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Newborn adipokines and birth outcomes

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Abstract

Background—Adipokines can serve as a measure of adipose tissue activity. Although birthweight correlates with neonatal adiposity, findings for cord blood levels of adipokines and birth outcomes have been conflicted. Therefore, we determined the cross-sectional associations between adipokines measured in newborn dried blood spots (DBS) and birth outcomes.

Methods—The Upstate KIDS study enrolled mothers and infants from 2008 to 2010. Among infants whose parents consented to use of residual DBS from Newborn Screening, 2397 singletons and 1240 twins had adipokine measurements from the Human Obesity Panel (R&D Systems) by Luminex. Odds ratios were estimated by multivariable logistic regression for risk of birth outcomes of preterm delivery (<37 weeks for singletons, <32 for twins) and small for gestational age (SGA <10th for singletons and <3rd for twins age and sex specific percentiles) by adipokine quintiles. Generalized estimating equations were applied to account for correlations between twins.

Results—Singletons in the lowest compared to the highest quintile of adiponectin were more likely preterm (adjusted odds ratio 3.26; 95% confidence interval: 1.99–5.34), and SGA (1.81; 1.18–2.77). Similar associations were observed among twins. Resistin was associated with preterm

birth (Q1 vs Q5: 2.08; 1.20-3.62) only among singletons. Adipsin had inconsistent associations after adjustment.

Conclusions—This large population based study demonstrates that newborn DBS measured adipokines are associated with birth outcomes, particularly preterm birth and SGA among those with lower adiponectin levels regardless of plurality.

Growth restriction and preterm birth are associated with an increased risk of obesity and cardiovascular disease later in life.¹ Alterations to adipocyte number and volume may be potential mechanisms for these associations.² Although there are no established biomarkers of adipocyte number and volume, adipose derived hormones measured at birth can serve as a good marker of adipose tissue function. An increased number of studies have revealed the active endocrine role that adipose tissue plays, including regulating satiety, reproductive function, and inflammation.³ The most well-studied adipokines are adiponectin and leptin, both of which have been shown to be related to obesity in adult populations.⁴ Adiponectin in particular may also be associated with adipocyte size.⁵

Although birthweight is correlated with neonatal fat mass⁶, conflicting findings have been observed between cord blood levels of measured adiponectin and leptin with birth size.⁷⁻¹⁰ In addition, very few studies have investigated resistin^{7,11,12} and adipsin¹³ at birth. Many previous studies have been small case-control studies investigating one birth outcome at a time or in selected populations (e.g., offspring of diabetic mothers) owing to the difficulties of collecting cord blood and the expense of assays. Studies also routinely excluded twins. Therefore, our study sought to investigate the associations between adipokines and birth size based on a large population study sample made possible by measurement of newborn dried blood spots. We hypothesize that small for gestational age and preterm birth would be associated with a different pattern of adipokine levels (i.e., lower levels of adiponectin).

Methods

Upstate KIDS is a birth cohort which recruited mothers and infants from New York State (excluding New York City) from 2008 to 2010.¹⁴ The study was originally designed to evaluate the long-term impact of infertility treatment on child health.¹⁴ As such, mothers were recruited based on birth certificate indication of infertility treatment.¹⁴ All mothers of singletons conceiving with infertility treatment and those who conceived without treatment frequency matched on region of birth at 1:3 ratio were recruited. In addition, all mothers of multiples were recruited regardless of mode of conception. Further details on the sampling and accuracy of using birth certificates have been discussed elsewhere.¹⁴ 3905 mothers of singletons (n=1011 conceived by infertility treatment, 2894 not conceived by treatment) and 1084 mothers of twins (n=286 conceived by infertility treatment, 798 not conceived by treatment) enrolled in the study. Mothers completed questionnaires regarding their pregnancy, infant's health and related information at enrollment approximately 4 months postpartum. The primary cohort of the study included all singletons and a random twin of the pair (n=4989). The secondary cohort consisted of the other twin in the pair (n=1066). The New York State Department of Health and the University of Albany (State University of New York) Institutional Review Boards (NYSDOH IRB #07-097; UAlbany #08-179)

approved the study and served as the IRB designated by the National Institutes of Health under a reliance agreement. All participants provided written informed consent.

Blood spot analyses

To better understand how prenatal exposures (such as infections and environmental chemicals) may affect the associations between infertility treatment and child health, newborn dried blood spot (DBS) analyses were conducted. Parental consent for using residual DBS specimens collected as part of the Newborn Screening Program for analyses was requested at 8 months postpartum. DBS cards of those with consent were retrieved from cold storage (4°C). Punches of the residual spots were extracted in a similar manner as previously described for other analytes.¹⁵ Eluants from the extraction of each 3.2mm punch were frozen at -80°C until analysis. Adipokines were measured as part of the Human Obesity Panel (R&D Systems, Minneapolis, MN) using a Luminex¹⁰⁰ analyzer with xPONENT 3.1 software (Luminex System, Austin, TX). Based on 3769 replicate measurements, the intra-assay coefficients of variation (CV) were 7-9% except for leptin which was 43% due to low sensitivity. Inter-assay CVs calculated at levels of DBS values ranged 6-9%. Due to the low detectability of leptin, its values were dichotomized at the median level (2.43 pg/ml).

Analyte levels were averaged from duplicate measures. Instrument reported values for those below the limit of detection (including samples where the instrument could not detect the presence of the analyte, and a value of zero was assigned) were used without censoring to prevent potential bias.¹⁶ For leptin, 3 panels failed to run the standard curve with at least 7 points and were excluded (n=108). In addition, for almost half of the participants leptin was not detected in either duplicate run (n=1791). Of the remaining (n=1864), 1495 (80%) had two non-zero replicate measurements and 369 (20%) had only one. Conversely, 29 participants had CRP above the upper limit of detection (>23,500 pg/ml). Aside from leptin, few participants (n=11) had a measurement equal to zero and all had one observable value which was averaged in analysis.

Birth size measures

Birthweight information came from birth certificates. Mothers reported birth length at baseline. Reported lengths exceeding the feasible range ± 3 cm by gestational age as reported from a US reference were excluded (n=95).¹⁷ Ponderal index was calculated as $100 \times [\text{birthweight (g)/length (cm}^3\text{)}]$. Gestational age was based on the clinical estimate collected from birth certificates. The birth attendant determined the best clinical estimate of gestational age by using all perinatal information including last menstrual period and ultrasound but not by measurements from the neonatal exam. Preterm birth was less than 37 weeks and early preterm was less than 32 weeks. Size for gestational age was computed using an external US population of singletons as reference.¹⁸ For singletons, large (LGA) and small for gestational age (SGA) were defined as $>90^{\text{th}}$ and $<10^{\text{th}}$ percentile, respectively. For twins, SGA was defined at a lower cut-point of $<3^{\text{rd}}$ percentile as $>30\%$ of the twins were $<10^{\text{th}}$ percentile by the singleton reference due to no twin reference being available.

Covariates

Parental ages, infertility treatment, private insurance use, marital status, neonatal intensive care unit (NICU) admission and parity were from birth certificates. Mothers reported on alcohol, smoking, and paternal anthropometrics. Race/ethnicity and education came from maternal report and relied on birth certificates as necessary. Prepregnancy weight and height information to derive body mass index (BMI) was from birth certificates and relied on maternal report if missing (n=53). Positive reports of gestational diabetes and hypertensive disorders in pregnancy were defined as indication from one of three sources, the hospital discharge codes from NYSDOH Statewide Planning and Research Cooperative System, birth certificate or maternal report.

Statistical analysis

Values were log transformed for normality. Quintiles (Q1-Q5) were generated to evaluate non-linear associations. Chi-square and analysis of variance were used to determine differences in baseline characteristics for categorical and continuous variables, respectively, by quintiles of birthweight. As parental information would be the same between twins, Table 1 shows the characteristics of all singletons and 1 random twin of a pair. Linear regression was used to determine the associations between the adipokines and continuous measures of birthweight z-scores, length, and ponderal index stratified by plurality, in keeping with the methods used in previous publications for comparability. Generalized estimating equations (GEE) were applied to account for the correlation between twins. However, to evaluate non-linear associations in this large cohort, risks of birth outcomes were conducted using plurality specific quintiles. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) for birth outcomes among singletons and GEE applied among twins. Maternal age, race, prepregnancy BMI, education, private insurance, marital status, paternal factors (age, race, BMI), infant gender, infertility treatment, parity, serpin and c-reactive protein (CRP) were evaluated as confounders. From these, maternal age, race, BMI, education, private insurance, infant gender, and CRP were retained as others did not meaningfully change results. Models with preterm birth were additionally adjusted for NICU admission. Models were run with mutual adjustment for the other adipokines. . For all regression models, sampling weights based on infertility treatment, twinning and New York State birth regions were applied to account for the study's sampling scheme; that is, recruitment of all who conceived with infertility treatment and of multiple gestation with a random group of unexposed singletons frequency matched on perinatal region of delivery.¹⁴ Weights were derived based on New York State birth certificate data of over 205,000 births, corresponding to the ~2-year recruitment period between 2008 and 2010. Analyses were completed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

We received parental consent to use residual Newborn Screening blood spots for 2388 singleton (61%) and 1237 twin (58%) infants. Among the infants with consent, 3637 (95.7%) had at least one measure of the 4 adipokines available (i.e., adiponectin, adipisin, resistin, leptin). Blood spots were collected median 2 days after birth for term deliveries (IQR 2-3 days) and 3 days after birth for preterm deliveries (IQR 2-4 days). Despite

significant positive associations with birthweight (Table 1), adipokines were not strongly correlated with each other with Spearman rank correlations ranging from 0.14 to 0.26. Maternal factors in association with higher birthweight observed were as expected and included increasing pre-pregnancy BMI, white race, being parous, married, and not having a hypertensive disorder during pregnancy. Accounting for plurality did not remove these associations with maternal characteristics (data not shown).

In adjusted models (Table 2), adiponectin was positively associated with birthweight z-scores, birth length, ponderal index and gestational age among twins but only birth length and gestational age among singletons. Resistin was positively associated with birth length and gestational age among singletons but no associations were observed for twins. Higher levels of leptin (dichotomized due to difficulty in measurement) were associated with greater birthweight z-scores regardless of plurality.

Table 3 shows the adjusted associations between increasing quintiles of adipokines and birth outcomes, with the highest quintile (Q5) being the reference group for comparisons. Among both singletons and twins, adiponectin was significantly associated with preterm birth and SGA. However, a trend was observed for preterm delivery among singletons whereas other associations remained significant only at the first or second quintiles. Adipsin was associated with early preterm among twins without a clear trend and sporadically associated with other outcomes. Resistin was associated with preterm birth but the association did not remain significant after additional adjustment for NICU admission (Q1 vs Q5: 1.52; 0.83-2.79). Low leptin levels were associated with higher odds of SGA and lower odds of LGA among singletons.

Comment

To our knowledge, this paper is the first to demonstrate associations between DBS measured adipokines and common birth outcomes at a population level. Several key findings were revealed in our analyses. First, apart from leptin, adipokines were reliably measured in all blood spots using multiplex despite the low volume of specimens. The feasibility of these measures shows that future studies can leverage biospecimens in a similar fashion. Second, while adiponectin may be influenced more by the timing of birth (i.e., gestational age), leptin is not and reflected birth size more closely. Third, adipsin and resistin are inconsistently associated with birth size after accounting for the other measures. Lastly, although we performed linear associations in keeping with other studies, we found that the lowest quintile of adipokines tended to be more discriminatory than the middle quintiles, perhaps explaining inconsistent results among smaller studies.

Adiponectin, an adipocyte specific hormone, regulates satiety and has insulin sensitizing effects in muscle and liver.¹⁹ Many studies have also observed a positive correlation with adiponectin levels measured in cord blood and birthweight.⁹ We here also found the most robust associations, regardless of plurality with adiponectin. Gestational age was found to be a strong predictor of higher cord blood adiponectin in a case control study investigating preterm birth from Spain.²⁰ A recent study found no association with birthweight but also suggested it may be due to gestational age having a greater influence on adiponectin levels

at birth.⁷ We confirm those observations in a larger cohort using DBS measures and that the associations remain after adjustment for other adipokines including leptin and important covariates. Single nucleotide polymorphisms of the adiponectin gene have been found to be associated with birthweight and cord blood levels, further supporting these associations.²¹ Similar to another large cross-sectional study of over 300 neonates, adiponectin levels were not associated with ponderal index after adjustment for covariates²², suggesting associations with birth size do not signify disproportional growth. In our observations, adiponectin had the most consistent inverse associations with preterm birth, which was similarly observed among twins with respect to early preterm delivery. A study of 43 term and 58 preterm infants suggests that adiponectin levels remained altered even after term-equivalent age.²³ Therefore, these adiponectin levels at birth could carry long-term implications regarding adipose tissue function.

Resistin, secreted by macrophages in adipose tissue, opposes the action of insulin and affects insulin sensitivity, with higher levels inconsistently observed among obese than lean adults.²⁴ Associations with resistin for birthweight and gestational age have also been conflicted. In a study of 120 Chinese newborns, resistin was not associated with birthweight or length nor other anthropometric measures;²⁵ Associations were also not observed with cord blood from 60 term newborns of diabetic mothers²⁶ or among full-term singletons from Generation XXI.⁷ The same study, which observed conflicting associations with adiponectin, also found higher umbilical cord serum resistin levels among those with fetal growth restriction and lower levels among those who were macrosomic.¹¹ Conflicting findings have been found regarding resistin and gestational age as well with a negative correlation in one study measuring cord blood²⁰ and a positive correlation in another study using resistin in venous samples collected within 2 hours after birth²⁷. Our findings are similar to the study collecting postpartum venous samples suggesting cord blood resistin may not represent neonatal expression due to placental production²⁸, although these studies differ on a number of factors including assay methods. We improved on these analyses with a larger sample size and the ability to control for other adipokines. Yet our findings still conflict with previous studies given that the lower resistin levels being associated (albeit non-significantly) with both low birthweight and LGA in adjusted models. Resistin may be more of an indicator of pregnancy complications that may lead to different birth size phenotypes (e.g. high resistin levels in LGA infants born to mothers with diabetes as well as for low birthweight infants born to those with hypertension), and of neonatal distress (as NICU adjustment removed associations). However, maternal levels of resistin may be needed to tease apart these mechanisms.

Adipsin, or complement factor D, functions by forming acylation stimulating protein which regulates lipid accumulation in adipose tissue.¹³ Adipsin's role in human adiposity remains unclear and has rarely been measured in newborns. Adipsin was increased in cord blood of obese mothers and in secretions from placental explants.¹³ Others reported a positive correlation with birthweight ($r=0.32$, $p<0.05$), which we also observed. However, when other adipokines are accounted for, no significant associations with birth outcomes and adipsin were found, making it less informative as a biomarker.

A meta-analysis of 44 studies found that positive associations between newborn leptin levels and birthweight did not differ by infant sex or race and that significant correlations were also demonstrated for birth length and ponderal index.²⁹ Although we had to dichotomize leptin values due to low detection, our findings using DBS were consistent with previous literature. Leptin was difficult to measure in eluants of DBSs possibly due to interference by the soluble leptin receptor or other factors released from lysed blood cells. Human leukocytes express leptin receptors³⁰, which could be released from the lysed leukocytes. In addition, different maternal or perinatal stresses associated with pregnancy and birth could affect leptin since stress, including oxidative stress, can modulate their expression.³¹ We tried to take these stresses into account through adjustment for CRP and NICU admission with little impact on results.

All adipokines except leptin were measurable in all DBSs. However, the mean levels of DBS adipokines differ from previously reported levels using cord or venous blood. The eluants from DBSs represent lysed blood cells and plasma and not serum or plasma alone. A previous study using blood spots to collect samples in children found high correlation between serum and blood spot levels of adiponectin measured by immunoassay despite the absolute mean levels differing.³² Even serum versus plasma levels of multiplex cytokines has been observed to differ.³³ Thus, major differences in absolute levels measured with the DBSs as compared to previously published literature for serum or plasma cord blood or venous levels are to be expected.

The strengths of our study include having a large sample size made possible by the use of DBSs for measurements and accounting for many covariates. However, we relied on the birth certificate for reports of birthweight and gestational age. Birth length is also well known to be difficult to measure and therefore maternal report may have been subject to error. However, these misclassifications would not be biased by adipokine levels. We also were limited in having to use a singleton reference due to lack of a good twin reference for birth weight z-scores and size for gestational age. We set lower cutoffs for twins with regard to SGA for such purposes. However, it remains difficult to distinguish between whether associations are pathological versus maturational; that is, whether adiponectin is associated with earlier delivery by itself or the pathological cause of the delivery. Our difficulties in measuring leptin may be due to use of multiplex which has less sensitivity than ELISA. Conflicting findings have been observed for effects of temperature on adipokine stability in DBS^{34,35} but we cannot rule out that the initial cold storage prior to freezing contributed to low measures of leptin. Though these random misclassifications may have decreased power to detect differences, that our findings are in line with those previously observed with cord or venous blood are reassuring.

Conclusion

Preterm birth and growth restriction are known risk factors for future cardiometabolic risk.¹ The development of adipocytes *in utero* may play a role downstream in the energy regulation of newborns as they grow. Our findings demonstrate associations between preterm birth and small for gestational age with adipokine levels and support the use of DBS

as an alternative to cord blood. Further analyses in this cohort will investigate how these levels at birth may be associated with infant growth and obesity risk.

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Table 1

Maternal and Offspring Characteristics by Quintiles of Birthweight from the Primary Cohort in Upstate KIDS (n=3033)

Characteristics ^a N	Quintile 1 (539-2692) 604	Quintile 2 (2693-3119) 606	Quintile 3 (3120-3409) 610	Quintile 4 (3410-3742) 613	Quintile 5 (3743-5255) 600
Birthweight (g)	2,163 (472)	2,925 (121)	3,269 (83)	3,572 (97)	4,050 (258)
Adiponectin (µg/ml) ^{b*}	0.35 (0.34-0.37)	0.42 (0.41-0.44)	0.45 (0.44-0.46)	0.46 (0.44-0.47)	0.45 (0.44-0.47)
Adipsin (µg/ml) ^{b*}	0.09 (0.08-0.09)	0.10 (0.10-0.11)	0.11 (0.10-0.11)	0.11 (0.10-0.11)	0.11 (0.10-0.11)
Resistin (ng/ml) ^{b*}	16.2 (15.3-17.2)	21.7 (20.6-22.8)	24.7 (23.5-25.9)	25.6 (24.4-27.0)	25.6 (24.4-26.8)
Leptin above median (n, %) [*]	246 (42)	275 (47)	316 (54)	328 (55)	353 (61)
C-reactive protein (ng/ml) ^{b*}	9.4 (8.3-10.5)	18.7 (16.9-20.6)	24.4 (22.1-27.0)	26.7 (24.2-29.4)	31.9 (29.0-35.0)
Serpin E1 (ng/ml) ^{b*}	2.57 (2.48-2.66)	2.72 (2.64-2.81)	2.81 (2.71-2.92)	2.87 (2.77-2.96)	2.74 (2.64-2.84)
Maternal age (years)	32 (6)	31 (6)	31 (6)	31 (6)	31 (6)
Paternal age (years)	34 (7)	33 (7)	34 (7)	33 (6)	34 (6)
Pre-pregnancy BMI (kg/m ²) [*]	26.51 (6.55)	26.70 (6.91)	26.56 (6.37)	26.59 (6.50)	28.68 (7.42)
Underweight [*]	19 (3)	15 (2)	13 (2)	13 (2)	6 (1)
Normal	291 (48)	301 (50)	295 (49)	304 (50)	215 (36)
Overweight	156 (26)	136 (22)	160 (26)	145 (24)	189 (32)
Obese	136 (23)	153 (25)	140 (23)	150 (25)	190 (32)
Paternal BMI (kg/m ²)	28.22 (5.46)	28.00 (5.32)	28.10 (5.34)	28.18 (5.60)	28.79 (5.51)
Underweight	5 (1)	7 (1)	5 (1)	1 (0)	2 (0)
Normal	148 (28)	141 (25)	148 (27)	157 (28)	130 (24)
Overweight	225 (42)	263 (47)	235 (43)	247 (44)	235 (43)
Obese	157 (29)	145 (26)	155 (29)	160 (28)	178 (33)
Maternal race [*]					
White	503 (83)	519 (86)	523 (86)	534 (87)	537 (90)
Black	34 (6)	28 (5)	24 (4)	18 (3)	16 (3)
Asian	24 (4)	12 (2)	24 (4)	20 (3)	12 (2)
Other	32 (5)	29 (5)	24 (4)	27 (4)	16 (3)
Mixed	11 (2)	18 (3)	15 (2)	14 (2)	19 (3)
Maternal education					
Less than high school	34 (6)	27 (4)	23 (4)	24 (4)	20 (3)
HS or GED equivalent	76 (13)	81 (13)	57 (9)	60 (10)	51 (9)
Some college	165 (27)	157 (26)	185 (30)	185 (30)	175 (29)
College	149 (25)	136 (22)	145 (24)	155 (25)	160 (27)
Advanced degree	180 (30)	205 (34)	200 (33)	189 (31)	194 (32)
Private insurance	474 (78)	487 (80)	477 (78)	487 (80)	486 (81)
Married [*]	512 (89)	535 (91)	526 (88)	558 (93)	539 (93)
Nulliparous [*]	309 (51)	288 (48)	301 (49)	255 (42)	246 (41)

Characteristics ^a N	Quintile 1 (539-2692) 604	Quintile 2 (2693-3119) 606	Quintile 3 (3120-3409) 610	Quintile 4 (3410-3742) 613	Quintile 5 (3743-5255) 600
Infertility treatment					
None	426 (71)	421 (69)	430 (70)	461 (75)	422 (70)
Drugs only	67 (11)	85 (14)	83 (14)	78 (13)	90 (15)
ART	104 (17)	95 (16)	90 (15)	64 (10)	82 (14)
Unknown Treatment	7 (1)	5 (1)	7 (1)	10 (2)	6 (1)
Gestational diabetes	63 (10)	58 (10)	48 (8)	50 (8)	69 (12)
Hypertensive disorders in pregnancy*	93 (15)	70 (12)	42 (7)	47 (8)	56 (9)
Any alcohol during pregnancy*	67 (12)	75 (13)	82 (14)	83 (14)	105 (18)
Smoking during pregnancy					
Never smoked	361 (63)	382 (65)	377 (64)	374 (63)	354 (62)
Not during pregnancy	143 (25)	131 (22)	133 (23)	158 (27)	168 (29)
Smoked during pregnancy	71 (12)	76 (13)	81 (14)	59 (10)	52 (9)
Singleton *	194 (32)	447 (74)	563 (92)	598 (98)	595 (99)
NICU admission *	293 (49)	47 (8)	20 (3)	22 (4)	29 (5)
Female infant gender*	328 (54)	302 (50)	320 (52)	281 (46)	239 (40)
Gestational age (weeks)*	34.98 (3.02)	38.06 (1.42)	38.84 (1.16)	39.19 (1.06)	39.42 (1.07)
Preterm (<37 weeks)*	398 (66)	87 (14)	20 (3)	4 (1)	5 (1)

^aNumber of participants missing information for variables: 11 adiponectin, 11 CRP, 32 serpin, 93 leptin, 6 prepregnancy BMI, 289 father's BMI, 2 insurance, 94 marital status, 21 previous pregnancy, 1 parity, 114 alcohol, 113 pregnancy smoking

^bGeometric means (95% CI) provided

* p<0.05

Table 2

Association (beta, 95% CI)^a between adipokines and continuous birth measures by multivariate linear regression among Singletons and Twins from Upstate KIDS

Singletons	Birth weight (z-score) ^b	Birth Length (cm) ^c	Ponderal Index (g/cm ³) ^c	Gestational age (weeks) ^b
Log Adiponectin	0.08 (−0.02-0.18)	0.36 (0.02-0.70)	0.03 (−0.02-0.07)	0.38 (0.23-0.54)
Log Adipsin	0.04 (−0.05-0.13)	0.12 (−0.19-0.42)	0.00 (−0.04-0.04)	−0.06 (−0.20-0.09)
Log Resistin	0.01 (−0.06-0.08)	0.30 (0.07-0.52)	−0.02 (−0.05-0.005)	0.16 (0.05-0.266)
Leptin (dichotomous)	0.30 (0.22-0.38)	0.53 (0.27-0.79)	0.03 (−0.01-0.06)	0.02 (−0.10-0.15)
Twins				
Log Adiponectin	0.14 (0.03-0.24)	1.51 (0.84-2.19)	0.05 (0.003-0.10)	0.22 (0.10-0.33)
Log Adipsin	0.07 (−0.05-0.18)	0.29 (−0.27-0.84)	0.0002 (−0.06-0.06)	0.05 (−0.05-0.16)
Log Resistin	0.02 (−0.07-0.10)	0.16 (−0.24-0.57)	0.004 (−0.04-0.05)	0.04 (−0.01-0.09)
Leptin (dichotomous)	0.16 (0.06-0.26)	0.23 (−0.26-0.73)	−0.02 (−0.08-0.04)	0.00 (−0.07-0.07)

Bolded values are statistically significant at p<0.05

^aModel 1 adjusted for all other adipokines, maternal age, maternal race, maternal BMI, infant gender, education, private insurance, and log CRP.

^bSample size: N= 2294 singletons and 1240 twins for birthweight and gestational age

^cSample size: N= 1997 singletons and 959 twins for length and ponderal index

Table 3

Odds ratios (95% CI) for DBS measured adipokines in association with birth outcomes stratified by plurality in Upstate KIDS

	Singletons (n=2295)			Twins (n=1240)	
	Preterm birth at <37 weeks	SGA at 10th percentile	LGA at 90th percentile	Early Preterm birth at <32w	SGA at 3rd percentile
N (cases)	180 (8%)	188 (9%)	246 (11%)	100 (8%)	111 (9%)
Adiponectin ^a					
Q1	3.26 (1.99-5.34)	1.81 (1.18-2.77)	1.34 (0.91-1.96)	1.23 (1.01-1.50)	1.95 (0.88-4.33)
Q2	1.69 (0.99-2.87)	1.04 (0.65-1.65)	0.90 (0.60-1.34)	0.97 (0.86-1.11)	2.73 (1.30-5.72)
Q3	1.53 (0.89-2.62)	1.05 (0.66-1.67)	1.19 (0.83-1.72)	0.88 (0.79-0.97)	1.48 (0.67-3.27)
Q4	1.21 (0.70-2.09)	1.37 (0.88-2.12)	0.98 (0.68-1.42)	0.96 (0.88-1.04)	0.81 (0.33-1.97)
Q5 (referent)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Adipsin ^a					
Q1	0.84 (0.51-1.40)	1.56 (0.99-2.47)	1.02 (0.68-1.52)	1.29 (1.08-1.54)	1.41 (0.63-3.15)
Q2	0.90 (0.54-1.49)	1.62 (1.04-2.52)	0.85 (0.57-1.26)	1.24 (1.08-1.42)	2.58 (1.23-5.41)
Q3	0.94 (0.56-1.57)	1.02 (0.64-1.63)	1.24 (0.86-1.77)	1.17 (1.06-1.29)	0.99 (0.45-2.16)
Q4	1.06 (0.64-1.76)	1.17 (0.75-1.81)	0.87 (0.60-1.27)	1.15 (1.06-1.25)	1.42 (0.65-3.09)
Q5 (referent)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Resistin ^a					
Q1	2.08 (1.20-3.62)	0.79 (0.51-1.24)	1.19 (0.80-1.78)	1.06 (0.92-1.23)	0.95 (0.46-1.98)
Q2	1.59 (0.90-2.80)	0.60 (0.38-0.96)	1.42 (0.98-2.07)	1.01 (0.89-1.15)	0.53 (0.28-1.03)
Q3	1.53 (0.86-2.72)	0.85 (0.57-1.29)	1.19 (0.81-1.74)	1.01 (0.90-1.14)	0.79 (0.37-1.72)
Q4	0.81 (0.43-1.54)	0.74 (0.49-1.12)	0.90 (0.61-1.33)	1.09 (0.98-1.20)	1.12 (0.56-2.22)
Q5 (referent)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Leptin ^b					
Low	1.09 (0.81-1.46)	2.29 (1.72-3.04)	0.56 (0.43-0.72)	0.96 (0.87-1.06)	1.43 (0.89-2.30)
High	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)

Models adjusted for all other adipokines, maternal age, maternal race, maternal BMI, infant gender, education, private insurance, log CRP (for twin models maternal race was dichotomized to white/non-white).

Bolded values are statistically significant at p<0.05.

^a Q1 is the lowest quintile whereas Q5 is the highest. Quintiles were calculated separately by plurality. Among singletons: mean levels from Q1 to Q5 for adiponectin were 0.26, 0.36, 0.45, 0.54, 0.77 µg/ml; for adipsin were 0.05, 0.08, 0.11, 0.14, 0.19 µg/ml; for resistin were 10.8, 19.0, 25.7, 34.8, 57.6 ng/ml. Among twins: for adiponectin were 0.20, 0.31, 0.39, 0.47, 0.71 µg/ml; for adipsin were 0.05, 0.07, 0.09, 0.11, 0.16 µg/ml; for resistin were 6.5, 11.6, 16.1, 22.1, 38.3 ng/ml.

^b Leptin dichotomized at the median of the cohort levels (2.43 µg/ml).