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ARTICLE

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IGF-I and IGFBP-3 concentrations at 2 years: associations with anthropometry and milk consumption in an Indian cohort

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Abstract

Background/objectives To ascertain associations between plasma insulin-like growth factor I (IGF-I), insulin-like growth factor-binding protein 3 (IGFBP-3) and their molar ratio at 2 y with neonatal size, infant growth, body composition at 2 y, and feeding practices in an Indian cohort.

Subjects/methods A cohort of 209 newborns, with 122 followed at 2 y. Anthropometry was conducted at birth and 2 y. IGF-I and IGFBP-3 concentrations were measured in cord blood and at 2 y. Maternal and child diet was assessed by food frequency questionnaires and maternal interviews. Multivariate regression was used to test for associations adjusting for confounding factors.

Results Mean 2 y plasma IGF-I and IGFBP-3 concentrations and IGF-I/IGFBP-3 were 49.4 ng/ml (95% CI: 44.1, 54.8), 1953.8 ng/ml (CI: 1870.6, 2036.9) ng/ml, and 0.088 (CI: 0.081, 0.095), respectively. IGF-I and IGF-I/IGFBP-3 were positively associated with current length, but not body mass index or adiposity. IGF-I was higher among those with greater change in length since birth. IGF-I concentrations were higher in children who drank the most milk (>500 vs. <250 ml per day: 65.6 vs. 42.8 ng/ml, $p < 0.04$), received other milk <6 months compared to ≥6 months (56.3 vs. 44.8 ng/ml, $p < 0.05$), and in those whose mothers consumed milk daily vs. less frequently in late pregnancy (56.4 vs. 42.7 ng/ml, $p < 0.01$). In multivariate regression, 2 y IGF-I concentration and IGF-I/IGFBP-3 were each positively associated with current length and milk intake. IGFBP-3 was not related to anthropometry or milk intake.

Conclusions Plasma IGF-I concentrations and IGF-I/IGFBP-3 at 2 y are positively associated with length at 2 y and current milk intake.

Introduction

Insulin-like growth factor I (IGF-I) is a small peptide that regulates growth and development [1]. It is produced in the liver and other tissues, and stimulates cell division and differentiation through endocrine, paracrine, and autocrine actions. Circulating concentrations of IGF-I are affected by both nutrition and pituitary growth hormone. IGF-I's binding proteins, especially IGFBP-3, may also independently regulate growth [1].

Plasma IGF-I concentrations are consistently associated with measures of length in cross-sectional studies in infancy and early childhood [2–6], as well as with prior linear growth rates [7–9]. Plasma IGF-I concentrations also predict subsequent growth rates in some [2, 3] but not all studies [7]. Associations between IGF-I and body mass index (BMI) or adiposity have been mixed [2, 8, 9]. IGF-I in early childhood has been negatively associated with birth weight [6, 7, 9]. Fetal IGF-I is responsive to maternal nutrition [10], and, in infancy, plasma IGF-I concentration is primarily regulated by diet [11]. Infants consuming formula or breastmilk have been reported to have lower IGF-I than those consuming cow's milk [12], and breastfed infants have lower IGF-I than those who consume formula [5, 9, 13], independent of weight [7, 9]. Animal protein intake,

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especially from cow's milk, has been positively correlated with plasma IGF-I concentration in children [4, 14].

We recently reported that maternal milk intake in the third trimester of pregnancy was positively related to IGF-I in cord blood, independent of neonatal size [15], and umbilical cord IGF-I was positively associated with neonatal adiposity, but not length, in Indian newborns [15]. We have followed up on children in this cohort, and now report plasma IGF-I and IGFBP-3 concentrations and their molar ratio (IGF-I/IGFBP-3), considered to be an indicator of bioactive IGF-I [16], at 2 y and their associations with neonatal and cord blood measurements and body size and composition at 2 y. We also investigated associations with breastfeeding practices and milk consumption patterns.

Subjects and methods

Details of the observational birth cohort at the Diabetes Unit, King Edward Memorial Hospital (KEM) Research Centre, Pune, India have been described elsewhere [17, 18]. Healthy pregnant women attending antenatal clinics in the urban and rural centres were enrolled between May 2004 and February 2006 to study maternal nutrition in pregnancy, fetal growth, and birth outcomes. Babies were followed up for measurements of growth, and mothers provided information on infant feeding practices.

Anthropometric measurements of mothers, neonates, and children were performed by trained personnel using standardized methods. The coefficient of variation between measures by different personnel was <2.0%. Neonatal weight was measured to the nearest 1 g (ATCO, Mumbai), and crown-heel length to the nearest mm (Pedobaby Babymeter, ETS J.M.B. Brussels), with the head touching the headboard and legs straightened. At 2 y child weight (to the nearest 0.005 kg, Conveigh, Mumbai) and length (to the nearest mm, Pedobaby Babymeter) were assessed. Triceps and subscapular skinfolds were measured on the left side to the nearest 0.2 mm using Harpenden skinfold calipers (CMS Instruments, London). Subscapular skinfold was measured at the inferior angle of the scapula. Triceps skinfold was measured midway between the tip of the acromion process and the tip of the olecranon. These measures were summed (sum-of-skinfolds) and divided by weight as a measure of child adiposity. Mid-upper arm circumference was measured at the same site to the nearest 0.1 mm using a fiberglass measuring tape (Chasmors, London). Maternal height was measured to the nearest mm at 34 weeks gestation (Harpenden Stadiometer, CMS Instruments, London).

Maternal milk intake at 34 weeks gestation and child diet at 2 y were assessed using a semi-quantitative food frequency questionnaire based on local practices. Maternal milk intake was classified into never/occasionally or ≥once daily. Milk consumption for 2-year-old children was classified by 250 ml increments and into three groups: <250 ml; ≥250 ml, and ≤500 ml; >500 ml per day. Macronutrient intake was calculated from a food nutrient database created from local foods [19]. At the 2 y visit, mothers reported duration of exclusive breastfeeding, age at weaning, age at introduction of other milk (cow, buffalo, or goat), and formula usage. At enrollment, socioeconomic status was assessed using the Standard of Living Index (SLI) devised by the National Family Health Survey of India [20].

At birth umbilical cord blood was collected from the placental end of the cord, centrifuged (4 °C, 2500 $g \times 15$ min) within an hour of collection, and plasma was stored at −70 °C. At 2 y a non-fasting venous blood sample was drawn, with the same preparation and storage procedure.

IGF-I and IGFBP-3 measurements were done on stored samples (−70 °C) using enzyme-linked immunosorbent assay (Mediagnost, Reutlingen, Germany), with a sensitivity of 0.09 ng/ml. All steps were performed as per the manufacturer's instructions. Calibration was done against the WHO International standard preparation. Inter- and intra-batch coefficients of variation were 8.5% and 2.8%, respectively.

Statistics

Descriptive statistics are reported using means and standard errors. *Z*-scores for size at birth and 2 y were computed from the WHO international standards using the STATA module [21]. Infants were categorized into those with catch up (*z*-score change ≥0.67), catch down (*z*-score change ≤ −0.67), or steady growth (*z*-score change > −0.67 and <0.67) in BMI-for-age and length-for-age *z*-scores from birth to 2 y [22]. The molar ratio IGF-I/IGFBP-3 was calculated as 1 ng/ml IGF-I = 0.13 nM and 1 ng/ml IGFBP-3 = 0.036 nM. All variables were normally distributed except for IGF-I and IGFBP-3 at birth and 2 y. Spearman correlations were calculated to test for associations between IGF-I, IGFBP-3, their molar ratio, and child anthropometric characteristics at birth and 2 y. The Wilcoxon rank sum test was used to assess differences in 2 y IGF-I, IGFBP-3, and IGF-I/IGFBP-3 by demographic and dietary categories; *t*-tests were used for anthropometric variables. Multiple linear regression was employed to test associations adjusting for confounders. For these analyses IGF-I, IGFBP-3, and their molar ratio were log transformed to better approximate normality, and results were back transformed for ease of interpretation. STATA SE15 was used for all statistical analysis [23]. Statistical significance was established at $p < 0.05$.

Results

Figure S1 illustrates the sample selection process. Of 209 total deliveries, 205 participants had maternal demographic, height, and milk consumption data in the third trimester. Of these, 153 had newborns with complete anthropometry and umbilical cord IGF-I and IGFBP-3 concentration measures. At 2 y 132 had anthropometric data, IGF-I, and IGFBP-3 concentrations, and 122 also had information on current diet and infant feeding practices. Compared to the 87 participants without complete data, those in the sample did not differ in anthropometry at birth or at 2 y, maternal milk intake, diet at 2 y or infant feeding patterns ($p > 0.05$ for all). Participants did have significantly higher SLI scores ($p < 0.05$). Average maternal height was 154.7 (0.5) cm and age at delivery was 23.0 (0.3) y.

Anthropometry and IGF-I and IGFBP-3 at birth and 2 y

Child characteristics are described in Table 1. Similar numbers of children came from rural (62) and urban (60) areas. Mean age was 24.6 months. Boys were heavier and longer than girls, while girls were more adipose. Thirty-seven percent had experienced catch-up growth in length and 25% experienced catch-down growth in length between birth and 2 y. For BMI, over half (52%) showed catch-up growth and 17% showed catch-down growth.

IGF-I concentration at 2 y was not different from IGF-I in umbilical cord blood. IGFBP-3 was higher at 2 y than at birth among both males and females, while the molar ratio was lower at 2 y than at birth. Females had higher IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratios at birth; at 2 y there were no sex differences in IGF-I or the molar ratio, but females retained higher IGFBP-3 concentrations. IGF-I, IGFBP-3, and IGF-I/IGFBP-3 at 2 y were each higher among urban than rural children (IGF-I: 57.7 vs. 41.4 ng/ml, $p < 0.01$; IGFBP-3: 2069 vs. 1842 ng/ml, $p < 0.01$; IGF-I/IGFBP-3: 0.097 vs. 0.079, $p < 0.04$).

As shown in Supplementary Table S1, IGF-I at 2 y was positively correlated with current weight, length, and Δlength z-score from birth to 2 y. These relationships were consistent among males and females. IGF-I at 2 y was not correlated with size at birth, cord IGF-I, or maternal height. IGFBP-3 at 2 y was not related to any measure of body size or 2 y but was correlated with cord IGF-I and IGFBP-3 concentration. At 2 y the molar ratio followed the same pattern as 2 y IGF-I, except that it was not related to change in length from birth to 2 y ($p < 0.07$). Cord IGF-I and the cord molar ratio were associated with a negative ΔBMI z-score from birth to 2 y.

Infant feeding practices and diet and IGF-I at 2 y

At 2 y children consumed an average of 3690 (138) kJ (882 [33] kcal) and 31 (1) g of protein, resulting in 14% of energy from protein. Breastmilk intake was not included in these totals, and 44% of children were still nursing at 2 y. Energy intake was below the 4435 kJ recommended by the National Institute of Nutrition/Indian Council for Medical Research for moderately active children age 1–3 y, while protein intake was sufficient (15–16 g protein per day recommended for children age 2–3 y) [24]. As Table 2 shows, all babies were breastfed. Twenty-two percent received infant formula, and only three received it exclusive of breastmilk. Forty percent received another form of milk before 6 months. Over 90% of children drank milk daily at 2 y, and the majority drank between 250 and 500 ml milk per day (mean = 379 ml). Approximately half drank cow milk and half drank buffalo milk; only 5 children drank goat milk. Over one-quarter consumed a vegetarian diet (no eggs, meat, or fish), but only seven children in the sample consumed these animal products at least once per day.

Children's milk intake did not vary by rural/urban residence or SLI. Children who drank more milk also had greater energy, protein, and fat intake ($r = 0.31$, 0.40, 0.51, respectively, $p < 0.001$). Those who received other milk early (<6 months) consumed more milk at 2 y than those who were supplemented with milk later [467 (59) vs. 320 (19) ml per day; $p < 0.01$]. Duration of breastfeeding was positively correlated with the age at which another source of milk was added to the diet ($r = 0.31$, $p < 0.001$).

In late pregnancy, almost half of women reported daily milk intake, but children's milk intake at 2 y did not differ between mothers who drank milk daily vs. less frequently.

IGF-I and IGF-I/IGFBP-3 at 2 y were higher among children who drank more milk at 2 y, received other milk at <6 months, drank cow's milk compared to buffalo milk, consumed a non-vegetarian diet (including eggs, meat, and fish), and whose mothers drank milk daily in late pregnancy (Table 2). IGFBP-3 was higher among children who drank cow's milk compared to buffalo milk but did not vary by other dietary practices. IGF-I, IGFBP-3, and IGF-I/IGFBP-3 at 2 y did not differ by breastfeeding practices or formula usage and were not correlated with current energy or protein intake. Children who were nursed 18+ months or who were received other milk after 6 months were shorter than those who were breastfed less or received other milk within the first 6 months. Length and BMI at 2 y did not differ by levels of current milk intake. Reported frequency of non-vegetarian food intake was not correlated with any anthropometric measure.

To ascertain whether associations between 2 y IGF-I, length, and milk intake were independent of confounding

Table 1 Body size and IGF measurements at birth and 2 y of age

2 y children	All participants ($n = 122$)	Males ($n = 65$)	Females ($n = 57$)
Age (months)	24.6 (0.1)	24.6 (0.1)	24.5 (0.1)
Length (cm)	85.0 (0.3)	85.9 (0.4)	83.9 (0.4)**
Weight (kg)	10.4 (0.1)	10.8 (0.1)	10.1 (0.2)***
BMI (kg/m^2)	14.5 (0.1)	14.6 (0.1)	14.3 (0.1)
MUAC (cm)	14.9 (0.1)	15.0 (0.2)	14.7 (0.1)
Triceps skinfold (mm)	8.5 (1.2)	8.3 (0.2)	8.8 (0.2)
Subscapular skinfold (mm)	6.3 (0.1)	6.2 (0.2)	6.5 (0.2)
Sum of skinfolds/weight (mm/kg)	1.4 (0.02)	1.3 (0.03)	1.5 (0.03)***
IGF-I (ng/ml)	49.4 (2.7)	46.6 (3.5)	52.6 (4.2)
IGFBP-3 (ng/ml)	1953.8 (42.0)	1872.5 (61.8)	2046.4 (53.7)*
IGF-I/IGFBP-3 (molar ratio)	0.088 (0.004)	0.087 (0.005)	0.089 (0.005)
Birth weight (g)	2819.9 (35.5)	2839.5 (50.1)	2797.4 (50.4)
Birth length (cm)	48.7 (0.2)	49.2 (0.2)	48.2 (0.2)**
Growth: birth to 2 y			
Δ length *z*-score	0.27 (0.1)	0.36 (0.2)	0.18 (0.1)
Δ BMI *z*-score	0.74 (0.1)	0.81 (0.2)	0.65 (0.2)
Cord IGF-I (ng/ml)	47.4 (2.4)	43.8 (3.4)	51.5 (3.3)*
Cord IGFBP-3 (ng/ml)	1261.7 (47.9)[a]	1219.7(70.3)[a]	1309.6 (63.9)*[a]
Cord IGF-I/IGFBP-3 (molar ratio)	0.136 (0.005)[a]	0.129 (0.008)[a]	0.145 (0.007)*[a]

Data are means (SEM). Sex differences evaluated by *t*-test for anthropometric variables and Wilcoxon rank sum test for IGF-I, IGFBP-3, and IGF-I/IGFBP-3

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

[a]Cord IGFBP-3 and IGF-I/IGFBP-3 significantly different from 2 y IGFBP-3 and IGF-I/IGFBP-3, respectively, $p < 0.001$ for all, based on Wilcoxon signed rank test

variables, we conducted multiple linear regression analysis incorporating maternal height, sex, birth weight, cord IGF-I, non-vegetarian diet, rural/urban residence, and SLI in the base model. Of these, only urban residence was significantly associated with 2 y IGF-I. We then added current length (Table 3, Model 1). Birth weight was negatively associated with IGF-I, while cord IGF-I concentration and current length were positively associated with the outcome; urban residence was no longer significant. In Model 2 we added current milk intake, and all relationships from Model 1 remained. IGF-I at 2 y was higher among children consuming >500 ml milk per day at 2 y compared to those consuming <250 ml (52.9 vs. 37.2 ng/ml, $p < 0.04$). Further adjustment for total calorie and protein intake (Model 3) did not alter these relationships. Consumption of other milk <6 months and daily maternal milk intake in late pregnancy was not significantly related to 2 y IGF-I when added to Model 1 ($\beta = 0.147$, $p < 0.11$, $\beta = 0.157$, $p < 0.07$, respectively).

We ran the same models 1–3 using the molar ratio IGF-I/IGFBP-3 as the dependent variable, with similar outcomes to the IGF-I analysis. In Model 1, birth weight had a negative association with the molar ratio while current length had a positive association. In Model 2, current length and milk intake >500 ml each contributed to a higher molar ratio, and further adjustment for total calories and protein (Model 3) did not change those relationships. Compared to children drinking <250 ml milk, those consuming >500 ml had higher IGF-I/IGFBP-3 in Model 3 (0.099 vs. 0.071, $p < 0.04$). Running Models 1–3 with 2 y IGFBP-3 as the dependent variable yielded no relationships with child length or milk consumption.

To assess whether the observed differences in IGF-I concentration at 2 y between children drinking cow's or buffalo milk daily were independent of other factors, we ran Model 4 (Table 3) adding type of milk as a covariate ($n = 100$). Those who drank cow's milk had higher IGF-I than those who drank buffalo milk (47.6 vs. 34.7 ng/ml, $p < 0.02$), independent of quantity of milk intake, which became insignificant. IGFBP-3 and the IGF-I/IGFBP-3 molar ratio were each higher among children drinking cow's milk compared to buffalo milk when Model 4 was run using each as the dependent variable (IGFBP-3: 2027.8 vs. 1818.5 ng/ml, $p < 0.04$; molar ratio IGF-I/IGFBP-3: 0.085 vs. 0.069, $p < 0.05$).

IGF-I concentration at 2 y was greater among children with catch-up growth in length compared to those with catch-down growth (46.8 vs. 33.8 ng/ml, $p < 0.03$), after adjusting for birth weight, cord IGF-I, gender, maternal height, place of residence, SLI, vegetarian diet, and total

Table 2 Dietary characteristics, anthropometry, and IGF-I, IGFBP-3, IGF-1/IGFBP-3 at 2 y

	n	IGF-I (ng/ml)	IGFBP-3 (ng/ml)	IGF-I/ IGFBP-3	Length (cm)
Maternal milk intake at 34 weeks					
Never or occasionally	62	42.7 (2.9)	1907.8 (63.7)	0.079 (0.004)	84.9 (0.4)
At least once daily	60	56.4 (4.4)*	2001.3 (54.3)	0.097 (0.006)*	85.0 (0.5)
Children					
Total duration of breastfeeding					
≤12 months	30	52.1 (6.1)	1898.5 (67.3)	0.094 (0.008)	85.8 (0.7)
>12 months & ≤18 months	30	47.8 (4.9)	1845.9 (60.5)	0.089 (0.007)	85.5 (0.6)
>18 months or still nursing @ 2 y visit	54	46.3 (3.5)	2022.2 (75.8)	0.081 (0.005)	84.0 (0.5)*[a]
don't know, but no longer nursing	8	66.2 (16)	2103.5 (167.4)	0.106 (0.020)	86.4 (1.2)
Introduction of other milk					
<6 months	49	56.3 (4.8)*	1964.9 (57.4)	0.028 (0.002)**	86.0 (0.6)**
≥6 months	73	44.8 (3.0)	1937.3 (60.9)	0.022 (0.001)	84.3 (0.4)
Current milk intake (ml)					
<250 ml	31	42.8 (4.2)	1976.3 (118.2)	0.076 (0.005)	84.3 (0.6)
>250 ml & ≤500 ml	74	48.5 (3.6)	1911.9 (44.7)	0.087 (0.005)	85.1 (0.4)
>500 ml	17	65.6 (6.9)*	2095.2 (80.3)	0.111 (0.010)*	85.6 (1.2)
Formula usage					
Yes	27	53.6 (7.7)	2035.2 (99.6)	0.089 (0.009)	84.8 (0.7)
No	95	48.2 (2.7)	1930.6 (45.9)	0.088 (0.004)	85.0 (0.4)
Drinking cow's milk daily	51	55.1 (4.6)**	2089.6 (73.8)**	0.092 (0.006)*	84.8 (0.5)
Drinking buffalo milk daily	49	40.7 (3.3)	1842.3 (50.9)	0.077 (0.005)	85.7 (0.5)
Vegetarian diet at 2 y	32	41.7 (4.5)	1885.0 (102.2)	0.091 (0.004)	85.0 (0.7)
Non-vegetarian diet at 2 y	90	52.2 (3.3)*	1978.2 (43.9)	0.078 (0.007)	85.0 (0.4)

Data are means (SEM)

*$p < 0.05$; **$p < 0.01$, differences in IGF-I, IGFBP-3, and molar ratio evaluated by Wilcoxon ranked sum or Kruskal–Wallis test; height differences by *t*-test or ANOVA

[a]Compared to <12 months

energy and protein intake. This difference became non-significant after adjusting for current length and milk intake, each of which was positively associated with IGF-I. Children with high milk intake (>500 ml) had greater IGF-I compared to those whose milk intake was <250 ml (55.9 vs. 36.9, $p < 0.05$) (see Fig. 1a). Higher IGFBP-3 at 2 y was found among children with steady growth in length compared to catch down growth after adjusting for the same initial covariates (1997.3 vs. 1747.2 ng/ml, $p < 0.02$). This difference was somewhat attenuated after inclusion of current length (1896.8 vs. 1793.7 ng/ml, $p < 0.05$). Milk intake was not related to IGFBP-3 in this analysis (Fig. 1b). Children experiencing catch-up growth in length did not have higher IGFBP-3 compared to catch-down or steady growth. The molar ratio followed a similar pattern as IGF-I after adjustment for the same covariates (Fig. 1c).

Discussion

IGF-I at 2 y was positively correlated with length and greater linear growth since birth, but not current adiposity. IGF-I/IGFBP-3 at 2 y was positively related to length but not prior growth. IGF-I and IGF-I/IGFBP-3 were higher among children who drank more milk, independent of current length and macronutrient intake. Higher IGF-I and IGF-I/IGFBP-3 among children displaying catch-up growth in length were related to differences in milk intake.

We had previously demonstrated that neonatal adiposity, but not length, was correlated with cord IGF-I in this cohort [15], and suggested that IGF-I could contribute to the intrauterine development of the 'thin-fat' Indian phenotype [25]. Cord IGF-I was associated with a negative ΔBMI *z*-score from birth to 2 y, which was consistent with research in European populations showing that higher IGF-I in early

Table 3 Standardized beta coefficients from multiple regression predictors of IGF-I at 2 y

	Birth weight (kg)	Log (cord IGF-I, ng/ml)	Female gender	Child 2 y length (cm)	Milk intake >500 ml[a]	Cow milk vs. buffalo milk[b]
Model 1	−0.241*	0.210*	0.123	0.331**		
Model 2	−0.214*	0.195*	0.110	0.298**	0.211*	
Model 3	−0.218*	0.202*	0.121	0.314**	0.254*	
Model 4	−0.185	0.144	0.163	0.358**	0.215	0.276*

All models adjusted for maternal height, rural/urban residence, SLI, and non-vegetarian diet (all NS in Models 1–4). Models 3 and 4 include adjustment for total energy and protein intake (coefficients NS). No interactions between categorical independent variables were detected

*$p < 0.05$; **$p < 0.01$

[a]Reference category is 2 y milk intake <250 ml

[b]Reference category is buffalo milk

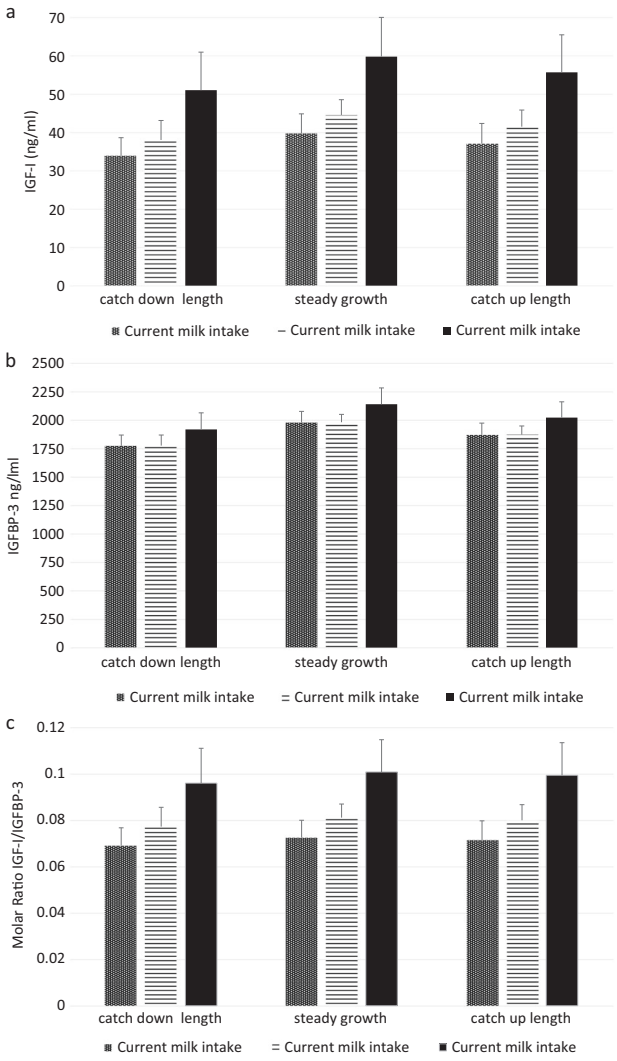

Fig. 1 **a** Differences in predicted IGF-I concentration at 2 y by milk intake and growth in length since birth. **b** Differences in predicted IGFBP-3 concentration at 2 y by milk intake and growth in length since birth. **c** Differences in the predicted IGF-I/IGFBP-3 molar ratio at 2 y by milk intake and growth in length since birth

infancy predicted lower adiposity during infancy and early childhood [3, 7]. Among participants in our study the relationship between IGF-I and body composition appears to shift from adiposity to linear growth between the pre- and post-natal periods. Our results support Ong et al.'s [3] suggestion that higher IGF-I contributes to the partitioning of weight gain from adiposity into linear growth over the course of infancy. It remains unclear what role IGFBP-3 plays in this process. IGFBP-3 at birth was associated with neonatal adiposity, but unrelated to size at 2 y; IGFBP-3 at 2 y was unrelated to body size or adiposity at 2 y. This contrasts with results from a recent study showing that IGFBP-3 was positively associated with central adiposity among 4-year-old South Asian children in the United Kingdom [26]. In our study, the molar ratio IGF-I/IGFBP-3 appeared to be associated with skeletal growth rather than adiposity.

In the main we found that IGF-I concentration and the molar ratio tended to have similar associations with diet or anthropometry. However, measurements of IGFBP-3 may be sensitive to the timing and glycemic index of a previous meal [27], and our measures of IGFBP-3 (and hence the molar ratio) may be biased since we did not calculate time since the prior meal or its glycemic index. IGF-I does not appear to be affected in the same way and so in this analysis, IGF-I results can be interpreted with greater certainty.

Unlike other studies [5, 7], we did not find relationships between breastfeeding practices, formula usage, and plasma IGF-I concentration. In our sample breastfeeding was universal and of long duration and formula usage occurred along with breastfeeding, rather than replacing it. Many infants received other milk before 6 months, and their higher IGF-I and IGF-I/IGFBP-3 at 2 y suggests that early milk intake may contribute to greater IGF-I activity in childhood. Similarly, in a cross-sectional study, male infants who were fed cow's milk had higher IGF-I compared to those consuming formula or breastmilk [12]. In contrast, IGFBP-3 does not appear to be related to

breastfeeding practices or quantity of milk intake, which is consistent with other studies [3, 12, 28].

An unexpected finding from this study was that children who consumed cow's milk had significantly higher IGF-I, IGFBP-3, and IGF-I/IGFBP-3 at 2 y than those who drank buffalo milk. This was the only dietary behavior associated with IGFBP-3 in the study. These differences remained significant in multivariate analysis and were independent of quantity of milk intake. Both milks are widely available and consumed in India. Buffalo milk is much higher in fat than cow's milk (6.9% vs. 3.4%), but is similar in protein and lactose content [29]. The cause of these differences remains unclear, and needs to be verified by additional studies in India.

Average IGF-I concentration at 2 y was lower than that found in most studies, which primarily are from European populations [30], although it was within the ±2 SD range [31]. Compared to a study of 2.5-year-old Danish children that showed positive associations between IGF-I, height, and milk intake [4], IGF-I concentrations were much lower in our study (46.6 and 52.6 ng/ml vs. 86.1 and 132.0 ng/ml for males and females, respectively), with no difference by sex. The Indian children were also substantially shorter (87.5 vs. 92.8 cm and 85.6 vs. 93.2 cm for males and females, respectively), with lower average BMIs (14.3 vs. 16.2 kg/m^2 and 14.0 vs.16.3 kg/m^2 for males and females, respectively), after standardizing for age to match the Danish sample. They drank similar amounts of milk as the Danish children and a similar percentage of their energy intake came from protein, although overall intake of both protein and energy was lower. Thus, the smaller size of children in our cohort and the high frequency of catch-down growth in length (25% of the participants) may contribute to their low IGF-I concentrations compared to the Danish children, although their IGF-I concentrations relative to their weight or length were also much lower.

Compared to IGF-I/IGFBP-3 data from multiple European and Canadian cohorts used to develop reference intervals for the molar ratio across the lifespan [16], our cohort's IGF-I/IGFBP-3 averages (0.087 and 0.089 for males and females, respectively) fell between the 2.5 and 50th percentile of 2-year-olds (0.042, 0.110 for males; 0.048, 0.108 for females). However, our means were similar to the molar ratio calculated from IGF-I and IGFBP-3 data reported for 12-month breastfed babies from the United Kingdom [3] (males = 0.078; females = 0.096) and 36-month-old Danish children [2] (males = 0.074; females = 0.093).

In our study greater maternal milk intake at 34 weeks gestation was associated with higher IGF-I and IGF-I/IGFBP-3 among children at 2 y, although the association weakened in multivariate analysis. This raises the possibility that a mother's milk consumption may exert a long-term influence ('programming') on IGF-I concentration and bioactivity in her offspring. On the other hand, others have reported that adults who drank more milk in early childhood or whose mothers consumed more milk during pregnancy exhibited lower IGF-I in adulthood [32, 33]. The children in our cohort are being followed up, and we will be able to evaluate if the maternal influences on offspring IGF-I axis persist later in life.

Strengths of our study include a longitudinal intergenerational design and serial follow-up into childhood, with collection of relevant physical and biomarker information in pregnancy (in utero), at birth, and in early childhood. Limitations of this study include the small sample size, and possible bias toward children of families with higher standards of living. Because of the observational study design, we could not establish the causal relationships between milk intake, length, and IGF-I. Although we conducted multivariate analyses, other unmeasured variables may be responsible for the relationships we observed between IGF-I and milk consumption.

In summary, we found that IGF-I has different relationships with body composition in utero (measured at birth) and in early childhood. The associations changed from adiposity to linear growth. Our results highlight the possibility of programming of the child's IGF-I axis by milk intake during pregnancy, in addition to the well-documented association with child's own milk consumption and linear growth. Cow's milk may have a stronger effect on the IGF-I axis compared to buffalo milk. We have now followed this cohort to 6 y of age, and are conducting a longitudinal analysis of IGF-I, IGFBP-3, milk consumption, body composition, and cardiovascular and metabolic disease risk factors.

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Author contributions CSY designed research; HGL, DSB, NSM, and DAR conducted research; ASW provided IGF-I analysis kits; ASW and SMJ analyzed data; ASW wrote paper; ASW, SMJ, and CSY had primary responsibility for the final content. ASW and CSY have equal authorship status.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics The study was approved by the KEM Hospital Research Centre Ethics Committee, Pune, India and informed written consent was obtained from mothers in the study. Analysis of the umbilical cord and 2-y blood samples for IGF-I concentration was approved by the Institutional Review Board at Indiana University, Bloomington, IN, USA.

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