Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

Does fetal leptin and adiponectin influence children's lung function and risk of wheeze?

Blanche C. Ip[1], Nan Li[2], Medina Jackson-Browne[3], Melissa Eliot[2], Yingying Xu[4], Aimin Chen[5,6], Bruce P. Lanphear[7,8], Adam J. Spanier[9] and Joseph M. Braun[2]

[1]Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI, USA; [2]Department of Epidemiology, Brown University, Providence, RI, USA; [3]College of Health Sciences, University of Delaware, Newark, DE, USA; [4]Cincinnati Children's Hospital Medical Center, Division of General and Community Pediatrics, Department of Pediatrics, Cincinnati, OH, USA; [5]Division of Epidemiology, Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, OH, USA; [6]Department of Biostatistics, Epidemiology and Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [7]Faculty of Health Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; [8]Child and Family Research Institute, BC Children's Hospital, Vancouver, British Columbia, Canada and [9]Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

Adipocytokines, which are secreted during fetal development by both mothers and fetuses, may influence fetal lung development, but little human data are available. We used data from the HOME Study to investigate the associations of cord blood adipocytokine concentrations with children's lung forced expiratory volume (FEV1; $N = 160$) and their risk of wheeze ($N = 281$). We measured umbilical cord serum adipocytokine concentrations using enzyme-linked immunosorbent assays and FEV1 using a portable spirometer at ages 4 and 5 to calculate the percent predicted FEV1 (%FEV1). Parents completed standardized questionnaires of their child's wheeze symptoms every 6 months from birth to age 5, then again at ages 6 and 8. We used multivariable linear mixed models and modified Poisson regression with generalized estimating equations to estimate associations of adipocytokine concentrations ($\log_2$-transformed) with children's %FEV1 and the risk of wheeze, respectively, adjusting for sociodemographic, perinatal, and child factors. Cord serum leptin was not associated with children's %FEV1. Higher cord serum adiponectin concentrations were associated with higher %FEV1 in girls ($\beta = 3.1$, 95% confidence interval [CI]: 0.6, 5.6), but not in boys ($\beta = -1.3$, 95% CI: −5.9, 3.3) (sex × adiponectin p-value = 0.05). Higher leptin was associated with lower risk of wheeze in girls (RR = 0.74, 95% CI: 0.66, 0.84), but not boys (RR = 0.87, 95% CI: 0.69, 1.11) (sex × leptin p-value = 0.01). In contrast, higher adiponectin concentrations were associated with lower risk of wheeze (RR = 0.84, 95% CI: 0.73, 0.96) in both boys and girls. These data suggest that fetal adipocytokines may impact lung development and function in early childhood. Future studies are needed to confirm these findings and explore the mechanisms underlying these associations.

Introduction

Wheezing is common in early childhood. Almost half of children experience wheezing during the first year of life and 10% of children have asthma after 6 years of age.[1,2] Early life exposures, including respiratory infections and airway inflammation, may lead to wheezing episodes in early childhood[3] and contribute to recurrent wheezing.[4] However, the *in utero* environment including the levels of circulatory adipocytokine may impact lung and immune system development to influence children's lung function, capacity to fight respiratory infections, and ability to resolve airway inflammation. Thus, identifying prenatal determinants of respiratory health is critical to develop novel interventions that lead to better respiratory health throughout life.[5]

Adipocytokines, which includes leptin and adiponectin, are secreted by both the mother and the fetus during fetal development, largely by adipose tissue, and may have developmental and immunoregulatory effects important to lung development.[6] Leptin is produced by adipose cells and lung tissues in the fetus,[7,8] as well as by the placental trophoblasts,[9] and may influence lung function and the risk of lung diseases in early childhood. The sharp rise in cord serum leptin after 34-week gestation coincides with the rapid increase in the production of fetal lung surfactant,[10] a complex mixture of phospholipids and proteins that is critical for gas exchange at the surface of lung alveolus,[11] and its defective metabolism results in respiratory distress. In addition, animal and *in vitro* studies suggest that fetal leptin modulates pulmonary development by enhancing lung maturity,[12,13] increasing lung weight, and stimulating surfactant protein synthesis.[14,15] Thus fetal leptin concentrations may have direct impact on lung function later in life.

Adiponectin, which is primarily produced by fetal subcutaneous adipose tissue,[16] has antioxidant and anti-inflammatory functions in adults.[6,14,15] Less is known about the impact of

Original Article

Cite this article: Ip BC, Li N, Jackson-Browne M, Eliot M, Xu Y, Chen A, Lanphear BP, Spanier AJ, and Braun JM. (2021) Does fetal leptin and adiponectin influence children's lung function and risk of wheeze? *Journal of Developmental Origins of Health and Disease* **12**: 570–577. doi: 10.1017/S2040174420000951

Received: 18 March 2020
Revised: 24 August 2020
Accepted: 21 September 2020
First published online: 27 October 2020

Keywords:
Adipocytokines; children; lung function; wheeze

Address for correspondence:
Blanche C. Ip, BioMed Box G-B, Brown University, Providence, RI 02912, USA.
Email: blanche_ip@brown.edu

© The Author(s), 2020. Published by Cambridge University Press in association with International Society for Developmental Origins of Health and Disease.

human fetal adiponectin on lung development. Adiponectin-deficient mice exhibited an emphysema-like phenotype and increased levels of pro-inflammatory mediators that may contribute to the pathogenesis of inflammatory lung conditions.[17] In contrast, the lungs of mice that over-expressed adiponectin from conception onward were protected from oxidative and inflammatory injury in later life.[18,19] Moreover, infants born preterm or small for gestational age, who have low concentrations of circulating adiponectin, are at increased risk for developing bronchopulmonary dysplasia from oxidative stress and inflammation,[20] although it is unclear whether *in utero* adiponectin has a direct impact on lung function and the development of lung diseases is independent of being born preterm or small for gestational age.

Little is known about the role of *in utero* adipocytokine concentrations on lung function during human childhood. Brenner *et al.* found no associations between cord blood adipocytokines and risk of wheezing disorders in early childhood in children of mothers with no history of atopy,[21] and reported that in children of mothers with a history of atopy, cord blood concentrations of adiponectin, but not leptin, were positively associated with risk of wheezing disorders in early childhood. However, we are unaware of any studies investigating whether *in utero* adipocytokines affect the risk for respiratory illness in later childhood or whether these two adipokines are associated with lung function. Thus, we investigated the associations of cord blood adiponectin and leptin with lung function and risk of wheezing disorders from ages 6 months to 8 years in a pregnancy and birth cohort.

Methods

We used data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort designed to assess the impact of early life chemical exposures on child growth and development.[22] From 2003 to 2006, study staff recruited women in the second trimester of pregnancy from nine prenatal care clinics affiliated with three hospitals in the greater Cincinnati, Ohio area. Inclusion criteria, recruitment, and follow-up have been described previously.[22] Of 468 women enrolled, 389 women remained in the study until delivery of a singleton live birth. We conducted follow-up visits with participating children at ages 4 weeks, 1 through 5 years, and again at 8 years. The HOME Study protocols were approved by the institutional review boards of their participating institutions. All participating mothers provided written informed consent for themselves and their children.

Serum adipocytokine measurements

Umbilical cord venous blood was collected at delivery as previously described.[23] Leptin and adiponectin concentrations in umbilical cord serum samples were measured using an enzyme-linked immunosorbent assay (ELISA) and BioTeckmicrotiterELx 808 plate reader. Each analytic batch included reagent blanks and low- and high-concentration quality control (QC) samples. The coefficient of variation (CV) of repeated QC measurements for leptin and adiponectin was approximately 11% and 17%, respectively. The limit of detections (LODs) were 0.8 ηg/ml and <2 μg/ml, for leptin and adiponectin, respectively. We used the machine-reading values for the seven samples below the LOD for leptin. All samples were above the LOD for adiponectin.

Childhood lung function

When children were 4 and 5 years old, trained research assistants attempted to collect at least three acceptable FEV1 measurements using a Piko-1 portable spirometer (nSpire Health Inc., Longmont, CO, USA). FEV1 was recorded in liters (resolution 0.01 l). We calculated the %FEV1 from the mean of three FEV1 measurements and multiplied the calculated %FEV1 value by 0.9 for children whose mothers reported their race as Black due to the established racial difference in FEV1.[24–26] A total of 160 children had complete data available for cord serum leptin and adiponectin concentrations, covariates, and at least one measurement of lung function (FEV1).

Childhood wheeze

Trained research staff surveyed parents every 6 months from birth to age 5, then again at ages 6 and 8 to assess childhood wheeze.[22] We used a question from the National Health and Nutrition Examination Survey, asking, "Has (child's name) had wheezing or whistling in his/her chest, in the last 6 months?".[27] A previous study has shown that 83.5% of parents correctly identified "whistling or squeaking" as the definition of wheeze.[28] A total of 281 children had complete data available for cord blood serum leptin and adiponectin concentrations, covariates, and at least one wheeze questionnaire.

Covariate assessment

We identified potential confounders of our exposure-outcome association based on a directed acyclic graph.[29] Trained research staff assessed maternal age, education, household income, parity, household income, maternal asthma, paternal asthma, maternal allergies, paternal allergies, pet ownership, living community at birth (rural, suburban, urban), and children's race using standardized interviews. We abstracted children's sex, birth weight, and gestational age from hospital medical charts. We calculated pre-pregnancy body mass index (BMI) using self-reported weight and height (or imputed weight if data were missing).[30,31] We calculated gestational weight gain by taking the difference between weight at the last visit prior to delivery and self-reported pre-pregnancy weight, and converting it to weight gain for gestational duration *z*-scores using data from a contemporary cohort of US women.[32] We measured children's weight and height during follow-up visits and calculated children's BMI *z*-scores based on World Health Organization age- and sex-specific standard data.[33] We used cord serum cotinine concentration or maternal gestational serum concentrations of cotinine at birth (if cord serum cotinine was not available; 11%) to assess gestational tobacco smoke exposure. We measured child serum cotinine concentrations using previously described methods at ages 1–4 years to assess child secondhand tobacco smoke exposure.[34–36] The LOD for serum cotinine was 0.015 ηg/ml with a CV ranging from 3% to 4% at high concentrations (1 ηg/ml) to 10% at low concentrations (0.1 ηg/ml). Gestational tobacco smoke exposure was categorized as unexposed (< LOD), secondhand exposure (LOD to 3 ηg/ml), and active exposure (> 3 ηg/ml).[34,37]

Statistical analyses

We began by describing cord serum adipocytokine and outcome measures (%FEV1 at ages 4 or 5 years; wheeze at birth through ages 5, 6, and 8 years: any wheeze ever vs. never reported wheeze, excluding those who did not respond) according to potential confounders of our exposure-outcome association. Then, we estimated the associations between continuous $\log_2$-transformed adipocytokine concentrations and %FEV1 outcomes using multivariable linear mixed models to account for the within-person correlation of

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

repeated lung function measures. Next, we estimated the relative risk of wheeze with increasing continuous log_2-transformed adipocytokine concentrations using modified Poisson regression with generalized estimating equations to account for the within-person correlation of repeated wheeze assessments.[38] We analyzed each exposure (leptin, adiponectin) and outcome (%FEV1; risk of wheeze) separately. We adjusted for child sex, maternal education, maternal race, parity, pre-pregnancy BMI, cord serum cotinine (when available, or maternal serum cotinine at birth), child birthweight percentile, and gestational age in all models. Finally, we examined the dose–response relation of the associations between adipocytokines and respiratory outcomes using 3-knot restricted cubic polynomial splines.[39]

Secondary analyses and sensitivity analyses

We included adipocytokine × sex interaction terms in our models to determine whether associations between adipocytokines and respiratory outcomes were modified by child sex because cord serum leptin concentrations have been reported to vary by child sex.[40,41] We also included adipocytokine × visit interaction terms in our models to determine whether associations between adipocytokines and %FEV1 were modified by the time when FEV1 was measured. We considered *p*-values for interaction terms <0.20 as an indication that the association varied by sex or by visit. In addition, we adjusted for baseline (prior or at birth) gestational weight gain *z*-score, maternal asthma, maternal allergies, paternal asthma, paternal allergies, and living community at birth (rural, suburban, or urban), as well as child serum cotinine concentrations (averaged concentrations at ages 1–4 years) and pet ownership (time-varying at ages 1–5 years). However, the presence or directionality of the associations of some of these covariates with exposure and outcome is unclear. We conducted all statistical analyses using SAS version 9.4 (SAS Institute Inc. Cary, NC, USA).

Results

In the HOME Study, median (25th, 75th) cord blood serum leptin and adiponectin concentrations were 9.1 (5, 15) ηg/ml and 41 (28, 53) μg/ml, respectively (Table 1). Median cord serum leptin concentrations were higher among girls, mothers who were overweight or obese (BMI ≥ 25 kg/m^2), and younger mothers (18–25 years). Median cord serum leptin concentrations were higher among non-Hispanic White than non-Hispanic black children. Average cord serum adiponectin concentrations were higher among children who were born to households with income greater than $80,000 per annum, but lower among children born to women who were married or higher parity. Our analytic sample included more non-Hispanic Whites (69% vs. 49%), less non-Hispanic Blacks (27% vs. 44%), more married mothers (74% vs. 54%), and more college graduates (56% vs. 44%) than the full HOME Study sample.

For the %FEV1 analysis, 160 children had 273 repeated spirometry measurements at ages 4 and 5 years. Forty-two percent of the children were males, and 69% were non-Hispanic Whites (Table 2), and 96% of children were born in urban or suburban areas. After adjusting for covariates, cord serum leptin was not associated with children's %FEV1 (Table 3). The associations between leptin and %FEV1 did not differ by sex or by visit for %FEV1 measurements. Cord serum adiponectin was positively associated with %FEV1 among all children, but the 95% CI of the point estimate included the null value. The associations between adiponectin and %FEV1 did not differ by visit, but differed by child sex, where adiponectin concentrations were positively associated with %FEV1 among girls (β = 3.1, 95% CI: 0.6, 5.6), but not boys (β = −1.3, CI: −5.9, 3.3) (sex × adiponectin interaction *p*-value = 0.05) (Table 3). We observed linear associations of cord serum leptin and adiponectin with %FEV1 (*P*-value for non-linearity = 0.66 and 0.95, respectively).

A total of 281 children had 2317 repeated measurements of wheeze every 6 months for the first 5 years, and at 6 and 8 years of age. Supplemental Table 2 describes the risk of wheeze for each of the 12 visits. Forty-three percent of the children were males, 70% were non-Hispanic Whites (Table 2), and 97% of children were born in urban or suburban areas. After adjusting for covariates, higher cord serum leptin was associated with lower risk of wheeze among all children (RR: 0.79; 95% CI: 0.71, 0.87) (Table 4), but the association was stronger in girls (RR = 0.74, CI: 0.66, 0.84) than boys (RR = 0.87, CI: 0.69, 1.11) (sex × leptin interaction *p*-value = 0.01) (Table 4). Cord serum adiponectin was associated with lower risk of wheeze in children (RR = 0.84, CI: 0.73, 0.96) (Table 3). Child sex did not modify this association (sex × adiponectin interaction *p*-value = 0.812). We observed linear associations of both cord serum leptin and adiponectin with RR for wheeze (*P*-values for non-linearity = 0.37 and 0.94, respectively).

Additional adjustments for maternal asthma, maternal allergies, paternal asthma, paternal allergies, pet ownership, child serum cotinine, and living community at birth did not substantially alter the associations between cord blood leptin and %FEV (Supplemental Table 1), and risks for wheeze (Supplemental Table 3). Similar results were observed for the associations between cord blood adiponectin and %FEV (Supplemental Table 1), and risks for wheeze (Supplemental Table 3) except for child serum cotinine adjustment. Adjusting for child cotinine concentrations attenuated the association between cord serum adiponectin and %FEV in girls (Supplemental Table 1), but did not alter the relationship between cord blood adiponectin and the risks for wheeze (Supplemental Table 3).

Discussion

We investigated the associations of cord blood serum adipocytokine concentrations with school-aged children's respiratory function and risk of wheeze in the HOME Study. Leptin concentrations were not associated with %FEV1 in children, but higher leptin concentrations were associated with lower risk of wheeze in girls. In both boys and girls, higher adiponectin concentrations were associated with lower risk of wheeze and suggestively associated with higher %FEV1. However, the positive association between adiponectin and %FEV1 was stronger in girls compared to boys.

Our results differ from a prior study of German mother–child pairs that reported no associations of adiponectin and leptin concentrations in cord serum with risk of asthma or obstructive bronchitis among mothers who had no history of atopy.[21] These discrepancies may be due to the differences in the characteristics of the study populations (90% German; 28.9% of mother with pre-pregnancy BMI of 25 or higher), the age children were examined (within first 2 years of life) and the assessments of outcomes. The newborns in the HOME Study also had modestly higher concentrations of both cord serum leptin (9.1 vs. 7.9 ηg/ml) and adiponectin (41.2 vs. 30.2 μg/ml) concentrations than children in the German cohort.

Our results suggest that cord serum leptin is associated with lower risk of wheeze in children. Leptin, an adipocytokine produced by both the adipose tissue and the placental trophoblast, is involved in fetal lung development and in pulmonary surfactant secretion.[9] Pulmonary surfactant is a complex mixture of specific

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

Table 1. Descriptive statistics of cord serum leptin and adiponectin concentrations in the Health Outcomes and Measures of the Environment (HOME) Study children

		Cord blood leptin (ηg/ml)					Cord blood adiponectin (ug/ml)				
		N	%	Median	25th	75th	*N*	%	Median	25th	75th
Overall		280	100	9	5	15	295	100	41	28	53
Child sex	Girls	151	62	11	7	18	162	55	41	28	53
	Boys	129	38	8	4	14	133	45	41	27	53
Child race	Non-Hispanic White	178	69	10	5	17	188	68	44	34	56
	Non-Hispanic Black	77	27	9	6	15	81	25	34	26	48
	Others	22	5	7	2	10	23	7	31	26	45
Marital status	Not married	82	26	9	5	15	86	26	33	25	49
	Married	196	74	10	5	16	207	74	44	33	53
Mother's age	18–25	59	15	8	4	11	62	18	35	26	45
	25–35	180	68	10	5	17	187	66	43	30	53
	>35	41	16	9	7	15	46	15	42	28	55
Maternal education	High school graduate or less	59	20	9	6	16	61	20	36	26	55
	Tech school or some college	69	24	9	5	16	74	23	36	27	47
	College graduate or above	150	55	10	5	15	158	57	44	33	56
Pre-pregnancy BMI	<25	142	44	8	4	14	148	53	42	31	53
	25–30	77	33	10	7	17	81	29	43	31	55
	>30	51	22	13	7	21	56	18	34	22	51
Household income	0–40K	100	33	8	5	15	105	32	33	26	45
	40–80K	102	41	10	5	17	108	38	44	33	55
	>80K	78	26	9	5	15	82	30	45	36	56
Breast milk fed	No	47	16	9	6	15	50	16	34	24	48
	Yes	226	84	9	5	16	238	84	42	30	53
Parity number	0	123	46	9	5	16	130	46	43	30	54
	1	92	29	9	5	15	99	34	41	30	53
	≥2	65	25	10	6	17	66	20	35	24	52
Maternal serum cotinine at birth	Unexposed (<0.015 ηg/ml)	107	43	9	5	15	113	43	43	28	55
	SHS (0.015–3.0 ηg/ml)	127	49	9	5	16	131	48	42	32	52
	Active (>3.0 ηg/ml)	26	9	8	5	14	28	9	28	21	48
Cord blood cotinine	Unexposed (<0.015 ηg/ml)	117	48	10	5	16	118	47	44	30	55
	SHS (0.015–3.0 ηg/ml)	120	45	9	5	15	122	46	40	29	51
	Active (>3.0 ηg/ml)	22	7	8	5	13	23	7	26	22	40

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

Table 2. Descriptive statistics of forced expiratory volume (FEV1) and wheeze in the Health Outcomes and Measures of the Environment (HOME) Study Children

		FEV_1 at 4- or 5-year visit				Wheeze**			
						No		Yes	
		*N**	%	Mean	SD	*N*	%	*N*	%
Overall		273	100	67	21	123	44	158	56
Child sex	Girls	164	58	66	21	68	55	85	54
	Boys	113	42	69	22	55	45	73	46
Child race	Non-Hispanic White	185	69	70	22	77	63	101	69
	Non-Hispanic Black	73	23	59	20	35	28	39	25
	Others	19	7	71	17	11	9	11	7
Marital status	Not married	69	22	59	19	35	28	45	28
	Married	208	78	70	21	88	72	113	72
Mother's age	18–25	48	16	61	19	23	19	33	21
	25–35	186	68	68	21	78	63	102	65
	>35	43	16	70	23	22	18	23	14
Maternal education	High school graduate or less	49	16	62	20	25	20	30	19
	Tech school or some college	70	25	65	23	28	23	45	28
	College graduate or above	158	59	69	21	70	57	83	53
Pre-pregnancy BMI	<25	137	51	68	22	60	50	83	54
	25–30	72	26	66	18	38	32	39	25
	>30	62	22	66	23	21	18	33	21
Household income	0–40K	87	28	60	20	42	34	56	35
	40–80K	109	40	69	21	44	36	60	38
	>80K	81	31	72	21	37	30	42	26
Breast milk fed	No	40	14	66	22	27	22	20	13
	Yes	237	86	67	21	96	78	137	87
Parity number	0	132	46	65	19	58	47	67	43
	1	85	32	69	22	39	32	56	35
	≥2	60	22	68	25	26	21	35	22
Maternal serum cotinine at birth	Unexposed (<0.015 ηg/ml)	127	49	70	20	47	41	64	44
	SHS (0.015–3.0 ηg/ml)	120	44	66	21	57	50	68	47
	Active (>3.0 ηg/ml)	20	7	60	24	10	9	14	10
Cord blood cotinine	Unexposed (<0.015 ηg/ml)	135	53	70	21	50	44	64	47
	SHS (0.015–3.0 ηg/ml)	111	41	66	21	56	50	61	44
	Active (>3.0 ηg/ml)	18	6	58	24	7	6	12	9
Wheeze	No	221	81	67	21				
	Yes	53	19	66	22				

**N* for FEV1 at 4- or 5-year visit is number of repeats.
***Wheeze for any one of the visits (every 6 months for the first 5 years, and at 6 and 8 years of age).

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

Table 3. Unadjusted and adjusted difference in the children's calculated percent predicted forced expiratory volume (FEV) at ages 4 and 5, per log_2-transformed increase in cord serum leptin or adiponectin concentrations, stratified by child sex[a]

	Overall		Girls		Boys		Sex × Adipokine	Visit × Adipokine
	N	β and 95% CI	*N*	β and 95% CI	*N*	β and 95% CI	*p*-value	*p*-value
Leptin and PCT predicted mean FEV								
Unadjusted	262	−0.4 (−2.4, 1.6)	150	0.6 (−2.2, 3.3)	112	−0.9 (−4.0, 2.2)	0.507	0.248
Adjusted[1]	252	−0.7 (−3.1, 1.7)	145	0.3 (−2.8, 3.4)	107	−0.8 (−4.9, 3.3)	0.179	0.314
Adiponectin and PCT predicted mean FEV								
Unadjusted	273	1.3 (−1.0, 3.6)	160	3.0 (0.6, 5.4)	113	−2.8 (−7.1, 1.6)	0.025	0.552
Adjusted[1]	262	0.8 (−1.6, 3.2)	154	3.1 (0.6, 5.6)	108	−1.3 (−5.9, 3.3)	0.052	0.209

[a]Adjusted model: we adjusted for neonatal birthweight percentile (<10%, 10%–90%, > 90%), gestational age (term or pre-term), maternal education (high school graduate or less, tech school or some college, college graduate or above), pre-pregnancy BMI (<25, 25–30, ≥30 kg/m^2), parity (0, 1, ≥2), child's sex (male, female), mother's race (non-Hispanic White, non-Hispanic Black, others), and birth serum cotinine concentrations (<0.015, 0.015–0.3, >0.3 ηg/ml). *N* = no. of repeats. We considered *p*-values for interaction terms < 0.20 as an indication that the association varied by visit or by sex.

Table 4. Unadjusted and adjusted difference in the children's relative risks for wheeze every 6 months from birth to age 5 years, then at ages 6 and 8 years, per log_2-transformed increase in cord serum leptin or adiponectin concentrations, stratified by child sex[a]

	Overall		Girls		Boys		Sex × Adipokine
	N	RR and 95% CI	*N*	RR and 95% CI	*N*	RR and 95% CI	*p*-value
Leptin and any wheeze							
Unadjusted	281	0.90 (0.81, 1.00)	153	0.77 (0.66, 0.90)	128	1.02 (0.90, 1.16)	0.005
Adjusted[1]	281	0.79 (0.71, 0.87)	153	0.74 (0.66, 0.84)	128	0.87 (0.69, 1.11)	0.007
Adiponectin and any wheeze							
Unadjusted	281	0.85 (0.76, 0.95)	153	0.86 (0.77, 0.96)	128	0.84 (0.65, 1.10)	0.916
Adjusted[1]	281	0.84 (0.73, 0.96)	153	0.81 (0.72, 0.91)	128	0.86 (0.64, 1.15)	0.812

[a]Adjusted model: we adjusted for neonatal birthweight percentile (<10%, 10%–90%, > 90%), gestational age (term or pre-term), maternal education (high school graduate or less, tech school or some college, college graduate or above), pre-pregnancy BMI (<25, 25–30, ≥30 kg/m^2), parity (0, 1, ≥2), child's sex (male, female), mother's race (non-Hispanic White, non-Hispanic Black, others), and birth serum cotinine concentrations (<0.015, 0.015–0.3, >0.3 ηg/ml). We considered *p*-values for interaction terms < 0.20 as an indication that the association varied by sex.

lipids, proteins, and carbohydrates, which is produced in the lungs by type II alveolar epithelial cells and is critical to support gas exchange in the lung alveoli.[42] Premature infants are at risk for developing respiratory distress syndrome at birth in part due to surfactant deficiency.[42] Fetal lung lipofibroblasts produce fibroblast pneumonocyte factor, a 10,000–20,000 molecular weight peptide that stimulates surfactant phospholipid synthesis by fetal type II cells.[15] Leptin and its receptor are expressed by both fetal lung fibroblasts and type II cells in vivo. Leptin secreted by lung lipofibroblasts supports type II cell maturation and is critical for stimulating type II cell surfactant phospholipid synthesis via a paracrine signaling loop in rats.[15] Furthermore, fetal rat lung explants treated with 1 ηg/ml leptin had enhanced surfactant protein B production.[12]

Fetal cord blood leptin level rises rapidly after 34 weeks gestation in human fetus,[43] and the rapid increase in fetal surfactant production coincides with the rise in cord blood leptin.[10] In fetal baboons, the expression of leptin receptors on type II epithelial cells upregulates during late gestation and was associated with increase in surfactant production.[44] Furthermore, exposing ovine fetuses during late gestation to exogenous leptin for 5 days increased serum leptin by 4.2 ηg/ml, which in turn promoted surfactant production and certain aspects of lung maturation including the reduction in alveolar wall thickness (critical for optimizing gas exchange by reducing alveolar diffusion distance), the density of secondary septal crests, and elastin content in alveolar walls,[13] but did not alter static lung compliance, maximal lung volume, lung compartment volumes, or alveolar surface area.[13] These prior results suggest that fetal leptin within the range of concentrations observed in this study (0.15–94.53 ηg/ml) may be associated with lower risk for wheeze in childhood, but may not be related to lung function.

Since cord blood leptin has been shown to positively associated with both birth weight and gestation age,[43] we speculate that the association between fetal leptin and respiratory outcomes may be a confounding factor of the association between birth weight and gestation age with respiratory outcome. However, the association between cord serum leptin and risk of wheeze remained similar when adjusting for both birth weight and gestational age, suggesting that cord blood leptin may be an independent risk factor for wheeze.

There is much less known about the mechanism by which adiponectin is involved in fetal lung development. In contrast to leptin, adiponectin is not produced by the placenta.[16] Cord blood adiponectin concentrations increase 20-fold between 24 weeks and term gestation,[45] and do not follow the same trajectory during late gestation as cord blood leptin, suggesting that fetal adiponectin may have a differential impact on fetal lung development than fetal leptin. In mice, adiponectin is present in bronchoalveolar lavage fluid.[17] Genetically induced adiponectin deficiency from conception led to the development of emphysema-like phenotype, dilated airspace, and increased lung proinflammatory cytokines and matrix metalloproteinases expression,[17] suggesting that fetal adiponectin may play a critical role in the development of lung structure that can directly impact on lung function later in life. Furthermore, genetically induced adiponectin deficiency was shown exacerbate lipopolysaccharide-induced lung

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

injuries through exaggerated inflammatory response in pulmonary vascular endothelium.[18] In contrast, bovine lung endothelial cells treated with adiponectin (0.05–1.0 μg/ml) ameliorated hyperoxia-induced oxidative stress,[19] and genetically induced overexpression of adiponectin that led to a 70 ηg/ml increase in bronchoalveolar lavage fluid adiponectin protected mice from hyperoxic-induced lung injuries and inflammation.[19] Adiponectin has antioxidant and anti-inflammatory properties,[6] and systemic inflammation has been shown to be inversely associated with lung function in humans.[46–48] These previous work supports our findings that increasing cord blood adiponectin within the range of concentrations observed in this study (2.15–142.57 μg/ml) may be associated with increased lung function in early childhood. Furthermore, these prior findings also supported our observation that adjustment with child serum cotinine as a marker for secondhand smoke exposure attenuated the positive association between cord serum adiponectin and %FEV in girls, because secondhand smoke is associated with lung inflammation and function.[49,50]

Adjusting for the averaged child secondhand smoke exposure from 1 to 4 years of age attenuated the association between cord blood adiponectin and %FEV, but not between cord blood adiponectin and the risk for wheeze. One plausible explanation is that differential mechanisms are involved in the associations between cord blood adiponectin, lung function, and risk for wheeze in early childhood. In addition, it is also possible that this may be in part due to potential selection bias in the HOME Study participants with %FEV measurements ($N = 160$) as compared to participants with risk for wheeze data ($N = 281$). Children with %FEV measurements were less exposed to secondhand smoke than the children with risk for wheeze data (53% vs. 44% unexposed, respectively; Table 2).

We observed sex-specific associations between cord serum adiponectin and %FEV1, as well as cord serum leptin and wheeze. It is plausible that there exists a sex dimorphism in fetal adipocytokine concentrations throughout the gestational period, where the increase in fetal adiponectin and leptin might occur sooner in girls than in boys, thus leading to differential associations with lung function later in life. Our sex-specific associations between cord serum adipocytokine and risk of wheeze provide a potential reason for the sex-specific risks of childhood wheeze that have been observed in published work,[51,52] where boys more often developed childhood wheeze than girls.

Most of our participants were born in urban or suburban areas and most are non-Hispanic Whites, which may limit the generalizability of our findings. One of the limitations of our study is that this is a secondary analysis of an existing study with relatively small sample size, thus the analysis may be underpowered to detect small effect sizes. Another limitation of our study is that we only measured adipocytokine concentrations at birth. Since there are changes in adipocytokine concentrations and lung development during the pre- and postnatal periods, future studies should consider examining trajectories of adipocytokine concentrations from prenatal to childhood in relation to children's lung function and related outcomes. Nevertheless, cord blood adipocytokines of term neonates can differ significantly from maternal serum,[16,53] thus it is critical to define fetal adipocytokine exposure based on cord blood concentrations to investigate whether fetal adipocytokines may contribute to lung function and the risks for lung diseases later in life.

In summary, these results may provide insights into the potential impacts of fetal leptin and adiponectin exposure on lung development and function in early childhood. Cord blood leptin concentrations were associated with reduced risk of wheeze in girls. Cord serum adiponectin concentrations were associated with reduced risk of wheeze in both boys and girls, and possibly greater lung function, as measured by %FEV1. Future studies should confirm these findings, and laboratory or molecular epidemiology studies are needed to explore the underlying biological mechanisms. If our findings are confirmed, there is a potential to improve children's respiratory function and reduce their risk for wheeze by intervening on potential modifiable factors of adiponectin and leptin concentrations, including maternal diet.[54,55]

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S2040174420000951

Financial Support. This work was supported by NIEHS grants P01 ES011261, R01 ES024381, R01 ES025214, R01 ES020349, and R01 ES014575.

Conflicts of Interest. Dr. Braun was financially compensated for serving as an expert witness for plaintiffs in litigation related to tobacco smoke exposures and received an honoraria for serving on an advisory board to Quest Diagnostics. Dr. Braun served as an expert witness in litigation related to perfluorooctanonic acid contamination in drinking water in New Hampshire. Any funds I receive from this arrangement were/are paid to Brown University and cannot be used for my direct benefit (e.g., salary/fringe, travel, etc.).

The rest of the authors declare no competing financial interest.

Ethical standards. All women in the Health Outcomes and Measures of the Environment Study provided written informed consent for themselves and their children after the study protocols had been explained. The institutional review boards (IRBs) of Cincinnati Children's Hospital Medical Center and the cooperating delivery hospitals approved this study. The CDC IRB relied on the determinations made by the other IRBs.

References

1. Matricardi PM, Illi S, Gruber C, *et al.* Wheezing in childhood: incidence, longitudinal patterns and factors predicting persistence. *Eur Respir J.* 2008; 32(3), 585–592.
2. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med.* 1995; 332(3), 133–138.
3. Wright AL, Holberg CJ, Martinez FD, Morgan WJ, Taussig LM. Breast feeding and lower respiratory tract illness in the first year of life. Group Health Medical Associates. *BMJ.* 1989; 299(6705), 946–949.
4. Soh JE, Kim KM, Kwon JW, *et al.* Recurrent wheeze and its relationship with lung function and airway inflammation in preschool children: a cross-sectional study in South Korea. *BMJ Open.* 2017; 7(10), e018010.
5. Ly NP, Gold DR, Weiss ST, Celedon JC. Recurrent wheeze in early childhood and asthma among children at risk for atopy. *Pediatrics.* 2006; 117(6), e1132–1138.
6. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy ClinImmunol.* 2005; 115(5), 911–919; quiz 920.
7. Bruno A, Pace E, Chanez P, *et al.* Leptin and leptin receptor expression in asthma. *J Allergy ClinImmunol.* 2009; 124(2), 230–237, 237.e231–234.
8. Vernooy JH, Drummen NE, van Suylen RJ, *et al.* Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. *Thorax.* 2009; 64(1), 26–32.
9. Henson MC, Castracane VD. Leptin in pregnancy. *Biol Reprod.* 2000; 63(5), 1219–1228.
10. Shekhawat PS, Garland JS, Shivpuri C, *et al.* Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. *Pediatr Res.* 1998; 43(3), 338–343.
11. Nkadi PO, Merritt TA, Pillers DA. An overview of pulmonary surfactant in the neonate: genetics, metabolism, and the role of surfactant in health and disease. *Mol Genet Metab.* 2009; 97(2), 95–101.
12. Kirwin SM, Bhandari V, Dimatteo D, *et al.* Leptin enhances lung maturity in the fetal rat. *Pediatr Res.* 2006; 60(2), 200–204.
13. De Blasio MJ, Boije M, Kempster SL, *et al.* Leptin matures aspects of lung structure and function in the ovine fetus. *Endocrinology.* 2016; 157(1), 395–404.

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press

14. Torday JS, Rehan VK. Stretch-stimulated surfactant synthesis is coordinated by the paracrine actions of PTHrP and leptin. *Am J Physiol Lung Cell Mol Physiol.* 2002; 283(1), L130–135.
15. Torday JS, Sun H, Wang L, Torres E, Sunday ME, Rubin LP. Leptin mediates the parathyroid hormone-related protein paracrine stimulation of fetal lung maturation. *Am J Physiol Lung Cell Mol Physiol.* 2002; 282(3), L405–410.
16. Gauda EB, Master Z. Contribution of relative leptin and adiponectin deficiencies in premature infants to chronic intermittent hypoxia: exploring a new hypothesis. *Respir Physiol Neurobiol.* 2018; 256, 119–127.
17. Summer R, Little FF, Ouchi N, *et al.* Alveolar macrophage activation and an emphysema-like phenotype in adiponectin-deficient mice. *Am J Physiol Lung Cell Mol Physiol.* 2008; 294(6), L1035–1042.
18. Konter JM, Parker JL, Baez E, *et al.* Adiponectin attenuates lipopolysaccharide-induced acute lung injury through suppression of endothelial cell activation. *J Immunol.* 2012; 188(2), 854–863.
19. Sliman SM, Patel RB, Cruff JP, *et al.* Adiponectin protects against hyperoxic lung injury and vascular leak. *Cell Biochem Biophys.* 2013; 67(2), 399–414.
20. Suursalmi P, Korhonen P, Kopeli T, *et al.* Severe bronchopulmonary dysplasia, growth, nutrition, and adipokines at school age. *Glob Pediatr Health.* 2016; 3, 2333794x16637290.
21. Rothenbacher D, Weyermann M, Fantuzzi G, Brenner H. Adipokines in cord blood and risk of wheezing disorders within the first two years of life. *Clin Exp Allergy.* 2007; 37(8), 1143–1149.
22. Braun JM, Kalloo G, Chen A, *et al.* Cohort profile: the health outcomes and measures of the environment (HOME) study. *Int J Epidemiol.* 2017; 46(1), 24.
23. Hansen A, Forbes P, Buck R. Potential substitution of cord blood for infant blood in the neonatal sepsis evaluation. *Biol Neonate.* 2005; 88(1), 12–18.
24. Hsu KH, Jenkins DE, Hsi BP, *et al.* Ventilatory functions of normal children and young adults–Mexican-American, white, and black. I. Spirometry. *J Pediatr.* 1979; 95(1), 14–23.
25. Eigen H, Bieler H, Grant D, *et al.* Spirometric pulmonary function in healthy preschool children. *Am J RespirCrit Care Med.* 2001; 163(3 Pt 1), 619–623.
26. Spanier AJ, Kahn RS, Kunselman AR, *et al.* Bisphenola exposure and the development of wheeze and lung function in children through age 5 years. *JAMA Pediatr.* 2014; 168(12), 1131–1137.
27. Statistics NCfH. Plan and operation of the Third National Heaith and Nutrition Examination Survey, 1988-94. (ed. U.S. Department of Health and Human Services PHS, Centers for Disease Control and Prevention), 1994.
28. Michel G, Silverman M, Strippoli MP, *et al.* Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J.* 2006; 28(6), 1124–1130.
29. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol.* 2008; 8, 70.
30. Verner MA, Gaspar FW, Chevrier J, *et al.* Increasing sample size in prospective birth cohorts: back-extrapolating prenatal levels of persistent organic pollutants in newly enrolled children. *Environ Sci Technol.* 2015; 49(6), 3940–3948.
31. van der Laan MJ, Polley EC, Hubbard AE. Super learner. *Stat Appl Genet Mol Biol.* 2007; 6, Article 25.
32. Hutcheon JA, Platt RW, Abrams B, Himes KP, Simhan HN, Bodnar LM. A weight-gain-for-gestational-age z score chart for the assessment of maternal weight gain in pregnancy. *Am J ClinNutr.* 2013; 97(5), 1062–1067.
33. Group WMGRS. *WHOMulticentre Growth Reference Study Group. WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*, 2006. World Health Organization, Geneva.
34. Braun JM, Daniels JL, Poole C, *et al.* A prospective cohort study of biomarkers of prenatal tobacco smoke exposure: the correlation between serum and meconium and their association with infant birth weight. *Environ Health.* 2010; 9, 53.
35. Bernert JT, Jacob P, 3rd, Holiday DB, *et al.* Interlaboratory comparability of serum cotinine measurements at smoker and nonsmoker concentration levels: a round-robin study. *Nicotine Tob Res Off J Soc Res Nicotine Tob.* 2009; 11(12), 1458–1466.
36. Bernert JT, Jr., McGuffey JE, Morrison MA, Pirkle JL. Comparison of serum and salivary cotinine measurements by a sensitive high-performance liquid chromatography-tandem mass spectrometry method as an indicator of exposure to tobacco smoke among smokers and nonsmokers. *J Anal Toxicol.* 2000; 24(5), 333–339.
37. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. *Am J Epidemiol.* 2009; 169(2), 236–248.
38. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol.* 2004; 159(7), 702–706.
39. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med.* 2010; 29(9), 1037–1057.
40. Vriens A, Plusquin M, Baeyens W, *et al.* Cord blood leptin and insulin levels in association with mitochondrial DNA content. *J Transl Med.* 2018; 16(1), 224.
41. Matsuda J, Yokota I, Iida M, *et al.* Serum leptin concentration in cord blood: relationship to birth weight and gender. *J Clin Endocrinol Metab.* 1997; 82(5), 1642–1644.
42. Chakraborty M, Kotecha S. Pulmonary surfactant in newborn infants and children. *Breathe.* 2013; 9(6), 476–488.
43. Stoll-Becker S, Kreuder J, Reiss I, Etspuler J, Blum WF, Gortner L. Influence of gestational age and intrauterine growth on leptin concentrations in venous cord blood of human newborns. *KlinPadiatr.* 2003; 215(1), 3–8.
44. Henson MC, Swan KF, Edwards DE, Hoyle GW, Purcell J, Castracane VD. Leptin receptor expression in fetal lung increases in late gestation in the baboon: a model for human pregnancy. *Reproduction (Cambridge, England).* 2004; 127(1), 87–94.
45. Kajantie E, Hytinantti T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. *J Clin Endocrinol Metab.* 2004; 89(8), 4031–4036.
46. Hancox RJ, Gray AR, Sears MR, Poulton R. Systemic inflammation and lung function: a longitudinal analysis. *Respir Med.* 2016; 111, 54–59.
47. van Rooyen Y, Schutte AE, Huisman HW, *et al.* Inflammation as possible mediator for the relationship between lung and arterial function. *Lung.* 2016; 194(1), 107–115.
48. Hart JE, Goldstein R, Walia P, *et al.* FEV1 and FVC and systemic inflammation in a spinal cord injury cohort. *BMC Pulm Med.* 2017; 17(1), 113.
49. Birru RL, Di YP. Pathogenic mechanism of second hand smoke induced inflammation and COPD. *Front Physiol.* 2012; 3, 348.
50. Bhat TA, Kalathil SG, Bogner PN, *et al.* Secondhand smoke induces inflammation and impairs immunity to respiratory infections. *J Immunol.* 2018; 200(8), 2927–2940.
51. Tse SM, Coull BA, Sordillo JE, Datta S, Gold DR. Gender- and age-specific risk factors for wheeze from birth through adolescence. *Pediatr Pulmonol.* 2015; 50(10), 955–962.
52. Mandhane PJ, Greene JM, Cowan JO, Taylor DR, Sears MR. Sex differences in factors associated with childhood- and adolescent-onset wheeze. *Am J Respir Crit Care Med.* 2005; 172(1), 45–54.
53. Zhang ZQ, Lu QG, Huang J, Jiao CY, Huang SM, Mao LM. Maternal and cord blood adiponectin levels in relation to post-natal body size in infants in the first year of life: a prospective study. *BMC Pregnancy Childbirth.* 2016; 16(1), 189.
54. Ma L, Lu Q, Ouyang J, *et al.* How are maternal dietary patterns and maternal/fetal cytokines associated with birth weight? A path analysis. *Br J Nutr.* 2019; 121(10), 1178–1187.
55. England JA, Jain J, Holbrook BD, Schrader R, Qualls C, Mozurkewich E. Effect of prenatal EPA and DHA on maternal and cord blood insulin sensitivity: a secondary analysis of the mothers, omega 3, and mental health study. *BMC Pregnancy Childbirth.* 2019; 19(1), 452.

https://doi.org/10.1017/S2040174420000951 Published online by Cambridge University Press