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Acute Placental Inflammation and Growth Outcomes in Preterm Infants

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ABSTRACT

Background. Acute placental inflammation (API) has been associated with various adverse outcomes in preterm infants. However, little is known about how API affects their body composition and growth outcomes. This study aims to examine the association between API and growth outcomes in preterm infants.

Methods. We conducted a retrospective cohort study of mother-infant dyads born < $32^{6/7}$ weeks of gestation with fat mass (FM) accretion measured using air displacement plethysmography at term-equivalent age. API severity including maternal inflammatory response (MIR) and fetal inflammatory response (FIR) was staged according to criteria by Society for Pediatric Pathology based on placental pathology reports. We examined the differences in characteristics of mother-infant dyads by API severity using ANOVA or the Kruskall-Wallis test. The association between API severity and growth outcomes was analyzed using linear regression models and adjusted for statistically significant confounders. Mediation analyses were used to study the direct and indirect association of API and growth outcomes via gestational age.

Results. Among 375 mother-infant dyads analyzed, 104 (28%) dyads had MIR. FIR was found in 82% of dyads with MIR. Adjusted models indicated that API severity progression was positively associated with FM accretion. Gestational age partially (49%)

mediated the association between API severity and FM z score (indirect effect: β=0.12, 95% CI: 0.05-0.19; direct effect: β=0.12, 95% CI: 0.01-0.25).

Conclusions. API severity is associated with growth outcomes and helps to accurately predict body composition in preterm infants.

Keywords: Placental Inflammation, Chorioamnionitis, Body Composition, Growth, Preterm Infants

DEDICATION

I dedicate this thesis to my family. My heartfelt gratitude goes to the most loving parents, Edi, Feli, Rudi, and Inge for their unending support and prayers. To my best friend and beloved husband, Adrian, thank you for always believing in me.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF ABBREVIATIONS

ADP	Air Displacement Plethysmography
API	Acute Placental Inflammation
CI	Confidence Interval
FIR	Fetal Inflammatory Response
FM	Fat Mass
IUGR	Intrauterine Growth Restriction
IRB	Institutional Review Board
MIR	Maternal Inflammatory Response
MUAC	Mid-Upper Arm Circumference
N/A	Not Applicable
PMA	Post-menstrual Age
UAB	University of Alabama at Birmingham
%BF	Percent Fat

INTRODUCTION

Despite being the earliest and most important organ that develops following conception, the placenta remains understudied. The placenta is a vital organ that mediates the complex interaction between maternal and fetal physiology to facilitate optimum nutrient exchange for the growing fetus.[1] Throughout its relatively short lifespan, the placental morphology and function continuously evolve and adapt. Pathologies found in the placenta may help reveal in-utero insults and inform postnatal care.[2] Furthermore, there is mounting evidence that these pathologies have profound effects on maternal and neonatal outcomes.[3-5]

Acute placental inflammation (API) represents one of the most common placental pathologies that has been increasingly studied in recent years.[6] Goldstein et al. defined API as the histopathologic diagnosis of chorioamnionitis which could occur in the absence of clinical signs and symptoms.[7] Among preterm infants, exposure to inflammation may injure their developing organs. API is associated with nearly 2-fold higher odds of adverse outcomes, including sepsis,[8] respiratory disorders,[9,10] neurodevelopment impairment,[11-14] necrotizing enterocolitis,[15] and death,[7,16,17] in infants born <34 weeks of gestation regardless of exposure duration.[18] However, existing studies have not addressed the association of API and growth outcomes.

At term-equivalent age, preterm infants were found to have significantly higher percent-fat mass compared to their term-born counterparts suggesting postnatal catch-up

fat.[19] Although the optimal body composition of premature infants has yet to be defined, rapid fat mass (FM) accretion in preterm infants could indicate an adaptive postnatal response. Of note, their environment and nutritional supplies change dramatically when transitioning from intrauterine to extrauterine life. This adaptive mechanism might be essential in thermoregulation and fluid balance. While quality nutritional support has been shown to improve growth and fat-free mass accretion in preterm infants, FM accretion appears to be independent of intake.[20,21] It is possible that prenatal inflammation mediates such postnatal adaptation and influences FM accretion and overall postnatal growth.

Hence, this study aimed to examine the association between API and growth outcomes in preterm infants. We hypothesized that the association between API severity and growth outcomes is dose-dependent.

METHODS

Study Design, Setting, and Participants

We conducted a retrospective cohort study to evaluate the effects of API and growth outcomes in preterm infants using prospectively collected data of mother-infant dyads born < $32^{6/7}$ weeks of gestation admitted to the tertiary neonatal intensive care unit at the University of Alabama at Birmingham (UAB) between 2016 to 2022. To be eligible, placental pathology reports had to be available and infants had to undergo body composition measurements at approximately term-equivalent age. Infants with genetic abnormalities were excluded from the analysis. Waiver of authorization and informed consent were obtained from the UAB Institutional Review Board (IRB-300009890).

Growth Outcome Measures

Primary outcome of interest was FM accretion defined by FM and percent-fat (%BF). FM and %BF were assessed using air displacement plethysmography (ADP) with the PeaPod® at 36 weeks post-menstrual age (PMA) or prior to discharge, whichever occurred first. We also calculated FM-for age and %BF-for-age z scores using the LMS method.[22]

Secondary outcomes were mid-upper arm circumference (MUAC) at term-equivalent age and anthropometric growth rates from birth to 36 weeks PMA or discharge.

Anthropometric growth rates were measured in terms of changes in weight-for-age, length-for-age, and head circumference-for-age z scores along with weight, length, and head circumference gains. Weight gain was calculated using difference in grams, 2-point average and exponential methods.[23]

API Severity

Our primary exposure was severity of API based on placental pathology reports. Pathologic examination of the placenta is routinely performed in cases of preterm delivery at our institution. Depending on the location of inflammation, API is separated into maternal inflammatory response (MIR) and fetal inflammatory response (FIR). The severity of each was categorized according to staging criteria proposed by the Society for Pediatric Pathology.[24]

Potential Confounders

We included several neonatal, maternal, and placental variables as potential confounders. All infant and maternal variables were derived from the respective medical records while placental variables were obtained from placental pathology reports.

The following neonatal variables were examined: birth weight, sex, race, delivery mode, antibiotics administered at ≤ 72 hours prior to delivery, and antenatal steroid treatment doses. Race was classified as “Black” and “Non-Black”. We categorized antenatal steroid treatment doses as “None”, “1 dose”, and “≥2 doses”.

Maternal variables assessed were age, obesity, smoking status, substance use, as well as hypertension, and diabetes mellitus based on diagnosis codes. Obesity was classified according to maternal BMI at hospital admission using a threshold of 30.0 kg/m^2. Placental variables evaluated included placental weight and disk dimensions (length and width).

Statistical Analysis

Descriptive statistics were presented using frequencies and percentages for categorical data. Continuous data were summarized with means and standard deviations or medians with interquartile ranges. We summarized the demographic characteristics and growth outcomes by API severity. We evaluated differences in demographic characteristics and growth outcomes by API severity using one-way ANOVA with Tukey-Kramer's HSD for the post-hoc t-test or Kruskall-Wallis and Chi-square tests. The association between API and growth outcomes was analyzed using linear regression models. We adjusted models for significant confounders (i.e., variables resulting in a 10% change in the relationship between API and growth outcomes). Our final model included significant confounders birth weight, delivery mode, maternal BMI, and hypertension. Due to the possibility of unequal placental sharing among dyads with multiple gestation, we conducted a sensitivity analysis which only included mother-infant dyads with singleton birth. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and were performed at $\alpha=0.05$ significance level.

As preterm birth lies on the causal pathway, we further investigated if gestational age mediated the relationship between API and growth. Mediation analysis was conducted using the Valeri and VanderWeele SAS macro.[25]

RESULTS

There were 1,180 mother-infant dyads born < $32^{6/7}$ weeks of gestation admitted to UAB between 2016 to 2022. Of these, we excluded 804 dyads who did not have body composition measurements at approximately term-equivalent age and 1 dyad with no placental pathology report. Our final analytic sample consisted of 375 mother-infant dyads with slightly more Black (53%), and female (55%) infants. The mean birth weight was 1239 ± 405 grams and the median gestational age was 29 weeks (Table 1).

We identified 104 (28%) dyads with MIR indicating histologic chorioamnionitis. Funisitis, which corresponds to FIR, was present in 82% of histologic chorioamnionitis. MIR and FIR were strongly and positively correlated (r=0.87, p<0.0001). Birth weight, gestational age, multiple gestation, delivery mode, antibiotics administered prior to delivery, antenatal steroid treatment doses, maternal obesity, hypertension, and placental weight differed significantly by API severity (Table 1). Infants with stage 3 MIR had lower birth weight, had smaller gestational age, received antibiotics treatment prior to delivery, and had lower placental weight compared to those without MIR and those with lower MIR stages (Table 1). Conversely, compared to those with MIR, mothers without MIR were more likely to have obesity, hypertension and had Cesarean section delivery (Table 1). There were no significant differences in sex, race, maternal age, maternal smoking status, maternal substance use, maternal diabetes, and placental length and width by API severity.

Table 1. Demographic characteristics of mother-infant dyads by acute placental inflammation (API) severity

	Total (n=375)	**MIR None (n=271)**	**MIR Stage 1 (n=19)**	**MIR Stage 2 (n=57)**	**MIR Stage 3 (n=28)**	***p*-value**
Neonatal Variables						
Birth Weight, g	1239 ± 405†	1268 ± 405†	1432 ± 375†	1185 ± 368†	931 ± 334†	**<0.0001**
Gestational Age, weeks	29 (27-31)‡	30 (28-32)‡	30 (28-32)‡	28 (27-30)‡	26 (25-27)‡	**<0.0001**
Sex, n(%)						0.2618
Female	206 (55)	151 (56)	8 (42)	28 (49)	19 (68)	
Male	169 (45)	120 (44)	11 (58)	29 (51)	9 (32)	
Race, n(%)						0.0694
Black	197 (53)	134 (49)	14 (74)	30 (53)	19 (68)	
Non-Black*	178 (47)	137 (51)	5 (26)	27 (47)	9 (32)	
Multiple Gestation, n(%)						**0.0123**
Yes	100 (27)	84 (31)	4 (21)	6 (11)	6 (21)	
No	275 (73)	187 (69)	15 (79)	51 (89)	22 (79)	
Delivery Mode, n(%)						**<0.0001**
Vaginal	130 (35)	64 (24)	13 (68)	38 (67)	15 (54)	
C-section	245 (65)	207 (76)	6 (32)	19 (33)	13 (46)	
Antibiotics Prior to Delivery, n(%)						**<0.0001**
Yes	269 (72)	178 (66)	13 (68)	52 (91)	26 (93)	
No	106 (28)	93 (34)	6 (32)	5 (9)	2 (7)	
Antenatal Steroids, n(%)						**0.0255**
None	25 (7)	18 (7)	4 (21)	2 (3)	1 (4)	
1 dose	56 (15)	39 (14)	6 (32)	6 (11)	5 (18)	
≥ 2 doses	294 (78)	214 (79)	9 (47)	49 (86)	22 (78)	
Maternal Variables						
Age, years	28 (23-32)‡	28 (23-32)‡	27 (21-33)‡	26 (23-32)‡	30 (25-32)‡	0.5198
Obesity, n(%)						**0.0119**
Yes	243 (66)	190 (71)	9 (50)	27 (51)	17 (61)	
No	123 (34)	77 (29)	9 (50)	26 (49)	11 (39)	
Smoking Status, n(%)						0.2667
Yes	53 (14)	40 (15)	3 (16)	4 (7)	6 (23)	
No	313 (86)	226 (85)	16 (84)	51 (93)	20 (77)	
Substance Use, n(%)						0.3481
Yes	40 (11)	28 (11)	4 (21)	4 (7)	4 (15)	
No	324 (89)	236 (89)	15 (79)	51 (93)	22 (85)	
Hypertension, n(%)						**<0.0001**
Yes	230 (61)	202 (75)	3 (16)	13 (23)	12 (43)	
No	145 (39)	69 (25)	16 (84)	44 (77)	16 (57)	
Diabetes, n(%)						0.4752
Yes	71 (19)	53 (20)	1 (5)	11 (19)	6 (21)	
No	304 (81)	218 (80)	18 (95)	46 (81)	22 (79)	
Placental variables						**0.0227**
Weight, g	243 ± 83†	238 ± 84†	284 ± 64†	260 ± 89†	225 ± 68†	
Disk dimensions, cm						

Length	15.6 ± 2.3†	15.6 ± 2.4†	16.2 ± 1.3†	15.5 ± 2.3†	15.5 ± 2.3†	0.7232
Width	12.6 (11.0-14.1)‡	12.5 (11.0-14.0)‡	14.0 (13.0-15.0)‡	13.0 (11.5-14.5)‡	12.6 (9.9-13.8)‡	0.0524

MIR = Maternal Inflammatory Response
† Mean ± SD
‡ Median (IQR)
* "Non-Black" races include White, Asian and Hispanic ethnicity

We observed statistically significant differences in fat mass accretion, MUAC, and change in weight z score by API severity (Table 2). At term-equivalent age, infants without MIR had lower FM (0.32 kg vs Stage 3: 0.45 kg, $p<0.0001$ and lower FM z score (0.63 ± 1.10 vs Stage 3: 1.34 ± 1.05, $p=0.0001$) than those with MIR (Table 2). Infants with more severe MIR had higher %BF (Stage 3: 18.3% vs Stage 1: 14.0%, $p<0.0001$) and % BF z score (Stage 3: 2.08 ± 1.00 vs Stage 1: 1.17 ± 1.27, $p=0.0001$) than those without and Stage 1 MIR (Table 2). There were no significant differences in other anthropometric growth rates by API severity.

Table 2. Growth outcomes by maternal inflammatory response (MIR)

	Total (n=375)	**MIR None (n=271)**	**MIR Stage 1 (n=19)**	**MIR Stage 2 (n=57)**	**MIR Stage 3 (n=28)**	***p*-value**
Body Composition at term-equivalent age*						
Fat Mass, kg	0.33 (0.24-0.44)‡	0.32 (0.23-0.42)‡	0.34 (0.23-0.46)‡	0.37 (0.29-0.45)‡	0.45 (0.34-0.66)‡	**<0.0001**
Percent Fat, %	14.9 ± 4.8†	14.4 ± 4.8†	14.0 ± 5.5†	16.2 ± 4.1†	18.3 ± 4.4†	**<0.0001**
Fat Mass z score	0.78 ± 1.10†	0.63 ± 1.10†	0.84 ± 1.11†	1.18 ± 0.93†	1.34 ± 1.05†	**0.0001**
Percent Fat z score	1.39 ± 1.17†	1.25 ± 1.17†	1.17 ± 1.27†	1.77 ± 1.01†	2.08 ± 1.00†	**0.0001**
Mid-Upper Arm Circumference, cm	8.8 (8.0-9.3)‡	8.6 (8.0-9.1)‡	8.8 (8.3-9.0)‡	8.8 (8.0-9.3)‡	10.0 (9.0-10.3)‡	**0.0353**
Anthropometric Growth Rates**						

Change in Weight z score	-0.95 ± 0.59†	-0.90 ± 0.55†	-0.91 ± 0.59†	-1.02 ± 0.58†	-1.31 ± 0.87†	**0.0030**
Change in Length z score	-1.29 ± 0.93†	-1.23 ± 0.84†	-1.36 ± 1.10†	-1.44 ± 0.96†	-1.59 ± 1.38†	0.1189
Change in Head Circumference z score	-0.85 (-1.39 to -0.24)‡	-0.84 (-1.41 to -0.22)‡	-0.65 (-1.01 to 0.17)‡	-0.82 (-1.30 to -0.19)‡	-1.14 (-1.80 to -0.73)	0.0618
Weight gain, g/day	20 (16-24)‡	20 (16-24)‡	23 (14-26)‡	21 (17-25)‡	19 (14-23)‡	0.4883
Weight gain (2-point), g/kg/day	13 (10-14)‡	13 (10-14)‡	12 (10-14)‡	12 (11-14)‡	13 (12-14)‡	0.6988
Weight gain (Exponential), g/kg/day	13 (11-15)‡	13 (10-15)‡	12 (10-14)‡	13 (12-15)‡	14 (12-15)‡	0.3648
Length gain, cm/week	0.8 (0.6-1.0)‡	0.8 (0.6-1.0)‡	0.8 (0.3-1.0)‡	0.8 (0.6-1.1)‡	0.8 (0.6-1.0)‡	0.8672
Head Circumference gain, cm/week	0.7 (0.5-0.8)‡	0.7 (0.5-0.8)‡	0.6 (0.6-0.9)‡	0.7 (0.6-0.8)‡	0.7 (0.7-0.8)‡	0.1434

† Mean ± SD
‡ Median (IQR)
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

The Association between MIR and Growth Outcomes

Our crude model revealed that with each stage of MIR progression, FM at term-equivalent age increased by 0.05 kg, %BF increased by 1.1%, and MUAC increased by 0.2 cm (Table 3). MIR progression was also positively associated with FM and %BF z scores at term-equivalent age but negatively associated with change in weight z score from birth to 36-week PMA (Table 3).

Table 3. Association between maternal inflammatory response (MIR) and growth outcomes

	Model 1		**Model 2**	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
Body Composition at term-equivalent age*				
Fat Mass, kg	0.05 (0.03-0.07)	**<0.0001**	0.02 (0.00-0.04)	0.0964
Percent Fat, %	1.1 (0.6-1.6)	**<0.0001**	0.6 (0.1-1.1)	**0.0142**
Fat Mass z score	0.25 (0.15-0.36)	**<0.0001**	0.23 (0.11-0.35)	**0.0003**
Percent Fat z score	0.26 (0.14-0.38)	**<0.0001**	0.21 (0.09-0.34)	**0.0012**
Mid-Upper Arm Circumference, cm	0.2 (0.1-0.4)	**0.0168**	0.1 (-0.1 to 0.3)	0.3495

Anthropometric Growth Rate**				
Change in Weight z score	-0.10 (-0.16 to -0.04)	**0.0007**	-0.07 (-0.14 to -0.01)	**0.0327**

Model 1 = Crude
Model 2 = Adjusted for birth weight, delivery mode, maternal obesity, and hypertension
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

After adjustment for birth weight, delivery mode, maternal BMI, and hypertension, the association between MIR and %BF persisted. (Table 3). With each stage of MIR progression, %BF at term-equivalent age increased by 0.6% (Table 3). MIR progression remained positively associated with FM and %BF z scores at term-equivalent age but negatively associated with change in weight z score from birth to 36-week PMA (Table 3). Sensitivity analyses restricted to mother-infant dyads with singleton birth showed similar magnitude of association between MIR and various growth outcomes (Table 4).

Table 4. Association between maternal inflammatory response (MIR) and growth outcomes restricted to mother-infant dyads with singleton birth (n=275)

	Model 1		**Model 2**	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
Body Composition at term-equivalent age*				
Fat Mass, kg	0.05 (0.03-0.07)	**<0.0001**	0.03 (0.00-0.05)	0.0586
Percent Fat, %	1.1 (0.5-1.6)	**<0.0001**	0.6 (0.0-1.2)	0.0532
Fat Mass z score	0.25 (0.12-0.37)	**<0.0001**	0.17 (0.03-0.32)	**0.0212**
Percent Fat z score	0.25 (0.11-0.38)	**0.0003**	0.15 (0.00-0.30)	0.0545
Mid-Upper Arm Circumference, cm	0.23 (0.04-0.42)	**0.0201**	0.14 (-0.09 to 0.37)	0.2355
Anthropometric Growth Rate**				
Change in Weight z score	-0.13 (-0.20 to -0.06)	**0.0002**	-0.11 (-0.19 to -0.03)	**0.0097**

Model 1 = Crude/Unadjusted
Model 2 = Adjusted for birth weight, delivery mode, maternal obesity, and hypertension
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

As birth weight is strongly and positively correlated to gestational age (r=0.81, $p<0.0001$), our mediation models were adjusted for significant confounders excluding birth weight. We observed that gestational age significantly but partially (49%) mediated the association between MIR and FM z score at term-equivalent age (indirect effect: β=0.12, 95% CI: 0.05-0.19; direct effect: β=0.12, 95% CI: 0.01-0.25) adjusted for significant confounders (Table 5). Gestational age partially mediated the association between MIR and %BF (88%), %BF z score (58%), and change in weight z score (59%), although their respective direct coefficients were not significant.

Table 5. Mediation analysis measuring the direct effect of maternal inflammatory response (MIR) and indirect effect of gestational age on growth outcomes

	Total effect†				Direct effect†				Indirect effect†				% Mediated‡
	β	**95% CI**	**Bootstrap 95% CI**	***p*-value**	**β**	**95% CI**	**Bootstrap 95% CI**	***p*-value**	**β**	**95% CI**	**Bootstrap 95% CI**	***p*-value**	
Body Composition at term-equivalent age*													
Percent Fat, %	1.15	0.62-1.68	0.64-1.66	**<0.0001**	0.14	-0.33 to 0.62	-0.35 to 0.64	0.5576	1.01	0.68-1.33	0.66-1.36	**<0.0001**	87.6
Fat Mass z score	0.24	0.12-0.36	0.12-0.37	**<0.0001**	0.12	0.01-0.25	-0.03 to 0.28	**0.0463**	0.12	0.07-0.17	0.05-0.19	**<0.0001**	49.1
Percent Fat z score	0.29	0.16-0.42	0.16-0.42	**<0.0001**	0.12	-0.01 to 0.25	-0.02 to 0.27	0.0602	0.17	0.11-0.24	0.09-0.25	**<0.0001**	58.4
Anthropometric Growth Rate*													
Change in Weight z score	-0.09	-0.15 to -0.02	-0.17 to -0.01	**0.0084**	-0.04	-0.10 to 0.03	-0.12 to 0.05	0.3000	-0.05	-0.08 to -0.02	-0.08 to -0.02	**0.0002**	59.2

† Adjusted for delivery mode, maternal BMI, and hypertension
‡ Calculated by dividing the indirect effect by total effect
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

Funisitis

Similar to MIR, infants without FIR had significantly lower FM (0.33 kg vs Stage 2: 0.39 kg, p=0.0076), %BF (14.8% vs Stage 1: 16.8%, p=0.0182), FM z score (0.66 ± 1.10 vs Stage 3: 1.23 ± 0.80, p=0.0041) and %BF z scores (1.27 ± 1.16 vs Stage 1: 1.77 ± 1.34, p=0.0138) at term-equivalent age compared to those with FIR (Table 6). On multivariable linear regression analyses adjusted for the same confounders, FIR progression remained positively associated with FM and %BF z scores (Table 7). Gestational age partially mediated the association between FIR and FM z score (41%) and %BF z score (50%), although their direct effects were not significant (Table 8).

Table 6. Growth outcomes by fetal inflammatory response (FIR)

	Total (n=371)	**FIR None (n=286)**	**FIR Stage 1 (n=29)**	**FIR Stage 2 (n=41)**	**FIR Stage 3 (n=15)**	***p*-value**
Body Composition at term-equivalent age*						
Fat Mass, kg	0.33 (0.24-0.44)‡	0.33 (0.23-0.42)‡	0.39 (0.29-0.55)‡	0.39 (0.32-0.49)‡	0.33 (0.29-0.46)‡	**0.0076**
Percent Fat, %	15.2 (12.2-17.5)‡	14.8 (11.9-17.2)‡	16.8 (13.4-19.3)‡	16.3 (14.1-19.1)‡	15.2 (12.7-18.3)‡	**0.0182**
Fat Mass z score	0.77 ± 1.10†	0.66 ± 1.10†	1.18 ± 1.16†	1.10 ± 1.05†	1.23 ± 0.80†	**0.0041**
Percent Fat z score	1.37 ± 1.17†	1.27 ± 1.16†	1.77 ± 1.34†	1.70 ± 1.04†	1.76 ± 0.88†	**0.0138**
Mid-Upper Arm Circumference, cm	8.8 ± 1.3†	8.7 ± 1.4†	9.3 ± 1.3†	8.9 ± 1.1†	8.8 ± 0.9†	0.1988
Anthropometric Growth Rates**						
Change in Weight z score	-0.95 ± 0.59†	-0.91 ± 0.56†	-1.10 ± 0.60†	-1.00 ± 0.73†	-1.19 ± 0.64†	0.1081
Change in Length z score	-1.28 ± 0.92†	-1.24 ± 0.88†	-1.75 ± 0.84†	-1.23 ± 1.10†	-1.26 ± 1.04†	**0.0382**
Change in Head Circumference z score	-0.82 ± 1.02†	-0.78 ± 1.02†	-0.90 ± 0.79†	-1.02 ± 1.19†	-0.87 ± 0.94†	0.5211
Weight gain, g/day	20 ± 6†	20 ± 6†	20 ± 7†	21 ± 7†	20 ± 5†	0.9691
Weight gain (2-point), g/kg/day	12 (10-14)‡	13 (10-14)‡	12 (11-13)‡	12 (12-14)‡	12 (10-13)‡	0.5493

Weight gain (Exponential), g/kg/day	13 (11-15)‡	13 (10-15)‡	13 (12-14)‡	13 (12-15)‡	13 (10-14)‡	0.4653
Length gain, cm/week	0.8 ± 0.4†	0.8 ± 0.4†	0.8 ± 0.3†	0.8 ± 0.6†	0.8 ± 0.4†	0.9159
Head Circumference gain, cm/week	0.7 (0.5-0.8)‡	0.7 (0.5-0.8)‡	0.7 (0.6-0.8)‡	0.7 (0.6-0.8)‡	0.7 (0.6-0.9)‡	0.3757

† Mean ± SD
‡ Median (IQR)
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

Table 7. Association between fetal inflammatory response (FIR) and growth outcomes

	Model 1		**Model 2**	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
Body Composition at term-equivalent age*				
Fat Mass, kg	0.04 (0.01-0.06)	**0.0045**	0.01 (-0.01 to 0.04)	0.2681
Percent Fat, %	0.84 (0.26-1.42)	**0.0046**	0.50 (-0.06 to 1.07)	0.0810
Fat Mass z score	0.23 (0.09-0.36)	**0.0009**	0.19 (0.05-0.33)	**0.0094**
Percent Fat z score	0.21 (0.07-0.35)	**0.0032**	0.18 (0.03-0.32)	**0.0185**
Anthropometric Growth Rate**				
Change in Length z score	-0.03 (-0.14 to 0.08)	0.5652	N/A	N/A

Model 1 = Crude/Unadjusted
Model 2 = Adjusted for birth weight, delivery mode, maternal obesity, and hypertension
* Measured at 36 weeks post-menstrual age (PMA) or discharge
** Calculated between birth and 36 weeks PMA or discharge

Table 8. Mediation analysis measuring the direct effect of fetal inflammatory response (FIR) and indirect effect of gestational age on growth outcomes

	Total effect†				Direct effect†				Indirect effect†				% Mediated‡
	β	**95% CI**	**Bootstrap 95% CI**	***p*-value**	**β**	**95% CI**	**Bootstrap 95% CI**	***p*-value**	**β**	**95% CI**	**Bootstrap 95% CI**	***p*-value**	
Body Composition at term-equivalent age*													
Fat Mass z score	0.20	0.06-0.34	0.07-0.34	**0.0047**	0.12	-0.02 to 0.26	-0.02 to 0.26	0.0827	0.08	0.03-0.13	0.02-0.14	**0.0015**	40.6
Percent Fat z score	0.23	0.08-0.38	0.09-0.37	**0.0029**	0.12	-0.03 to 0.26	-0.03 to 0.26	0.1079	0.11	0.05-0.18	0.03-0.19	**0.0007**	49.6

† Adjusted for delivery mode, maternal BMI, and hypertension

‡ Calculated by dividing the indirect effect by total effect

* Measured at 36 weeks post-menstrual age (PMA) or discharge

** Calculated between birth and 36 weeks PMA or discharge

DISCUSSION

In this retrospective cohort study of mother-preterm infant dyads, we found dose-dependent associations of API severity and FM accretion, MUAC, as well as change in weight z score from birth to 36 weeks PMA. Our analysis showed that birth weight, delivery mode, maternal obesity, and hypertension confounded the relationships and influenced the magnitude of these dose-dependent associations. We also found that gestational age partially mediated the association between API severity and growth outcomes.

Prior studies investigating the association between API and growth outcomes were lacking. With regards to anthropometric growth rates, a study of 88 matched very preterm infants by González et al. found that histologic chorioamnionitis did not affect weight gain, changes in weight z scores, or extrauterine growth restriction when adjusted for gestational age, IUGR and neonatal complications.[26] Contrastingly, we found that the severity of histologic chorioamnionitis was negatively associated with change in weight z score at 36 weeks PMA or discharge. The association was partially (59%) mediated by gestational age although direct effect was not significant. Our study also found that API did not affect changes in length and head z scores as well as postnatal weight, length, and head circumference gains, which have not been reported previously.

There was a significant association between API severity and MUAC at 36 weeks PMA which became insignificant after adjusting for confounders. This finding suggests

the importance of body composition in providing a more accurate assessment of growth status especially in preterm infants. Although not exclusively in a preterm infant population, body composition curves illustrate that body composition is dynamic during infancy which may not be reflected by anthropometric growth measures.[22]

API increased FM accretion in preterm infants with a dose-dependent relationship. In our study, each stage of MIR progression was directly associated with increase in %BF at term-equivalent age by 0.6%. Prior studies have demonstrated that API progression is associated with systemic inflammation and higher cortisol levels in the first week of life in preterm infants exposed.[16,27,28] It is plausible that these mechanisms induce fat accumulation in these infants. Cortisol has been shown to promote visceral fat tissue deposition.[29] Visceral fat has high metabolic activity and contributes to systemic inflammation.[30] Meanwhile, systemic inflammation potentially alters insulin-signaling pathways and causes insulin resistance, which promotes further fat accumulations in general and perpetuates the cycle as observed in individuals with obesity.[31] Indeed, Uthaya et al. found that preterm infants had significantly higher intra-abdominal and lower subcutaneous adipose tissues compared to term-born infants at term-equivalent age, with illness severity as the principal predictor of intra-abdominal adiposity resulting in altered adipose tissue partitioning.[32] Therefore, to accurately predict body composition in preterm infants, we need more information about the presence or absence of API.

Our exploratory mediation analysis indicated a direct association between API and increase in FM z score at term-equivalent age. However, it is inconclusive whether API was directly associated with fat mass accretion in general, as there were non-significant

direct effects observed for %BF and %BF z score. Additional data is required to examine these questions as the current sample is likely underpowered.

Strengths of our study include the use of large longitudinal cohort consisting of preterm infants born in a tertiary center. This comprehensive approach allowed us to investigate the association between API with multiple growth outcomes, including FM accretion, and performed an exploratory mediation analysis to study the indirect effects of preterm birth. However, our study is not without limitations. First, this was a retrospective cohort study, so we are unable to determine a causal relationship between API severity and FM accretion. Secondly, our study only included preterm infants with body composition analysis performed using ADP. Because API is uncommon in term infants and placental pathology is rarely ordered for them, our findings are not generalizable to term infants. Furthermore, due to the impracticalities of placing infants into a separate air chamber,[33] ADP is limited to clinically stable preterm infants. Thus, it is possible that our findings predicted an association that is weaker than the actual relationship assuming that more infants exposed to API were unable to undergo ADP assessment. Another limitation is that mother-infant dyads were only recruited from a single referral academic hospital which may limit the generalizability of our findings.

CONCLUSION

To the best of our knowledge, this is the first study reporting the direct dose-dependent effect of API severity on FM z score, in conjunction with its indirect effect via preterm birth which has been widely recognized. Our findings indicate that accurate prediction of body composition in preterm infants requires consideration of API as well because API may partially explain rapid FM accretion as a potential adaptive mechanism in preterm infants. Future studies attempting to analyze causes of rapid fat-mass accretion in preterm infants should include information on API either through placental pathology reports or postnatal biomarkers, including cytokine profiles, and ideally should include multiple centers to enhance the external validity of the results. Additional research could further explore the associations of API and FM distributions as well as other body composition models.

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