velocity (SDS). To evaluate associations between IGF-I as well as IGFBP-3 with length and weight velocities, independently from confounding factors, 2 models of multiple regression analyses were used. Model 1 included length or weight velocity as the dependent variable and mean IGF-I (SDS), birth weight (SDS), feeding pattern 0 to 4 months, *PAPPA2* genotype, and target height (SDS) as explanatory variables. Model 2 was supplemented with mean body fat % (SDS) as an explanatory variable. The same models were used to evaluate mean IGFBP-3 (SDS) as an explanatory variable in additional analyses using the same models. We also performed the same models stratified for sex for the association between mean IGF-I (SDS) and length and weight velocities.

One-way ANOVA was performed to test for differences between feeding patterns and mean IGF-I SDS, mean length SDS, mean weight SDS, mean length velocity SDS, and mean weight velocity SDS. Tukey's test for post hoc analysis was performed following statistical significance in the ANOVA test.

All statistical analyses were generated using the SAS version 9.4 and IBM SPSS Statistics 22. $P \leq 0.05$ was considered statistically significant.

Ethical Considerations

The COPENHAGEN Minipuberty Study was approved by the Ethical Committee of at the Capital Region of Denmark (H-15014876) and the Danish Data Protection Agency (RH-2015-210 I-Suite nr. 04146). Written informed consent was obtained from all families included in this study.

Results

Descriptive birth characteristics, maternal characteristics, and feeding patterns according to sex are shown in Table 1. Figure 1 shows that length velocity declined after birth and stabilized during first year of life. To evaluate whether there was tracking of length velocity (ie, whether each child maintained their SD level over time), the cohort was divided into tertiles depending on the individual mean length velocity (SDS). This revealed that the SD level for each child remained relatively stable during the first year of life (Supplemental Figure 1A (27)). Weight velocity decreased with increasing age for both boys and girls (Fig. 2).

The reference curves for serum concentrations of IGF-I and IGFBP-3 are shown in Fig. 3 and the corresponding GAMLSS data for L, M, and S as well as SD values for the references are presented in Supplemental Table 1 (28) and Supplemental Table 2 (29). IGF-I showed an overall decreasing trend during first year of life in girls and boys (Fig. 3). At birth, IGF-I and IGFBP-3 concentrations were higher in girls compared with boys (Table 1). In boys, IGF-I concentrations increased from cord blood to the first 10 days of life (range, 4-14 days) mean difference 24.8 ± 34.1 SD µg/L, $P < 0.001$. Serum IGF-I concentrations did not differ between girls and boys at 12 months of age ($P = 0.49$). IGFBP-3 concentrations increased from cord blood to the first 10 days of life in both girls and boys (mean difference 444 ± 580 SD µg/L, $P < 0.01$, and mean difference 511 ± 465 SD µg/L, $P < 0.001$, respectively). A strong positive correlation was observed between IGF-I SDS and IGFBP-3 SDS ($r = 0.69$, $P < 0.001$).

As for length velocity, we evaluated tracking of the IGF-I concentrations during the first year of life by dividing the cohort into tertiles depending on their individual mean IGF-I (SDS) (Supplemental Figure 1B (27)). This revealed that IGF-I SD levels remained relatively stable during first year of life. To evaluate intraindividual variability of IGF-I and IGFBP-3, individual dispersions were presented (Fig. 4). The median dispersion of IGF-I and IGFBP-3 was 0.56 (0.37-0.81) SD and 0.46 (0.28-0.66) SD for girls and 0.45 (0.23-0.79) SD and 0.45 (0.26-0.74) SD for boys, respectively.

Mean length velocity (SDS) and mean weight velocity (SDS) were positively associated with mean IGF-I (SDS) (β = 0.12 (95% CI, -0.0001 to 0.24), $P = 0.05$ and β = 0.25 (95% CI, -0.14 to 0.08), $P < 0.001$). After adjusting for possible confounding variables, only mean weight velocity (SDS) remained positively associated with mean IGF-I (SDS) (Table 2). Including body fat percent (SDS) in the model did not change the association between mean IGF-I (SDS) and neither mean length velocity (SDS) nor mean weight velocity (SDS). Body fat percent (SDS) was positively associated with mean weight velocity (SDS), but not with length velocity. When stratified for sex, mean IGF-I (SDS) was only associated with mean weight velocity (SDS) in girls (β = 0.44 [95% CI, 0.22-0.66], $P = 0.0001$), but not in boys (β = 0.09 [95% CI, -0.06 to 0.25], $P = 0.25$). The estimates did not significantly change after adjusting for

Table 1. Basic characteristics according to sex

	n	Girls	n	Boys	*P*
		n = 114		n = 119	
Child characteristics					
Birth length, cm	114	52 (50-53)	119	53 (52-54)	<0.001
Birth weight, g	114	3455 (3116-3730)	119	3648 (3330-3930)	0.001
Gestational age, days	114	283 (276-287)	119	283 (280-290)	0.04
IGF-I, µg/L[a]	70	78.7 (65.4-100.9)	72	69.5 (56.6-90.1)	0.05
IGFBP-3, µg/L[a]	70	1601 (1393-1927)	72	1523 (1419-1639)	0.03
Target height, cm	82	169.5 (166.0-173.3)	95	181.5 (179.0-185.0)	<0.001
Maternal characteristics					
Prepregnancy BMI, kg/m^2	91	22.0 (20.2-24.1)	104	21.2 (19.8-23.1)	0.13
Parity	112		112		
First child, %		72.3		72.3	
Second or later child, %		27.7		27.7	
Feeding pattern					
Feeding 0-4 months:	94		104		
Exclusively breastfed, n (%)		64 (68.1)		77 (74)	
Exclusively formula-fed, n (%)		6 (6.4)		5 (4.8)	
Mixed feeding, n (%)		24 (25.5)		22 (21.2)	

Median (25th-75th percentile).
Abbreviations: BMI, body mass index; IGFBP-3, IGF binding protein 3.
[a]Umbilical cord concentrations.

possible confounders. Mean IGFBP-3 (SDS) was positively associated with mean weight velocity (SDS) (β = 0.16 [95% CI, 0.03-0.29], *P* = 0.01), but not with mean length velocity (SDS) β = 0.07 [95% CI, -0.04 to 0.19], *P* = 0.20). Similar results were observed when the same multivariate regression models were performed for mean IGFBP-3 (SDS): IGFBP-3 was positively associated with mean weight velocity (SDS) (β = 0.14 [95% CI, 0.01-0.28], *P* = 0.04, but not with mean length velocity (SDS) (β = 0.06 [95% CI, 0.05-0.17]).

Mean IGF-I SDS was higher in the exclusively formula-fed infants compared with breastfed infants (0.57 ± 0.68 SD vs 0.03 ± 0.86 SD, *P* = 0.01), but the mixed-feeding group did not differ from the other groups. There was no significant difference in mean length SDS or mean weight SDS between the feeding groups (data not shown). Mixed-fed infants had higher mean length velocity SDS and mean weight velocity compared with breastfed infants (0.36 ± 0.71 vs -0.17 ± 0.71, *P* < 0.001 and -0.35 ± 0.82 vs -0.14 ± 0.81, *P* = 0.001), but formula-fed infants did not differ from the other groups.

The genotypes were distributed as follows: *PAPPA2* rs1325598 C > T CC 27.3% (n = 61), CT 55.2% (n = 123), TT 17.5% (n = 39), *PAPPA* rs751543 A > G AA 46.4% (n = 104), AG 45.1% (n = 101), GG 8.5% (n = 19), and *STC2* rs889014 C > T CC 42.9% (n = 96), CT 47.3% (n = 106), TT 9.8% (n = 22). No significant associations were observed with mean length velocity (SDS) and mean weight velocity (SDS) for any of the 3 SNPs (data not shown).

Discussion

In this longitudinal study of 233 healthy infants with serial blood samples, we present detailed references curved for the dynamic changes in length, weight, and circulating IGF-I and IGFBP-3 concentrations during first year of life. Weight velocity was positively associated with IGF-I levels even after adjusting for feeding pattern, birth weight, target height, *PAPPA2* genotypes, and body fat percent. In addition, we present detailed reference curves for the dynamic changes in length, weight, and circulating IGF-I and IGFBP-3 concentrations during first year of life.

Children follow individual distinct growth trajectories and growth in infancy is a continuation of the rapid growth during fetal life. As expected, we found both high length and weight velocities at birth, which rapidly declined during infancy. In older children, deviations from an individual's growth trajectory may indicate the existence of underlying disease, but during infancy minor "catch-up" and "catch-down" growth patterns are usually regarded as early nutritional adaptations. A former study suggested that an increased growth rate in infancy reflects a mechanism to compensate for insufficient nutrition during fetal life (30). Thus, the majority of children born small for gestational age have spontaneous catch-up growth during

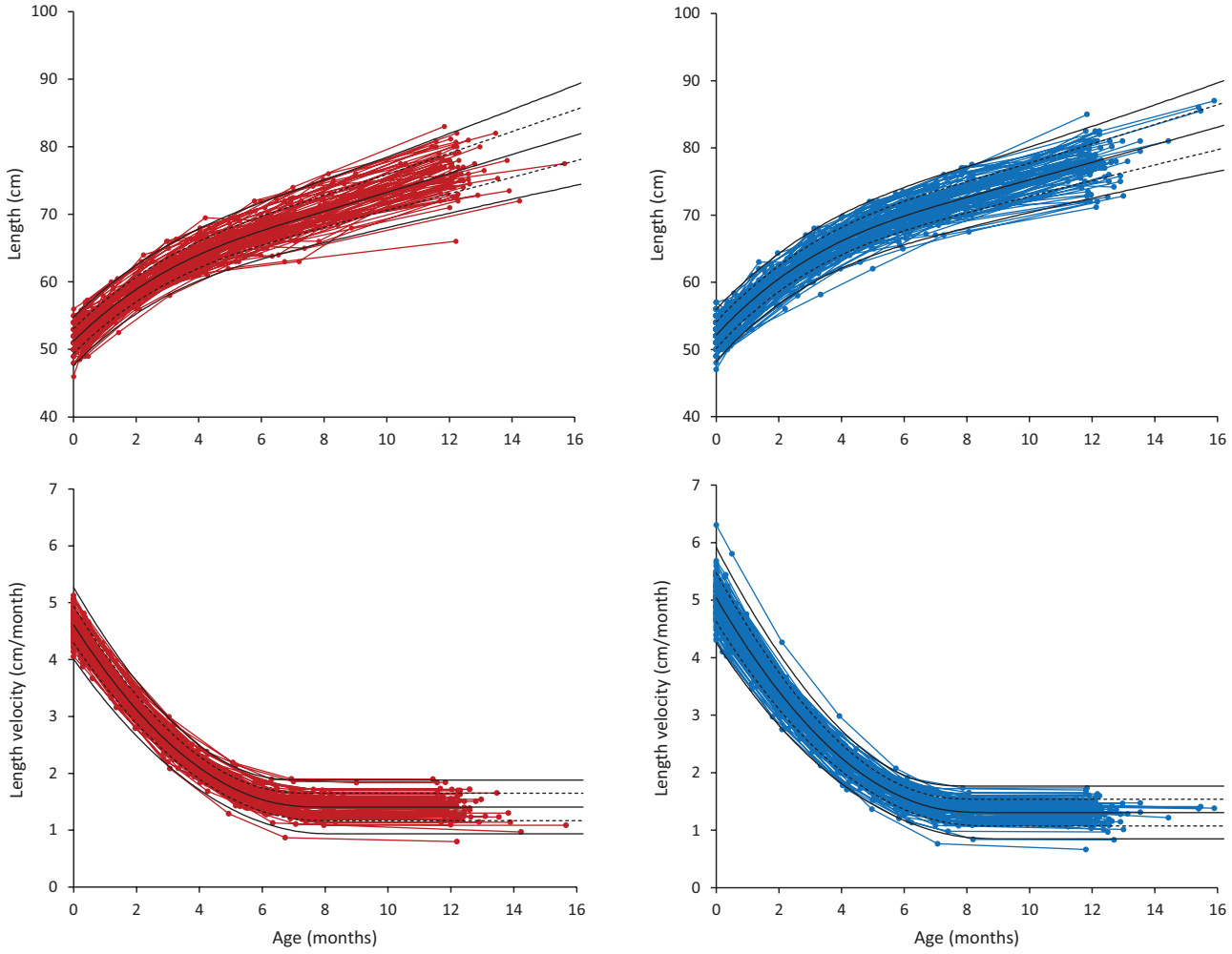

Figure 1. Individual growth trajectories for length (upper panel) and length velocity (lower panel) in girls (red lines) and boys (blue lines) according to age. Solid black lines represent +2 SD, median, and -2 SD and dotted black lines represent +1 SD and -1 SD, respectively.

infancy. Numerous studies have shown that children with rapid growth early in life have an increased risk of development of overweight and obesity in childhood that may have long-term deleterious health consequences (1). However, in the current cohort comprising healthy, term, and AGA infants, we found as expected a stable growth pattern during the first year of life.

Serum IGF-I and IGFBP-3 concentrations are believed to be important for growth during infancy (13, 14); however, the circulating levels are lower during this period relative to older children and adults. Overall, in this large longitudinal cohort, we found that IGF-I decreased during the first months of life followed by a plateau around 6 months of age in both boys and girls. Notably, this pattern closely resembled the shape of the length and weight velocity curves. Interestingly, we found that IGF-I levels increased from cord blood until 10 days of age in boys, but not in girls. In accordance, Kurtoğlu et al reported increasing IGF-I levels during the neonatal period (31). It could be speculated that the greater increase in IGF-I in boys during the first postnatal weeks reflects the higher postnatal growth velocity compared with girls. A previous study demonstrated a strong positive association between postnatal growth velocity and IGF-I concentrations (32). Only a few studies have reported on IGF-I concentrations at more than 1 timepoint during first year of life; nevertheless, the ranges of IGF-I concentration found in the present study are comparable with the majority of the previously published cross-sectional as well as longitudinal data (33-35) (for review see (36)).

Age- and sex-specific reference curves for IGF-I and IGFBP-3 are useful tools for the pediatric endocrinologist in evaluating growth-related conditions in children. However, to our knowledge, detailed reference ranges for children aged 0 to 1 years of age have not previously been reported. IGF-I and IGFBP-3 have been found valuable in the diagnostic workup of infants suspected of GH deficiency (37). However, we demonstrate that the lower reference range

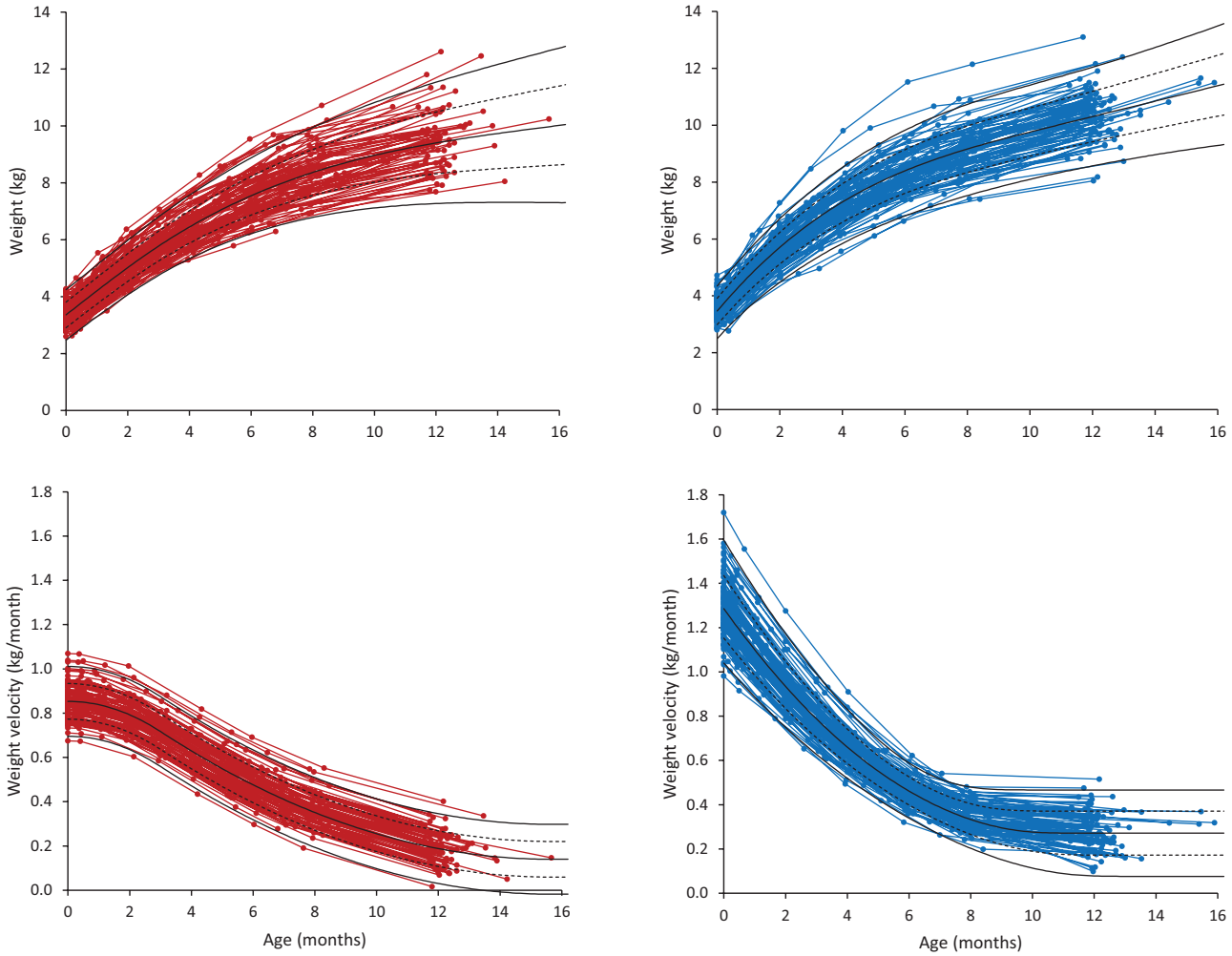

Figure 2. Individual growth trajectories for weight (upper panel) and weight velocity (lower panel) in girls (red lines) and boys (blue lines) according to age. Solid black lines represent +2 SD, median, and -2 SD and dotted black lines represent +1 SD and -1 SD, respectively.

corresponding to -2 SD for IGF-I in girls is very close to the detection limit of the assay. The lower part of the normal range for IGFBP-3 is at a proper distance from the detection limit of the assay enhancing the diagnostic value of low IGFBP-3 in infancy.

Postnatal weight gain, but not longitudinal growth, was positively associated with mean IGF-I during first year of life after adjustment for relevant confounders. Additional stratification for sex revealed that this association remained significant in girls exclusively. Previous studies have mainly evaluated the relationship between a single IGF-I measurement at a specific age and length and weight measurements before or after this time point. In a previous study, no association was found between IGF-I concentrations at 3 months and subsequent weight gain between ages 3 and 12 months, but a positive relation was found between IGF-I and length gain at this age (10). Another study, with IGF-I measurements at different ages during first year of life, found positive correlations between IGF-I and both current weight and length as well as positive correlations with the increase in both weight and length in the month following IGF-I measurement (38). Increases in body weight do not only reflect increases in fat mass, but also include lean mass. In our study, adjustment for body fat did not change the association between IGF-I and weight velocity, suggesting that the association between IGF-I and weight velocity was not primarily mediated through gain in fat mass. IGF-I promotes bone growth (5), increases muscle mass (6), and stimulates the differentiation of preadipocytes (7), which suggest a stimulatory effect on body mass. During the first year of life, longitudinal growth is dependent on nutrition, which is supported by the present study showing significant associations between feeding patterns and length as well as weight velocity. IGF-I was significantly higher in formula-fed children compared with breastfed children. According to the Infancy-Childhood-Puberty growth model, the transition from infancy to childhood growth occurs around 10 months of age (39) after

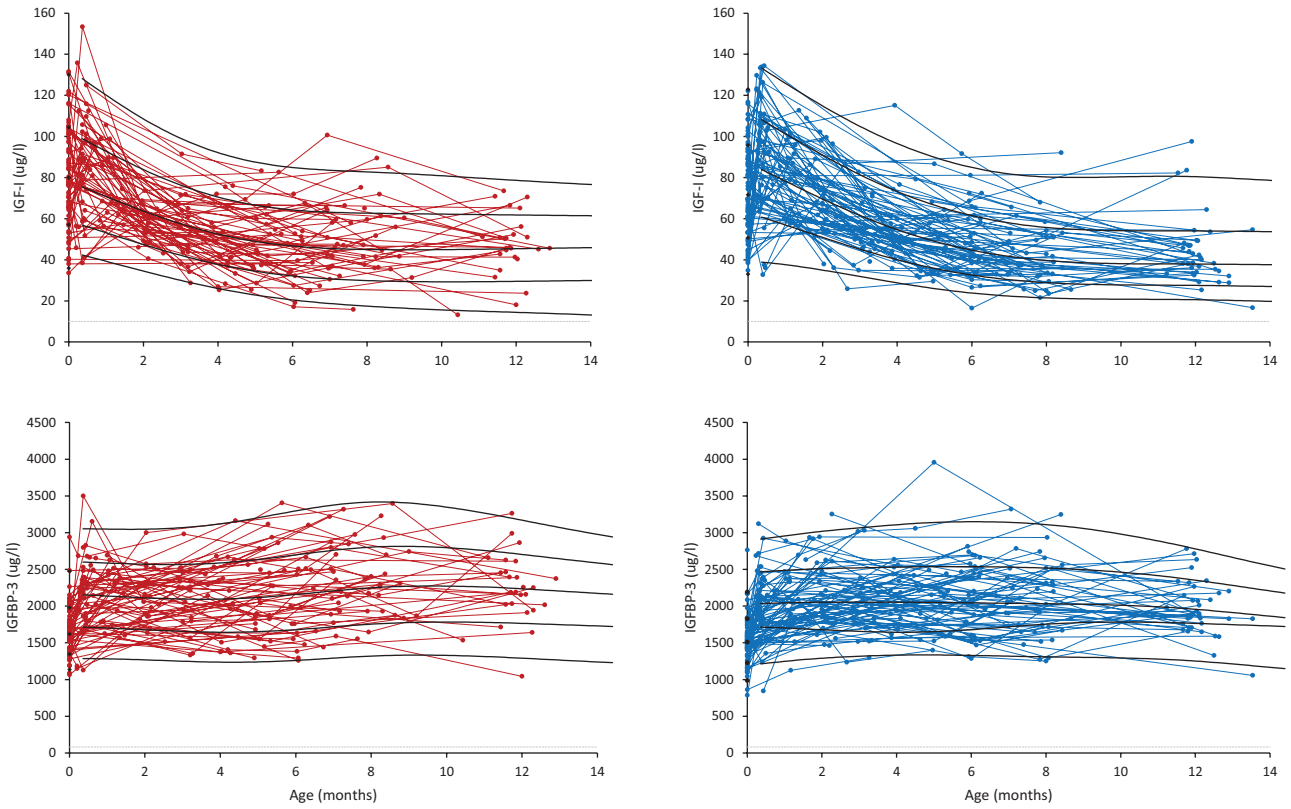

Figure 3. Longitudinal serum concentrations of IGF-I (upper panel) and IGFBP-3 (lower panel) in girls (red lines) and boys (blue lines) according to age. Black lines represent +2 SD, +1 SD, median, -1 SD, and -2 SD for the serum samples, respectively. Black dots represent the +2 SD, +1 SD, median, -1 SD, and -2 SD for the umbilical cord samples, respectively. The gray dotted line represents the detection limit of the assays. IGFBP-3, IGF binding protein 3.

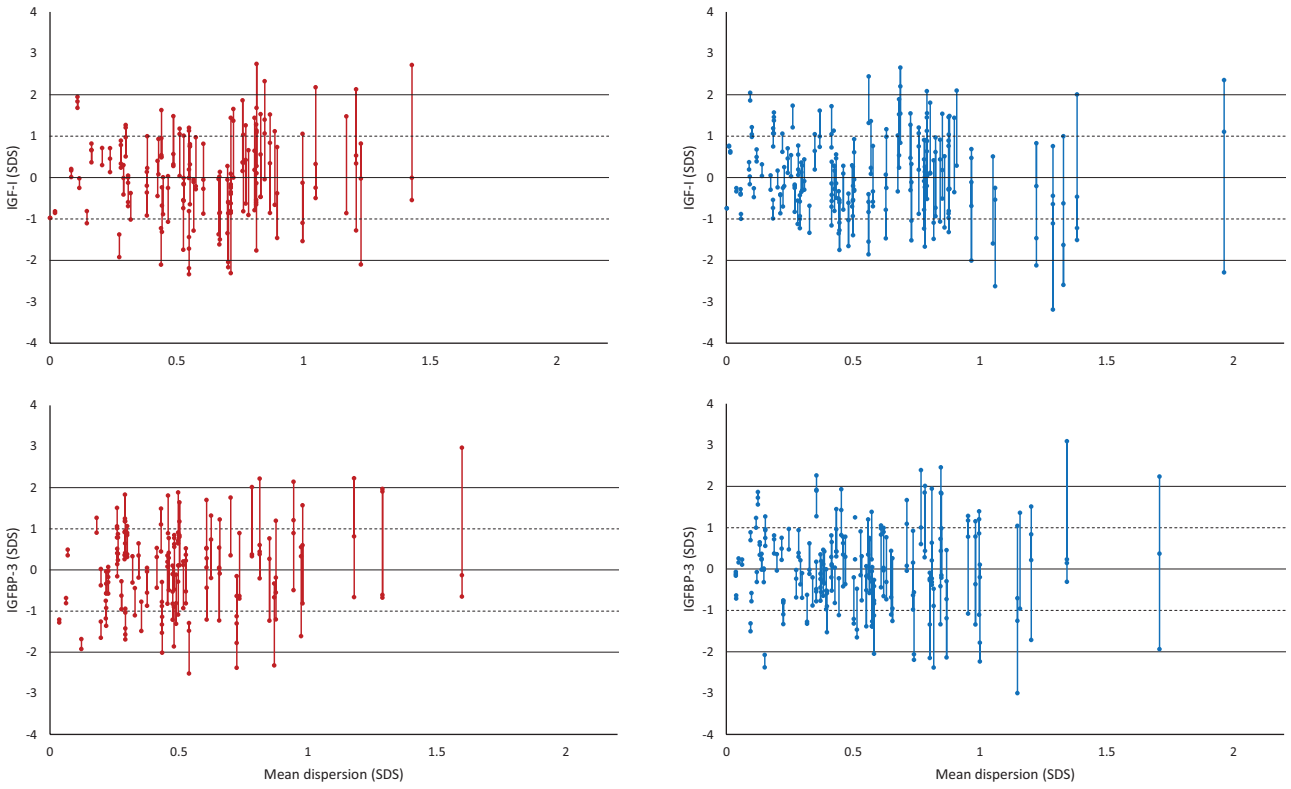

Figure 4. The intraindividual variation in serum IGF-I (upper panel) and IGFBP-3 (lower panel) concentration for girls (red lines) and boys (blue lines), respectively, expressed as SD scores for each individual sample according to the mean dispersion of each individual. IGFBP-3, IGF binding protein 3.

Table 2. Multiple regression analysis

	Length velocity (SDS)[a]		Weight velocity (SDS)[a]	
Model 1	r^2 = 33%		r^2 = 18%	
	Estimate (95% CI)	*P* value	Estimate (95% CI)	*P* value
IGF-I (SDS)[a]	0.09 (-0.03 to 0.21)	0.14	0.21 (0.06-0.36)	0.006
Birth weight (SDS)[b]	-0.18 (-0.31 to -0.06)	0.004	0.14 (-0.02 to 0.29)	0.09
Feeding pattern 0-4 months:				
Breastfed	Ref.		Ref.	
Formula-fed	0.49 (0.08-0.91)	0.02	0.28 (-0.25-0.80)	0.30
Mixed feeding	0.54 (0.32-0.77)	<0.0001	0.45 (0.17-0.73)	0.002
Target height (SDS)	0.42 (0.30-0.55)	<0.0001	0.27 (0.10-0.43)	0.002
PAPPA2 genotype				
CC (major allele)	Ref.		Ref.	
CT	0.005 (-0.21 to 0.22)	0.96	0.14 (-0.13 to 0.41)	0.30
TT (minor allele)	0.003 (-0.27 to 0.28)	0.98	-0.01 (-0.36 to 0.34)	0.96
Model 2	r^2 = 33%		r^2 = 28%	
	Estimate (95% CI)	*P* value	Estimate (95% CI)	*P* value
IGF-I (SDS)[a]	0.09 (-0.03 to 0.21)	0.14	0.19 (0.05-0.33)	0.007
Birth weight (SDS)[b]	-0.18 (-0.31 to -0.05)	0.006	0.04 (-0.11 to 0.20)	0.59
Feeding pattern 0-4 months:				
Breastfed	Ref.		Ref.	
Formula-fed	0.50 (0.08-0.92)	0.02	0.42 (-0.08 to 0.92)	0.10
Mixed feeding	0.54 (0.32-0.77)	<0.0001	0.46 (0.19-0.73)	0.001
Target height (SDS)	0.43 (0.29-0.56)	<0.0001	0.35 (0.19-0.51)	<0.0001
PAPP-A2 genotype				
CC (major allele)	Ref.		Ref.	
CT	0.005 (-0.21 to 0.22)	0.96	0.13 (-0.13 to 0.38)	0.33
TT (minor allele)	0.003 (-0.276 to 0.282)	0.98	-0.04 (-0.37 to 0.29)	0.80
Body fat (SDS)	0.005 (-0.14 to 0.16)	0.94	0.41 (0.23 to 0.59)	<0.0001

Abbreviations: PAPP-A2, pregnancy plasma protein-A2; SDS, SD score.
[a]Mean.
[b]Adjusted for gestational age.

which GH (and IGF-I) becomes increasingly important for growth. Likewise, regulation of IGF-I is highly dependent on nutrition in early life (9), whereas hormonal factors, mainly GH and thyroid hormones, regulate IGF-I in older children. In addition, food intake induces a rise in portal insulin that decreases IGFBP-1 secretion leading to a subsequent increase in bioavailable IGF-I. Furthermore, insulin promotes hepatic IGF-I synthesis (40), which implies a regulatory effect of insulin on the IGF-I concentration.

In our study, target height, calculated from parental heights, was strongly associated with both infant length and weight velocity, which supports a genetic influence of growth during the first year of life. Height is a highly heritable polygenetic trait and 60% of the variation can be explained by common variants in > 400 genetic loci (41), including SNPs in genes encoding the known IGF-I regulators PAPP-A2, PAPP-A, and STC2, which are significantly associated with adult height (19). Therefore, we studied these genetic variants in relation to growth in infancy but found no association between *PAPPA2* genotypes with neither length nor weight velocity. By contrast, we found an association between *PAPPA2* genotypes and childhood height in a recent study (20). However, our sample size is probably too small to detect statistically significant effect sizes of a single genetic variant.

A major strength of the present study is the prospective longitudinal design in combination with a well-characterized cohort. However, the lack of IGF-I and IGFBP-3 measurements in all infants at all timepoints is a minor limitation. This was not always possible for various reasons (eg, failed venipuncture, the parent declining blood sampling at a specific visit, insufficient blood volume for the analysis). All children were Caucasian, born at term and AGA, which could theoretically introduce bias. However, our serial IGF-I measurements are in agreement with observations obtained from

previous cross-sectional studies of different ethnicities (42) (for review see (36)). Thus, we believe our results based on Danish Caucasian infants are representative for other populations. Similarly, the presented reference curves for IGF-I and IGFBP-3 are laboratory and assay-specific and may not be directly applicable in other centers or settings. Finally, the number of children who were exclusively formula-fed was much smaller compared with the other groups.

In conclusion, we present individual growth trajectories for length, weight, length velocity, and weight velocity in 233 Danish infants. Our data showed that IGF-I decreased during infancy, whereas IGFBP-3 remained stable. Weight velocity, but not length velocity, was positively associated with circulating IGF-I levels in infant girls during the first year of life. This suggests that IGF-I during infancy promotes an overall gain in mass rather than a specific gain in longitudinal bone growth.

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Additional Information

Correspondence: Emmie N. Upners, MD, Department of Growth and Reproduction, GR-5064, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. Email: emmie.nicolina.upners.02@regionh.dk.

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Data Availability: Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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