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Association of circulating FGF-21 levels in the first week of life and postnatal growth in hospitalized preterm infants

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ABSTRACT

Background: The role of fetal and neonatal growth in the development of adult-onset diseases such as obesity and metabolic syndrome has become increasingly appreciated. Fibroblast growth factor-21 (FGF-21) is known as a regulator of glucose and lipid metabolism. FGF-21 levels are elevated in obese adults and children. The role of FGF-21 in neonatal growth in preterm infants is not known.
Objectives: We aimed to evaluate the association of circulating FGF-21 levels in the first week of life and neonatal growth parameters at the time of discharge from NICU.
Methods: We performed a longitudinal study of 25 preterm neonates admitted to NICU. Blood samples were collected at two time points: within 24 h of life (T1), and 24–96 h after the first blood draw (T2). FGF-21 levels were measured in plasma by ELISA. Weight, length, BMI and their Z-scores were measured at the time of birth and discharge.
Results: The FGF-21 levels were significantly higher at T2 than at T1 ($p < 0.001$). FGF-21 levels at both time points were positively correlated with gestational age ($r = 0.43$, $p = 0.03$). FGF-21 at T1 was positively associated with weight Z-score ($\beta = 0.19$, $p = 0.001$) and length Z-score at discharge ($\beta = 0.21$, $p = 0.03$).
Conclusions: Circulating FGF-21 levels increase significantly in the first week, and the FGF-21 levels within the first 24 h are positively associated with weight and length Z-scores at discharge in preterm infants. These results suggest that FGF-21 may be involved in growth and developmental maturation.

1. Introduction

The role of fetal and early postnatal growth and weight gain in the development of adult-onset diseases such as obesity and metabolic syndrome has become increasingly appreciated [1,2].

Fibroblast growth factor 21 (FGF-21), is a peptide hormone secreted predominantly by the liver [3–5], that stimulates insulin-independent glucose uptake and enhances lipid metabolism [3]. In a mouse model, FGF-21 showed a protective effect against diet-induced obesity [4]. By contrast, in adults and children, elevated circulating levels of FGF-21 were associated with obesity and type 2 diabetes mellitus [5–7].

The physiologic role of FGF-21 in the neonatal period, and the association between circulating FGF-21 levels and growth in neonates has not been widely studied in humans. We aimed to investigate the associations between the levels of circulating FGF-21 in the first week of life, and weight, length, body mass index (BMI) Z-

Abbreviations: FGF-21, fibroblast growth factor-21; BMI, body mass index; PMA, postmenstrual age; CI, confidence interval.

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scores at the time of discharge in hospitalized preterm newborn infants.

2. Methods

Preterm neonates born at 24–36 weeks of gestation and admitted to a level III Neonatal Intensive Care Unit at Morristown Medical Center, NJ, USA during December 2012–May 2014 were included in this study. Blood samples (0.5 mL) were collected at two time points: within the first 24hr of life (T1), and between 24 and 96 h after the first blood draw (T2). The timing of blood draw corresponded with clinically indicated blood collection in the NICU. A total of 25 infants were included in this study.

Blood samples were centrifuged at 4000 rpm for 15 min, and the plasma was stored in aliquots at −80 °C until the time of analysis. Infant weight and length at birth and at the time of discharge were measured by bedside nurses. A digital body weight scales was used after calibration for weight measurement, and a recumbent measuring board was used in a supine position to measure the length. Clinical data including infant sex, mode of delivery, gestational age, 1 and 5 min Apgar scores, feeding status were obtained from electronic medical records. Z-scores of weight, length, and body mass index (BMI) were obtained using a sex-specific US reference by Olsen et al. [8,9]. FGF-21 levels were measured by ELISA (EZHFGF21-19K Human FGF-21 ELISA kit; MilliporeSigma, Burlington MA). The intra- and inter-assay coefficients of variability were <6% and <9% respectively for FGF-21, and the standard curve range was 31.3–2000 pg/mL. In addition, we measured serum FGF-21 levels in 27 healthy adult donors (age: 33.8 ± 10.8 years) from BioreclamationIVT (Westbury, NY) for comparison of circulating FGF-21 levels between neonates and adults. Samples from adult and neonates were measured simultaneously.

Informed consent was obtained from primary caregivers of the infants, and the IRB at Atlantic Health System approved this protocol.

Wilcoxon signed-rank tests were used for comparison of neonatal FGF-21 levels between two time points. Comparisons between FGF-21 levels in adults and neonates at each time point were performed by Mann-Whitney *U* test. Spearman correlation analyses were performed to assess correlation between FGF-21 levels and growth parameters at birth. Multiple linear regression analysis was used to evaluate the associations between FGF-21 levels at each time point and the Z-scores of weight, length, and BMI at the time of discharge. Models were adjusted for gestational age at birth. We used SPSS 22 (Chicago, IL, USA) for statistical analysis.

3. Results

Median gestational age was 30.5 weeks (Interquartile range (IQR): 24.3–35.6 weeks), and the median birth weight was 1460 g (IQR: 913–2200 g). Eight infants (32%) were female. Noticeably, our patient population had relatively benign hospital course without significant complications such as necrotizing enterocolitis (n = 0), bronchopulmonary dysplasia (n = 0).

The circulating levels of FGF-21 were significantly higher at T2 compared to the levels at T1 (T1: 94.1 (IQR 0–213.6) vs. T2: 471.6 (IQR 159.6–786.1) pg/mL, $p < 0.001$). Eight out of 25 infants (32%) had undetectable levels of FGF-21 at T1 (therefore, was assigned the value of 0), however, all infants had detectable levels of FGF-21 at T2. Circulating levels of FGF-21 in neonates were significantly higher at T2 compared to the FGF-21 levels in adults (471.6 (159.6–786.1) vs. 102.6 (65.8–269.9) pg/mL, $p = 0.016$, Fig. 1).

At T1, 8 neonates (32%) were on enteral feeding, and 17 infants (68%) were on *not* per *oral* (NPO) status. Of note, all 8 infants who had undetectable FGF-21 levels at T1 were all on NPO status from birth to T1. And the FGF-21 levels at T1 in infants who were on enteral feeding trended higher compared to the FGF-21 levels in infants who were not fed, but this difference was not statistically significant (154.53 (interquartile range (IQR) 48.79–246.99) vs. 51.35 (0–213.64) pg/mL, p = 0.18).

Correlation between FGF-21 levels and gestational age, birth weight, length, and BMI at birth (Table 1A).

FGF-21 levels at both T1 and T2 were both positively correlated with gestational age (T1: $r = 0.43$, $p = 0.03$, T2: $r = 0.43$, $p = 0.03$). FGF-21 levels at T1 and T2 were also positively correlated with birth weight (T1: $r = 0.50$, $p = 0.01$, T2: $r = 0.43$, $p = 0.03$) and length (T1: $r = 0.55$, $p = 0.005$, T2: $r = 0.42$, $p = 0.04$). However, FGF-21 levels at neither T1 nor T2 correlated with Z-scores of birth weight and length, which reflect relative weight and length at their gestational age.

Association of FGF-21 and growth outcomes at the time of discharge (Table 1B).

Circulating FGF-21 levels at T1 were positively associated with weight Z-score (β = 0.19 (95% CI 0.10, 0,29), p = 0.001) and length Z-score (β = 0.21 (95% CI 0.03, 0.39), p = 0.03) at the time of discharge in multiple linear regression analyses adjusted for gestational age. Circulating FGF-21 levels at T2 were not significantly associated growth outcomes at discharge in adjusted models. Sensitivity analysis excluding 4 infants with neonatal sepsis (1), and infants of mothers with chorioamnionitis and positive group B streptococcus screen (2), and rupture of membrane ≥18 h (1) did not attenuate these results. Other maternal characteristics such as preeclampsia and early pregnancy BMI werenot associated with FGF-21 levels at neither T1 or T2.

4. Discussion

In this longitudinal study of preterm newborn infants, we found that circulating FGF-21 levels increase over time during the first week of life. Additionally, FGF-21 levels within the first 24 h after birth are positively associated with weight and length Z-score at the time of discharge. To our knowledge, there are no previous reports of longitudinal measurements of FGF-21 in preterm newborn infants during the first week of life, therefore, our results provide new insights into early neonatal growth.

An animal study in mice showed that initiation of sucking after birth enhanced mRNA expression of FGF-21 in liver, and that circulating levels of FGF-21 are low at birth and increase significantly in 2–6 days of life [10], which corresponds to what we saw in our human preterm infants. FGF-21 levels increase from week 1–5 after birth in very preterm infants [11], and there is a significant increase in serum FGF-21 levels from birth to 4 months of life in full term infants [11,12]. However, there has been no previous studies on longitudinal changes in circulating FGF-21 levels in the first week of life in preterm neonates.

FGF-21 is present in breast milk, and infusion of FGF-21 directly into neonatal gut via orogastric tube resulted in increased lactase activity in the intestine of newborn mice [13]. In our study, all infants who had undetectable levels of FGF-21 in the first 24 h of life were on NPO status, and there was a trend toward higher FGF-21 levels in enterally fed infants compared to the infants who were on NPO status. However, a subgroup analysis of infants on NPO vs infants who were fed showed that, regardless of feeding status, the infants' FGF-21 levels increased significantly from T1 to T2.

Fisher et al. reported that obese mice have elevated levels of FGF-21 and showed attenuated response to exogenous FGF-21 [14], which suggests that an FGF-21-resistance state is associated with obesity. In human clinical studies, previous reports in children and adults showed that elevated levels of FGF-21 are associated with

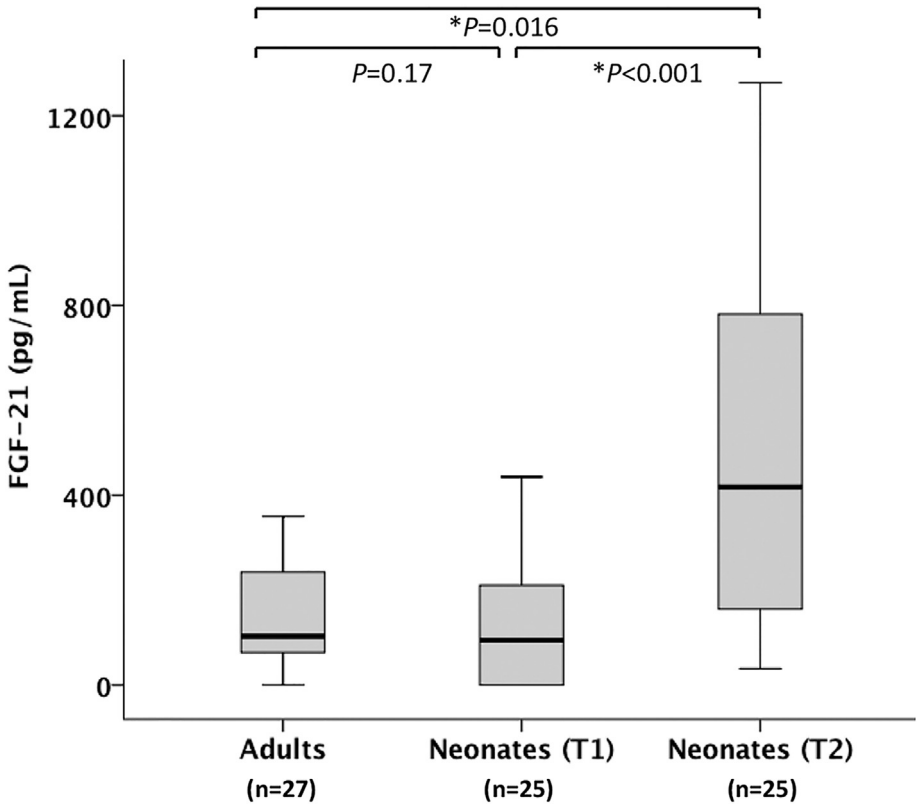

Fig. 1. Comparison between FGF-21 levels of neonates at T1 (within 24 h), T2 (24–96 h after T1), and adults. Data presented as median and IQR (box), and 95% confidence interval (Whiskers). Wilcoxon Signed Rank Test for comparison of neonatal FGF-21 levels at T1 and T2, Mann-Whitney *U* test for comparison of adult Vs. neonatal FGF-21 at each time point. *$p < 0.017$ (0.05/3).

Table 1A
Correlation analysis between the levels of FGF-21, gestational age, and the growth parameters at birth.

	FGF-21, T1 (pg/mL)	FGF-21, T2 (pg/mL)	Gestational Age (weeks)	Birth weight (g)	Birth Weight Z-score	Birth Length (cm)	Birth Length Z-score	BMI (Kg/m^2)	BMI Z-score
FGF-21, T1 (pg/mL)	1	$r = 0.15$ $p = 0.47$	***r* =0.43*** ***p*=0.03**	***r*=0.50*** ***p*=0.01**	$r = 0.15$ $p = 0.49$	***r*=0.55**** ***p*=0.005**	$r = 0.34$ $p = 0.10$	$r = 0.33$ $p = 0.11$	$r = 0.06$ $p = 0.79$
FGF-21, T2 (pg/mL)		1	***r* =0.43*** ***p* =0.03**	***r*=0.43*** ***p*=0.03**	$r = 0.18$ p 0.39	***r*=0.42*** ***p*=0.04**	$r = -0.01$ $p = 0.95$	***r*=0.41*** ***p*=0.04**	$r = 0.03$ $p = 0.88$
Gestational Age (weeks)			1	***r*=0.94**** ***p*<0.001**	$r = 0.05$ $p = 0.80$	***r*=0.94**** ***p*<0.001**	$r =$ **0.19**** ***p*<0.001**	***r*=0.78**** ***p*<0.001**	$r = -0.15$ $p = 0.47$
Birth weight (g)				1	$r = 0.29$ $p = 0.16$	***r*=0.95**** ***p*<0.001**	$r = 0.30$ $p = 0.14$	***r*=0.88**** ***p*<0.001**	$r = 0.10$ $p = 0.62$
Birth Weight Z-score					1	$r = 0.17$ $p = 0.41$	***r*=0.50*** ***p*=0.01**	***r*=0.48*** ***p*=0.02**	***r*=0.70**** ***p*<0.001**
Birth Length (cm)						1	$r = 0.43$ $p = 0.03$	***r*=0.73**** ***p*<0.001**	$r = -0.11$ $p = 0.59$
Birth Length Z-score							1	$r = 0.08$ $p = 0.71$	$r = -0.05$ $p = 0.82$
BMI (Kg/m^2)								1	$r = 0.49$ $p = 0.01$
BMI Z-score									1

Abbreviations: FGF-21, Fibroblast Growth Factor-21; BMI, Body Mass Index. Spearman correlation coefficient, *$p < 0.05$, **$p < 0.01$.

higher BMI and obesity [7,15,16]. While our study suggests that FGF-21 levels within 24 h of life are positively associated with weight and length Z-score at the time of discharge for preterm newborn infants, it did not correlate with BMI Z-score. Whether abnormal FGF-21 levels in early life are associated with obesity and other metabolic complications over the long-term remains to be further investigated.

FGF-21 is expressed in chondrocytes, therefore, FGF-21 could have an important role in the control of growth and development in children [17]. The accurate assessment of FGF-21's role in linear growth of preterm infants needs to be performed by further long-term follow up studies.

Strengths of our study are the longitudinal analysis of FGF-21 at two different time points in the early neonatal period. Limitations of our study include its relatively small sample size and potential unmeasured confounding factors, such as maternal diet and nutritional status. Although there is positive association between FGF-21 and neonatal growth, whether there is a causal relationship

Table 1B
Multiple regression analysis between FGF-21 levels in the first week and Z-scores of weight, length, BMI at the time of discharge.

		Weight Z-score			Length Z-score			BMI Z-score		
		β	95% C.I.	*P*	β	95% C.I.	*P*	β	95% C.I.	*P*
FGF-21, T1 (per 100pg/mL)	Unadjusted model	**0.19****	**0.10, 0.28**	**<0.001**	**0.27***	**0.09, 0.45**	**0.01**	0.01	−0.18, 0.19	0.93
	Adjusted model	**0.19****	**0.10, 0.29**	**0.001**	**0.21***	**0.03, 0.39**	**0.03**	0.07	−0.11, 0.26	0.42
FGF-21, T2 (per 100pg/mL)	Unadjusted model	0.05	−0.001, 0.10	0.05	**0.09***	**0.01, 0.18**	**0.03**	−0.02	−0.10, 0.06	0.55
	Adjusted model	0.05	−0.11, 0.12	0.11	0.05	−0.04, 0.14	0.27	0.02	−0.07, 0.10	0.69
FGF-21, average (per 100pg/mL)	Unadjusted model	**0.10****	**0.03, 0.17**	**0.007**	**0.17***	**0.05, 0.29**	**0.01**	−0.03	−0.15, 0.10	0.67
	Adjusted model	**0.11***	**0.02, 0.19**	**0.01**	0.12	−0.02, 0.26	0.09	0.04	−0.10, 0.18	0.55

Abbreviations: FGF-21, Fibroblast Growth Factor-21; BMI, Body Mass Index; C·I., Confidence Interval; PMA, post-menstrual age.
Adjusted models: adjusted for gestational age at birth. *$p < 0.05$, **$p < 0.01$.

needs to be proven in further studies.

In conclusion, FGF-21 significantly increases over time during the first week of life. Circulating FGF-21 levels within 24 h of life are positively correlated neonatal growth parameters of weight and length Z-scores, but not the BMI Z-score (that reflects obesity) at the time of discharge. These results give insights into the potential role of FGF-21 in early neonatal period into postnatal growth and developmental maturation.

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Declaration of competing interest

Authors have no conflicts of interests.

CRediT authorship contribution statement

Kyoung Eun Joung: Formal analysis, Writing - original draft. **Dana Clausen:** Writing - original draft. **Aimee Herdt:** Writing - review & editing. **Amy Presti:** Writing - review & editing. **Ruth Snyder:** Writing - review & editing. **Caryn Peters:** Writing - review & editing. **Helen Christou:** Writing - review & editing. **Christos S. Mantzoros:** Writing - review & editing.

References

[1] Hofman PL, Cutfield WS. Insulin sensitivity in people born pre-term, with low or very low birth weight and small for gestational age. J Endocrinol Invest 2006;29:2–8.
[2] Thompson JA, Regnault TR. In utero origins of adult insulin resistance and vascular dysfunction. Semin Reprod Med 2011;29:211–24.
[3] Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta 2000;1492:203–6.
[4] Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627–35.
[5] Babaknejad N, Nayeri H, Hemmati R, Bahrami S, Esmaillzadeh A. An overview of FGF19 and FGF21: the therapeutic role in the treatment of the metabolic disorders and obesity. Horm Metab Res 2018;50:441–52.
[6] Pyrzak B, Demkow U, Kucharska AM. Brown adipose tissue and browning agents: irisin and FGF21 in the development of obesity in children and adolescents. Adv Exp Med Biol 2015;866:25–34.
[7] Reinehr T, Woelfle J, Wunsch R, Roth CL. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. J Clin Endocrinol Metab 2012;97:2143–50.
[8] Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. New intrauterine growth curves based on United States data. Pediatrics 2010;125:e214–24.
[9] Olsen IE, Lawson ML, Ferguson AN, Cantrell R, Grabich SC, Zemel BS, et al. BMI curves for preterm infants. Pediatrics 2015;135:e572–81.
[10] Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F. Hepatic FGF21 expression is induced at birth via PPARalpha in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metabol 2010;11:206–12.
[11] Guasti L, Silvennoinen S, Bulstrode NW, Ferretti P, Sankilampi U, Dunkel L. Elevated FGF21 leads to attenuated postnatal linear growth in preterm infants through GH resistance in chondrocytes. J Clin Endocrinol Metab 2014;99:E2198–206.
[12] Sanchez-Infantes D, Gallego-Escuredo JM, Diaz M, Aragones G, Sebastiani G, Lopez-Bermejo A, et al. Circulating FGF19 and FGF21 surge in early infancy from infra- to supra-adult concentrations. Int J Obes 2015;39:742–6.
[13] Gavalda-Navarro A, Hondares E, Giralt M, Mampel T, Iglesias R, Villarroya F. Fibroblast growth factor 21 in breast milk controls neonatal intestine function. Sci Rep 2015;5:13717.
[14] Fisher FM, Chui PC, Antonellis PJ, Bina HA, Kharitonenkov A, Flier JS, et al. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 2010;59:2781–9.
[15] Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 2008;57:1246–53.
[16] Reinehr T, Karges B, Meissner T, Wiegand S, Fritsch M, Holl RW, et al. Fibroblast growth factor 21 and fetuin-A in obese adolescents with and without type 2 diabetes. J Clin Endocrinol Metab 2015;100:3004–10.
[17] Millward DJ. Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. Nutr Res Rev 2017;30:50–72.