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Investigating the IGF axis as a pathway for intergenerational effects

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Original Article

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Abstract

Early nutritional and growth experiences can impact development, metabolic function, and reproductive outcomes in adulthood, influencing health trajectories in the next generation. The insulin-like growth factor (IGF) axis regulates growth, metabolism, and energetic investment, but whether it plays a role in the pathway linking maternal experience with offspring prenatal development is unclear. To test this, we investigated patterns of maternal developmental weight gain (a proxy of early nutrition), young adult energy stores, age, and parity as predictors of biomarkers of the pregnancy IGF axis ($n = 36$) using data from the Cebu Longitudinal Health and Nutrition Survey in Metro Cebu, Philippines. We analyzed maternal conditional weight measures at 2, 8, and 22 years of age and leptin at age 22 (a marker of body fat/energy stores) in relation to free IGF-1 and IGFBP-3 in mid/late pregnancy (mean age = 27). Maternal IGF axis measures were also assessed as predictors of offspring fetal growth. Maternal age, parity, and age 22 leptin were associated with pregnancy free IGF-1, offspring birth weight, and offspring skinfold thickness. We find that free IGF-1 levels in pregnancy are more closely related to nutritional status in early adulthood than to preadult developmental nutrition and demonstrate significant effects of young adult leptin on offspring fetal fat mass deposition. We suggest that the previously documented finding that maternal developmental nutrition predicts offspring birth size likely operates through pathways other than the maternal IGF axis, which reflects more recent energy status.

Introduction

Child growth and nutrition are powerful predictors of health outcomes, with effects that extend into adulthood and beyond. Early undernutrition has been demonstrated to impact immune function, metabolic regulation, and cognitive development later in life.[1–3] Furthermore, evidence suggests that children who are undernourished or stunted experience more adverse cardiometabolic symptoms as adults, achieve lower adult heights, and give birth to smaller babies with greater risk of being stunted themselves.[3–8] Thus, poor early nutrition and subsequent growth alterations can induce permanent changes to individual health trajectories that also collectively shape the health of populations and further perpetuate global health disparities. Given the clear intergenerational effects of early undernutrition and growth stunting, the pathways through which these relationships manifest are therefore of significant interest to researchers, practitioners, and health policy advocates.

In contrast to the robust evidence demonstrating associations between early growth/nutrition, adult health, and reproductive outcomes across populations, there is less consensus on the biological systems driving these effects. Mechanisms of intergenerational transmission of nutrition have attracted growing research interest as important pathways for chronic disease trends in low- and middle-income countries, particularly metabolic syndrome.[2,3,6,9] Undernutrition during early life is associated with a range of metabolic alterations that have been proposed to shift biological trajectories toward an energy-conserving phenotype, such as reduced long bone growth and stature, a greater propensity to store energy in adipose tissue, and reduced insulin sensitivity.[1,3,5,10] We recently demonstrated that relative growth patterns throughout infancy, childhood, and adolescence predicted offspring birth size in a longitudinal birth cohort from the Philippines.[10] Those findings are in accordance with other studies demonstrating associations between maternal childhood nutritional supplementation,[11,12] relative growth,[7] leg length,[13] weight patterns,[8,11,15] and stature[4–17] and offspring fetal growth across populations. Notably, maternal height, leg length, and leg-to-height percentage have also been shown to predict glucose tolerance in pregnancy.[18] Taken together, these results point to an important role of metabolic changes in the link between chronic maternal growth experience and later reproductive outcomes.

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A potential candidate mechanism connecting early nutritional and growth effects with persistent metabolic alterations is the insulin-like growth factor (IGF) axis, a key regulator of energy throughout the life course. The IGF axis is comprised of IGF-1, IGF-2, at least 6 IGF-binding proteins (IGFBP-1–6), and several proteases, although IGF-1 and IGFBP-3 are most directly involved in postnatal growth and metabolism. Hepatic IGF-1 synthesis is stimulated by pituitary growth hormone (GH) and regulates GH through negative feedback via GH-inhibiting somatostatin.[19] The majority of total IGF-1 in circulation is bound to IGFBPs, which have greater affinity than the IGF-1 receptor (IGF1-R).[20] Upon binding to IGF1-R, IGF-1 increases insulin sensitivity in skeletal muscle and promotes anabolic processes, cell differentiation, and cell proliferation via the PI3K-AKT and MAPK signaling pathways.[21] Over 90% of total IGF-1 in circulation is bound to IGFBP-3, which is saturated under physiologic conditions, making it a key modulator of free IGF-1 bioavailability.[22] IGF axis activity is particularly sensitive to nutritional status in childhood and is dysregulated by chronic malnutrition, contributing to linear growth stunting.[23,24] In chronic undernutrition, children show decreased levels of both total IGF-1 and IGFBP-3,[25,26] while transient exposure to a 6-month famine in Dutch children was associated with increased total IGF-1 and IGFBP-3 in middle age, potentially indicating a post-famine overcorrection of IGF axis activity and dysregulation after nutrition became abundant again.[27] Importantly, early dysregulation of the IGF axis can also exert lasting effects on energy metabolism and body weight regulation into adulthood.[6]

Evidence suggests that past maternal stunting predicts lower offspring birth weight,[7] and recent findings from Cebu demonstrated that maternal patterns of early life weight gain are the strongest predictors of offspring birth measures that have traditionally been viewed as relatively canalized, including length and head circumference, while the more labile traits of birth weight and skinfolds were less related to markers of the mother's prior nutrition and growth.[10] The possible mechanisms underlying these relationships remain unclear, although we speculate that markers of maternal chronic nutritional status are potential sources of information about past resource availability and the average energetic environment that informs fetal development.[28–30] Given that the IGF axis is sensitive to chronic energy/nutritional status and has important roles in growth, somatic function, and pregnancy metabolism, including maternal adaptation to fetal demands, it is a possible candidate for these long-term nutritional cues. In pregnancy, maternal total and free IGF-1 rise to facilitate the metabolic and anabolic requirements of fetal growth.[31] High, sustained placental GH and placental lactogen induce maternal insulin resistance and drive increases in maternal IGF-1 throughout pregnancy to increase substrate availability for the fetus.[32] In addition to systemic effects on maternal metabolism that indirectly support fetal growth, maternal IGF-1 directly stimulates placental trophoblast proliferation and increases glucose uptake by the placenta.[31] Maternal serum total IGF-1 is inversely associated with intrauterine growth restriction or small for gestational age (SGA) infants;[33–35] however, fewer studies have observed associations between maternal IGF axis measures and fetal growth in uncomplicated pregnancies.[36–38]

Due to the direct influence of current maternal energy status on offspring prenatal growth, investigation of long-term nutritional cues requires isolating the effects of recent or periconceptional energy status from developmental nutrition. Leptin, an adipocyte-derived hormone that signals fat stores to the hypothalamus, is strongly correlated with adiposity and may be viewed as a measure of the body's current energetic reserves.[39,40] Here, we assess the maternal IGF axis as a potential pathway for the intergenerational transmission of nutritional signals from infancy through young adulthood with data collected in a sample from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) in Metro Cebu, Philippines. We first test the hypothesis that preadult conditional weight (CW) gain, a proxy for past developmental nutrition, predicts maternal free IGF-1 and IGBFP-3 in late pregnancy. Second, we test the hypothesis that maternal IGF axis measures are associated with offspring neonatal anthropometrics, a proxy for fetal growth. We use leptin measured at approximately age 22, 4–8 years before pregnancy, to account for the direct influence of maternal fat reserves on fetal growth, allowing us to isolate any potential effects of growth experience throughout infancy, childhood, and adolescence on pregnancy metabolism.

Methods

Study population and data collection

Data and samples come from the CLHNS in Metropolitan Cebu, Philippines.[41] In 1983–84, 3327 gravidas were recruited, and 3080 singleton offspring were tracked from birth to 24 months, with additional surveys in 1991, 1994, 1998, and 2005. Data collection included maternal interviews with CLHNS project staff and nutritional and anthropometric measures. The 2005 follow-up survey also collected fasting whole blood samples for metabolic analysis from 808 female participants (mean age = 21.5 years). Between 2009 and 2014, a new study tracked 507 pregnancies among 383 of the now-adult index children.[42] In-home interviews and dried blood spot (DBS) sample collection were conducted in the third trimester and postpartum. The average gestational age at the time of pregnancy interview and DBS collection was 29.9 weeks from the last menstrual period, with 90% of visits falling between 26 and 36 weeks. Height and weight were measured by CLHNS researchers using standard procedures.[43] DBS were collected on filter paper (Whatman #903, GE Healthcare, Piscataway, NJ) using a sterile microlancet, allowed to dry, and stored at −30°C. Additional details of DBS collection in this sample are given elsewhere.[34] Neonatal anthropometric measurement was conducted in participants' homes shortly after delivery by project staff using standardized procedures.[43] Birth weight, length, head circumference, and a sum of five skinfold thicknesses (triceps, subscapular, suprailiac, bicep, and calf) were measured. The median interval between birth and survey measurement was 3 d (mean 4.7). All study protocols were conducted with informed consent and approved by the Institutional Review Board of Northwestern University.

Maternal conditional weight measures

To characterize age-specific patterns of maternal weight gain, CW variables were estimated for the following intervals: 0–2 years, 2–8 years, and 8–22 years. CWs are uncorrelated residuals derived by regressing current weight on current age, age ^ 2, and all previous weights, representing measures of relative change in weight gain trajectory given past weight measures.[8,44] For details on the calculation of these CW measures, see Ragsdale *et al.* 2023.[10]

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Biomarker measurements

Venous whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes in 2005, centrifuged to separate plasma, and then shipped on dry ice to the United States, where they were stored at −80°C. Fasting plasma leptin was assayed in duplicate using the Linco Human Leptin ELISA kit (Catalog # EZHL-80SK, Linco Research, St Charles, MO) at the Laboratory for Human Biology Research at Northwestern University.[45] Participants who were pregnant at the time of the 2005 blood draw were excluded from these analyses.

IGF axis measures were quantified in DBS from the 2009 to 2014 pregnancy tracking study using electrochemiluminescent (ECL) assays with Meso Scale Discovery reagents and reader (Meso QuickPlex SQ 120 MM instrument, MSD, Rockville, MD) in the Laboratory for Human Biology Research. All samples were analyzed in duplicate by the same technician using a single lot of reagents, and controls were included in all runs to monitor variability across plates.

Free IGF-1: Using two 5 mm discs per well, DBS samples and controls were eluted in 50 µL of buffer (phosphate buffered saline, 0.1% Tween-20) overnight in duplicate and then transferred to a coated MSD GOLD 96-well Small Spot Streptavidin SECTOR plate (MSD, Kit Catalog # K1519DR). Protocols were carried out according to manufacturer instructions. Omitting a preliminary acid-extraction step to unbind IGF-1 from IGF-binding proteins results in a measure of free rather than total IGF-1. The assay detection range was 13 pg/ml–40,000 pg/ml. Inter-assay variability (% coefficient of variation) for high, middle, and low controls was 2.5%, 8.9%, and 4.9%, respectively.

IGFBP-3: Using a 3.2 mm disc, DBS samples and controls were eluted in 400 µL of buffer (phosphate buffered saline, 0.1% Tween-20) overnight and then transferred to a coated U-PLEX 2-Assay, 96-well SECTOR Plate (MSD, Kit Catalog # K15227N). Protocols were carried out according to manufacturer instructions. The assay detection range was 15 pg/ml–80,000 pg/ml. Inter-assay variability (% coefficient of variation) for high, middle, and low controls was 14.6%, 1.5%, and 11.1%, respectively.

Serum-equivalent concentrations, transformations, and gestational age correction

Serum-equivalent concentrations for free IGF-1 and IGFBP-3 were estimated using Passing–Bablok regression equations obtained from validation testing. Both assays were validated for DBS using 47 matched plasma and DBS samples collected in mid/late pregnancy (mean gestational age = 28.6 weeks) from a Chicago-area cohort. P–B equation for serum-equivalent free IGF-1 (pg/ml): (6.025038 × DBS) – 38,028.49. P–B equation for serum-equivalent IGFBP-3 (pg/ml): (415.2039 × DBS) – 170,085.9. Free IGF-1 and IGFBP-3 distributions were skewed and non-normal, so both were transformed using zero-skewness Box-Cox procedures (bcskew0 command in Stata). Free IGF-1 concentration was adjusted for gestational age at measurement to account for changes throughout pregnancy, and the residuals were used in analyses. IGFBP-3 was unrelated to gestational age.

The ratio of free IGF-1 to IGFBP-3 was calculated from molar concentrations. Estimated serum concentrations were converted to SI units (nmol/L) using the following equations: IGF-1 nmol/L = IGF-1 ng/ml × 0.1307; IGFBP-3 nmol/L = IGFBP-3 ng/ml × 0.0348.[46]

Table 1. Descriptive statistics for participants ($n = 35$) and their offspring ($n = 36$)

Maternal variables	Mean	SD
Age	27.0	1.0
Parity	2.0	1.3
Height (cm)	150.0	5.5
Prepregnancy BMI	20.5	4.0
Gestational timing at DBS collection (weeks)	29.5	4.8
Offspring variables	**Mean**	**SD**
Birth weight (g)	3101	507
Birth length (cm)	48.4	1.8
Head circumference (cm)	32.9	1.3
Gestational age at birth (weeks)	38.7	3.1
Days between birth and measurement	4.7	4.7

DBS, dried blood spot.

Data analysis

Statistical analysis was conducted using Stata/SE 16.1 for Windows (College Station, TX). Due to the limited remaining DBS from the 2009–2014 pregnancy tracking study, free IGF-1 and IGFBP-3 were measured in a small subset of the original sample, leading to a final sample size of 36 pregnancies. To isolate the effects of developmental nutrition from those of adult nutritional status, we used CW gain in infancy, childhood, and adolescence and serum leptin measured in early adulthood to quantify energy stores prior to pregnancy. Descriptive statistics and pairwise correlations were performed prior to analysis. Relationships between maternal CW gain, adult prepregnancy energy status, and pregnancy IGF axis measures were assessed using a series of three multiple regression models. Cluster-robust standard errors were specified (vce(cluster) option in Stata) to account for one participant with two tracked pregnancies. Independent variables in Model 1included maternal age, parity, and prepregnancy leptin. Independent variables in Model 2 included maternal gestational age-adjusted birth weight and CWs at 2 years (CW_{2y}), 8 years (CW_{8y}), and 22 years (CW_{22y}). Model 3 included all predictors from Models 1 and 2 together. Dependent variables were free IGF-1, IGFBP-3, and the IGF-1/IGFBP-3 molar ratio in mid/late pregnancy. Free IGF-1 and IGFBP-3 concentrations were also evaluated as independent predictors of birth outcomes in separate multiple regression models including maternal age, parity, leptin, and days after birth of measurement as covariates. Offspring birth outcome variables were residuals obtained from a regression of raw anthropometrics on gestational age at birth.

Results

The mean age of participants during their third-trimester interview was 27 years (range: 25.6–29.2). In general, participants were lean and short-statured, with an average prepregnancy BMI of 20.5 kg/m^2 and average height of 150 cm (Table 1). Median serum leptin (at age 22) was 16.0 ng/ml (IQR 10.8, 26.1). Leptin was significantly correlated with prepregnancy BMI ($p < 0.001$). The median unadjusted free IGF-1 concentration was 63.2 ng/ml (IQR 43.2, 84.6), and the median IGFBP-3 concentration was

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Table 2. Results from a series of multiple regression analyses predicting maternal pregnancy free IGF-1, IGFBP-3, and the molar ratio of free IGF-1/IGFBP-3. Model 1 predictors: age in pregnancy, parity, and nonpregnant leptin. Model 2 predictors: gestational age-adjusted birth weight, conditional weight at 2 years, conditional weight at 8 years, and conditional weight at 22 years. Model 3 includes all predictor variables from Models 1 and 2. **p < 0.01 *p < 0.05 ~p < 0.1

	Model 1	Model 2	Model 3
Free IGF-1 (ng/ml)			
Age	5.75 (2.25)*		6.11 (2.41)*
Parity	−5.29 (1.50)**		−4.93 (2.31)*
Leptin, 22y	0.46 (0.18)*		0.76 (0.31)*
Birth weight		−0.70 (7.54	3.11 (7.53)
Condtnl. weight, 2y		0.19 (2.23)	−3.65 (2.38)
Condtnl. weight, 8y		−2.29 (1.80)	0.15 (1.68)
Condtnl. weight, 22y		0.44 (0.53)	−0.38 (0.57)
Model adjusted R^2	0.29	−0.07	0.26
IGFBP-3 (ng/ml)			
Age	−1.23 (6.06)		0.29 (7.08)
Parity	7.14 (4.77)		4.92 (6.91)
Leptin, 22y	0.70 (0.57)		0.39 (1.09)
Birth weight		11.40 (16.36)	8.54 (16.90)
Condtnl. weight, 2y		−2.22 (5.99)	−2.16 (6.40)
Condtnl. weight, 8y		2.88 (4.59)	1.46 (5.48)
Condtnl. weight, 22y		1.18 (1.02)	0.62 (1.55)
Model adjusted R^2	0.00	−0.04	−0.12
Free IGF-1/IGFBP-3 ratio			
Age	0.33 (0.21)		0.36 (0.26)
Parity	−0.45 (0.16)*		−0.49 (0.23)*
Leptin, 22y	−0.00 (0.02)		−0.01 (0.03)
Birth weight		−0.20 (0.43)	0.16 (0.47)
Condtnl. weight, 2y		0.03 (0.18)	−0.10 (0.20)
Condtnl. weight, 8y		−0.20 (0.14)	−0.02 (0.14)
Condtnl. weight, 22y		0.00 (0.03)	0.02 (0.04)
Model adjusted R^2	0.17	−0.06	0.07

Values shown are regression coefficients (robust standard errors).
All models control for gestational timing at measurement.

374.7 ng/ml (IQR 183.9, 611.1). Free IGF-1 and IGFBP-3 were unrelated to maternal prepregnancy BMI, height, or offspring sex in pairwise correlations (not shown). Mean offspring birth weight was 3101 g, and the average gestational age at birth was 38.7 weeks (Table 1).

IGF axis measures

Maternal age and prepregnancy leptin were significantly, positively related to free IGF-1 in Models 1 and 3 (Model 1: age ($p = 0.015$), leptin ($p = 0.015$); Model 3: age ($p = 0.016$), leptin ($p = 0.018$)), while parity was significantly, inversely related to free IGF-1 in these models (Model 1: $p < 0.001$; Model 3: $p = 0.040$) (Table 2). In the full model (Model 3), each additional year of maternal age was associated with a 6.1 ng/ml increase in free IGF-1, while each additional pregnancy predicted a 4.93 ng/ml decrease in free IGF-1. Holding age and other covariates constant, IGF-1 concentration during a sixth pregnancy is expected to be 29.58 ng/ml lower than during a first pregnancy. IGFBP-3 was not related to any maternal variables across all three models, and higher maternal parity significantly predicted lower pregnancy IGF-1/IGFBP-3 ratio in Models 1 and 3 (Model 1: $p = 0.010$; Model 3: $p = 0.042$). Maternal CWs did not predict any IGF axis measures in regression models.

Birth outcomes

Analyses were run to determine whether maternal traits or biomarkers predicted offspring birth size parameters in this sample. Free IGF-1, IGFBP-3, and their ratio were included as predictors of birth outcomes in separate multiple regression models along with maternal age, parity, and nonpregnant leptin. Maternal free IGF-1, IGFBP-3, and the IGF-1/IGFBP-3 ratio were not significantly associated with neonatal anthropometrics in any models (Table 3). There was, however, a positive association between maternal free IGF-1 and offspring skinfold thickness that fell just short of statistical significance ($p = 0.057$). In contrast, maternal leptin at age 22 was significantly, positively associated with offspring birth weight (free IGF-1 model: $p = 0.045$, IGFBP-3 model: $p = 0.026$, IGF-1/IGFBP-3 ratio model: $p = 0.024$) and skinfold thickness (free IGF-1 model: $p = 0.025$; IGFBP-3 model: $p = 0.004$; IGF-1/IGFBP-3 ratio model: $p = 0.005$). Each 1 ng/ml increase in serum leptin predicted approximately 20 g of birth weight, with a 306 g difference between the 25th and 75th percentiles of leptin, and 0.17–0.21 cm increase in the sum of skinfolds, with a difference of 2.9 cm between the lower and upper quartiles of leptin concentration. Leptin was also significantly and positively related to offspring head circumference in the IGF-1/IGFBP-3 ratio model ($p = 0.032$) and moderately associated with head circumference in the free IGF-1 ($p = 0.068$) and IGFBP-3 models ($p = 0.056$), although these were not statistically significant. Maternal age was inversely related to offspring birth weight in the free IGF-1, IGFBP-3, and ratio models, but the association was only statistically significant in the model including the IGF-1/IGFBP-3 ratio ($p = 0.037$). Across free IGF-1, IGFBP-3, and IGF-1/IGFBP-3 ratio predictor models, R^2 values were highest for offspring skinfold thickness, then birth weight, and lowest for birth length and head circumference (Table 3).

Discussion

This analysis investigated the role of the IGF axis in the relationship between maternal early growth patterns and offspring fetal growth in a pregnancy cohort from the Philippines. We found that maternal age, parity, and recent/adult nutritional status, but not developmental weight gain, predicted free IGF-1 in mid/late pregnancy. While higher maternal age and leptin at age 22 both predicted higher free IGF-1 in pregnancy, increasing parity was associated with lower free IGF-1 and lower free IGF-1 relative to IGFBP-3 (Table 2). There were no associations between maternal variables and IGFBP-3 or between IGFBP-3 and offspring birth size. Maternal free IGF-1 was not a statistically significant predictor of offspring birth outcomes, although there was a marginally positive relationship between free IGF-1 and offspring skinfold thickness ($p = 0.057$). In contrast, higher maternal leptin at age 22 significantly predicted increased offspring birth weight, skinfold thickness, and head circumference in models including

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Table 3. Results from separate multiple regression models predicting neonatal anthropometrics. Birth outcome variables are residuals after adjusting for days after birth of measurement. **p < 0.01 *p < 0.05 ~p < 0.1

	β (RSE)	*p*-value	sig.		β (RSE)	*p*-value	sig.		β (RSE)	*p*-value	sig.
	Offspring birth weight				*Offspring birth weight*				*Offspring birth weight*		
Free IGF-1	−0.49 (6.78)	0.942		IGFBP-3	−1.31 (2.06)	0.530		IGF1/BP3 ratio	90.07 (63.88)	0.168	
Age	−145.06 (82.77)	0.089	~	Age	−149.5 (83.07)	0.081	~	Age	−178.7 (82.4)	0.037	*
Parity	44.13 (77.86)	0.575		Parity	56.11 (69.41)	0.424		Parity	82.17 (79.88)	0.311	
Leptin	20.02 (9.60)	0.045	*	Leptin	20.71 (8.86)	0.026	*	Leptin	19.9 (8.4)	0.024	*
Model R^2	0.26			Model R^2	0.27			Model R^2	0.29		
	Offspring birth length				*Offspring birth length*				*Offspring birth length*		
Free IGF-1	0.00 (0.02)	0.888		IGFBP-3	0.01 (0.01)	0.154		IGF1/BP3 ratio	−0.4 (0.3)	0.194	
Age	−0.07 (0.48)	0.881		Age	−0.04 (0.43)	0.928		Age	0.08 (0.47)	0.860	
Parity	0.09 (0.35)	0.808		Parity	−0.01 (0.31)	0.965		Parity	−0.09 (0.33)	0.796	
Leptin	0.07 (0.04)	0.140		Leptin	0.06 (0.04)	0.137		Leptin	0.07 (0.04)	0.090	~
Model R^2	0.12			Model R^2	0.16			Model R^2	0.16		
	Offspring sum of skinfolds				*Offspring sum of skinfolds*				*Offspring sum of skinfolds*		
Free IGF-1	0.08 (0.04)	0.057	~	IGFBP-3	0.00 (0.02)	0.940		IGF1/BP3 ratio	0.75 (0.49)	0.138	
Age	−0.25 (0.75)	0.743		Age	0.20 (0.73)	0.786		Age	−0.06 (0.74)	0.939	
Parity	0.98 (0.58)	0.100		Parity	0.59 (0.49)	0.235		Parity	0.9 (0.59)	0.134	
Leptin	0.17 (0.07)	0.025	*	Leptin	0.21 (0.07)	0.004	**	Leptin	0.21 (0.07)	0.005	**
Model R^2	0.38			Model R^2	0.35			Model R^2	0.35		
	Offspring head circumference				*Offspring head circumference*				*Offspring head circumference*		
Free IGF-1	0.00 (0.02)	0.936		IGFBP-3	0.01 (0.01)	0.348		IGF1/BP3 ratio	−0.14 (0.23)	0.539	
Age	0.05 (0.32)	0.866		Age	0.07 (0.28)	0.805		Age	0.11 (0.31)	0.723	
Parity	0.12 (0.25)	0.631		Parity	0.07 (0.21)	0.751		Parity	0.06 (0.23)	0.805	
Leptin	0.05 (0.03)	0.068	~	Leptin	0.04 (0.02)	0.056	~	Leptin	0.05 (0.02)	0.032	*
Model R^2	0.14			Model R^2	0.17			Model R^2	0.15		

Values shown are regression coefficients (robust standard errors). All models control for gestational timing at blood sampling.

maternal age, parity, and IGF axis measures. Our results suggest that the maternal IGF axis is more closely related to recent nutritional status than to patterns of relative weight gain in infancy, childhood, and adolescence.

In this sample from the Philippines, leptin measured at age 22 predicted free IGF-1 concentrations during pregnancy, 4–8 years later (Table 2). Leptin released by adipocytes serves as an energetic signal to the hypothalamus and is a key participant in glucose homeostasis.[39,40] It is closely associated with fat mass percentage and thus reflects the amount of stored energy.[47–49] These results contrast with those of Qiu et al., in which the authors reported inverse correlations of prepregnancy BMI and leptin with maternal free IGF-1.[50] Although we can only speculate on explanations for this inconsistency, there were differences in the timing of collection and measurement that could be relevant: Qiu and colleagues measured free and total IGF-1 in early pregnancy (mean: 13 weeks gestation; IQR: 8–16 weeks), while our blood samples were collected around 30 weeks of gestation (90% between 26 and 36 weeks). Between 9 and 14 weeks of gestation, the placental growth hormone variant (GH-v) begins to replace pituitary GH as the main stimulator of maternal IGF-1 for the remainder of pregnancy.[32,51,52] As such, maternal IGF-1 levels during early pregnancy are lower and may be stimulated by either maternal pituitary GH or placental GH-v depending on the stage of placental development, potentially contributing to the differing results. Little past work has looked at prepregnancy leptin as a predictor of birth outcomes, limiting our ability to compare our findings with prior analyses. Other studies of leptin in relation to fetal growth have reported no associations between pregnancy leptin and offspring birth size;[53,54] however, pregnancy is known to induce a state of leptin resistance that leads to a temporary elevation in circulating leptin.[55]

While maternal nutritional status in early adulthood was associated with free IGF-1 in this analysis, developmental nutrition, reflected in CW measures from birth into adulthood, was not related to the pregnancy IGF axis. In this sample, free IGF-1 and the ratio of free IGF-1 to IGFBP-3 in pregnancy are more associated with recent nutritional status or fat stores than long-term growth experiences, counter to our initial predictions. In experimental and epidemiological studies, breastfeeding status, growth stunting, and early exposure to famine have been linked to alterations in the IGF axis later in life.[25,27,56] Further, work in rats has demonstrated that maternal undernutrition and high-fat diets both induce altered metabolic phenotypes and IGF axis profiles in adult offspring regardless of postnatal nutrition.[57] These effects have led to the proposition that IGF axis activity and regulation reflect a cumulative record of energy/nutritional experience, which influence adult metabolic strategy and thus the gestational environment.[58] The findings of this analysis do not support this hypothesis, although there are several factors that may contribute to our results. Given the degree of fetal control over maternal metabolism and IGF axis activity by mid/late pregnancy,[32,59] it is likely that the influence of fetoplacental hormones masks or overrides preexisting regulatory set points of the maternal IGF axis. Without IGF axis measures prior to pregnancy we are unable to test this suggestion in our sample. In addition, some nutrition-induced changes to IGF axis regulation appear to be reversible with improvements in energy status,[25,26,60,61] and it is currently unclear how the timing and severity of nutritional exposures may affect long-term regulation.

Notably, increasing maternal age predicted higher free IGF-1 in pregnancy, while increasing parity predicted lower free IGF-1 and a lower IGF-1/IGFBP-3 ratio (Table 2). These relationships are consistent with the perspective of life history theory, as energetic investment in a pregnancy is predicted to increase across the reproductive span (e.g. as future reproduction becomes a shrinking contributor to total lifetime fertility), while also decreasing in relation to the number of existing dependent offspring.[62–64] As a stimulator of mitogenic and metabolic processes that is closely tied to nutritional status, free IGF-1 is one potential signal of this reproductive investment. There are relatively few analyses of age, parity, and the pregnancy IGF axis; however, one study of SGA and control pregnancies in the UK found that maternal parity was negatively associated with total IGF-1 measured in early pregnancy.[33] Higher parity may also be related to lower total IGF-1 and IGFBP-3 outside of pregnancy, although the relationships were not statistically significant in a large cohort.[65] Given that total IGF-1 and IGFBP-3 peak in adolescence and then slowly decline over the life span,[66,67] it is interesting that free IGF-1 in pregnancy was positively associated with maternal age. This suggests that aspects of the dyadic interaction between maternal and fetoplacental hormones may change with successive pregnancies if later-born offspring stimulate relatively larger changes in the maternal IGF axis compared with earlier-born offspring.

In regression models predicting neonatal anthropometrics, age 22 leptin and age in pregnancy were the only maternal variables significantly associated with birth outcomes. Higher maternal leptin predicted greater offspring skinfold thickness, birth weight, and head circumference, while higher maternal age predicted lower birth weight (Table 3). Significance values for regressions of maternal IGF measures and covariates on birth outcomes varied strongly in relation to the specific birth measure of interest, patterns which are broadly relevant to this area of inquiry and have been demonstrated in analyses with other maternal predictors of neonatal anthropometrics.[10] Maternal free IGF-1 showed a modest association with offspring skinfold thickness ($p = 0.057$), in contrast to p-values > 0.8 for weight, length, and head circumference. These results agree with most of the existing literature on the maternal IGF axis.[68] One study of maternal IGF axis measures in Jamaica reported positive associations between total IGF-1 in mid/late pregnancy and offspring birth weight and skinfold thickness;[69] however, these findings have not been replicated. The lack of relationships between maternal IGFBP-3 and offspring birth measures is also consistent with other studies.[33,52,70] Taken together, this evidence does not indicate a central role of the maternal IGF axis in facilitating intergenerational phenotypic effects.

This study draws on a rich longitudinal dataset to isolate effects from many potentially contributing factors in our analyses. We have quantified maternal relative energy status from birth into adulthood with discrete, uncorrelated CW measures.[8,36] Our study also benefits from the use of two IGF axis measures: free IGF-1 and IGFBP-3. Unlike total IGF-1, free IGF-1 is not correlated with IGFBP-3 ($r = 0.04$) and is a more precise (although not exact) estimate of bioactive IGF-1 in maternal circulation. Despite these strengths, our findings are subject to some limitations. Due to the blood sample volume required to measure both IGF-1 and IGFBP-3 in each pregnancy and several participants missing one or more growth measures in the dataset, our final sample was restricted to 36 pregnancies. While smaller sample sizes are not uncommon in clinical endocrinology, intergenerational analyses of growth effects tend to use larger cohorts. Our study sample is likely underpowered with respect to these intergenerational models, and therefore it is possible that some true relationships were not detected. While many of the associations in our models were

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strongly nonsignificant, several had p-values < 0.15 that could potentially shift to significance in a larger study sample. Further, although CWs are responsive to common experiences of early life nutritional stress in this population, such as infant infectious disease, they are only proxy measures of nutritional status that may be influenced by factors other than nutrition, including genetic and epigenetic effects. Finally, quantification of free IGF-1 is biochemically complex due to the presence of multiple binding partners at high concentrations and the short half-life of unbound IGF-1 in circulation. Most commercially available assay kits are unable to confirm a total lack of interference by other binding proteins above certain thresholds and some protein fragments.[71,72] Our ECL assay uses a recombinant human IGF-1 standard with a neutralizing capture antibody and is therefore specific to the free form of IGF-1, although there is potential for some measurement error related to protein degradation.

Conclusion

The present study tested the prediction that the maternal IGF axis influences offspring development in relation to maternal developmental nutritional history. Our findings do not support this hypothesis and instead suggest that the pregnancy IGF axis is more closely related to recent nutritional status than developmental energetic experience. We identified significant associations between maternal age, parity, and prepregnancy fat stores and the IGF axis, as well as between maternal age, fat stores, and offspring measures most related to fat mass. These relationships between maternal fat stores and more labile offspring traits complement previous work in this cohort showing that maternal lifetime growth patterns predict more canalized features of fetal growth, like head circumference and length, and suggest that the maternal IGF axis is not likely a pathway for the transmission of energetic signals through the matriline. The mechanisms of intergenerational phenotypic inertia in humans remain unclear and will require additional research to elucidate.

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