

# Supplemental Material

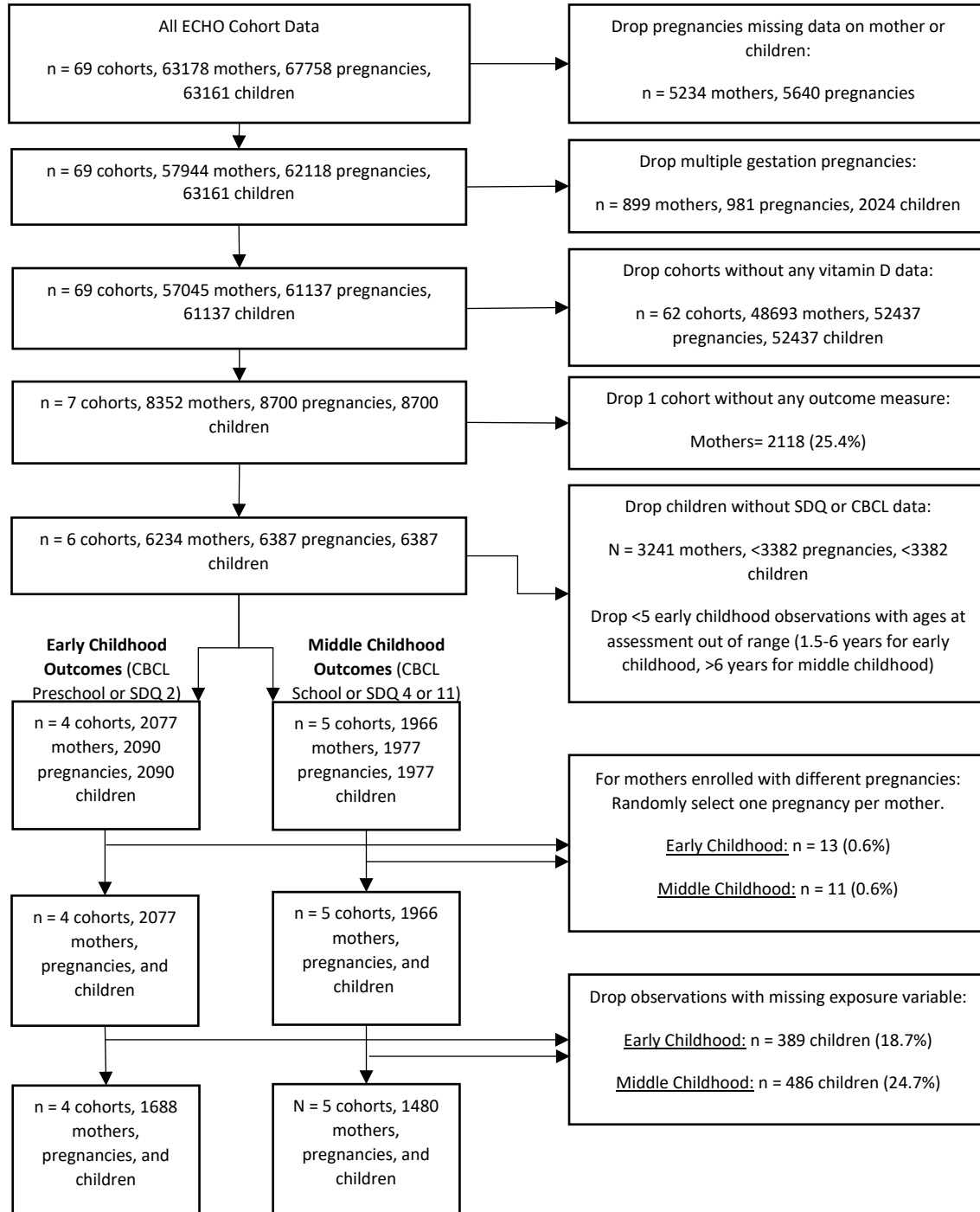
## Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program

Melissa M. Melough et al.

### Table of Contents

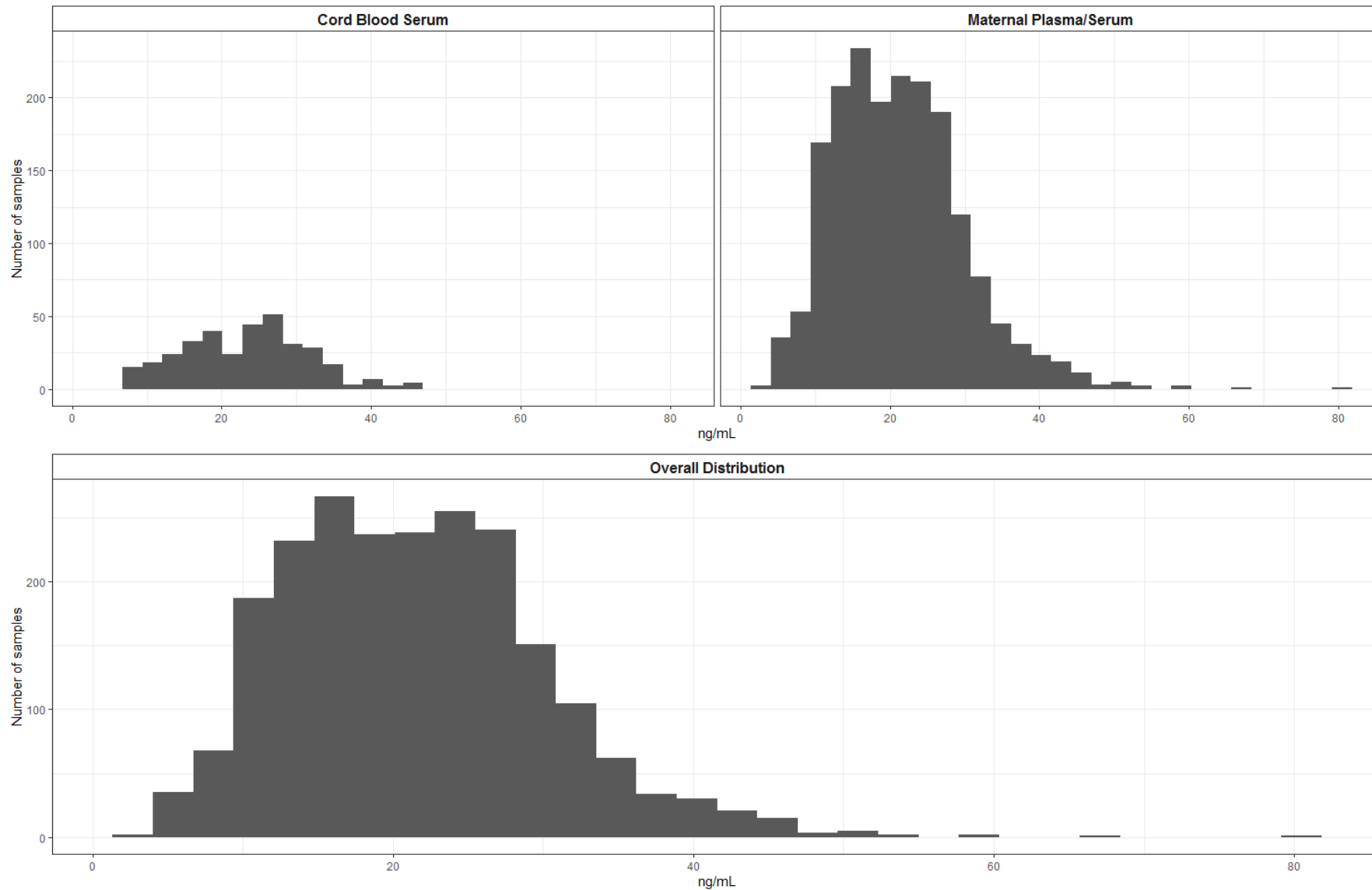
<b>Supplemental Figure 1.</b> Flowchart of ECHO Program participants included in the study. ....	2
<b>Supplemental Figure 2.</b> Distribution of 25-hydroxyvitamin D concentrations in maternal and cord blood samples. ....	3
<b>Supplemental Figure 3.</b> Sensitivity analysis: Associations of gestational vitamin D with (a) early childhood and (b) middle childhood behavioral problems among children in all cohorts and after omission of each cohort individually. ....	4
<b>Supplemental Table 1.</b> Type and timing of biospecimen collection for 25-hydroxyvitamin D measurement across cohorts .....	5
<b>Supplemental Table 2.</b> Analytical details for 25-hydroxyvitamin D assessment across cohorts .....	6
<b>Supplemental Table 3.</b> Covariate data availability across cohorts in the analysis of early childhood behavioral outcomes .....	7
<b>Supplemental Table 4.</b> Covariate data availability across cohorts in analysis of middle childhood behavioral outcomes .....	7
<b>Supplemental Table 5.</b> Concentration of 25(OH)D in maternal or cord blood samples according to timing of biospecimen collection.....	8
<b>Supplemental Table 6.</b> Sensitivity analysis: Relationships between second trimester 25(OH)D concentrations and behavioral problem T-scores in early and middle childhood in the ECHO program 8	
<b>Supplemental Table 7.</b> Sensitivity analysis: Estimated regression coefficients for associations of model terms with early childhood behavioral problem T-scores with added term for outcome instrument used.....	9
<b>Supplemental Table 8.</b> Sensitivity analysis: Estimated regression coefficients for associations of model terms with middle childhood behavioral problem T-scores with added term for outcome instrument used.....	10
<b>Supplemental Table 9.</b> Estimated risk difference of borderline or clinical CBCL scores for 25(OH)D exposure of $\geq 20$ ng/mL or $\geq 30$ ng/mL based on inverse probability of treatment weighting analysis..	11

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)



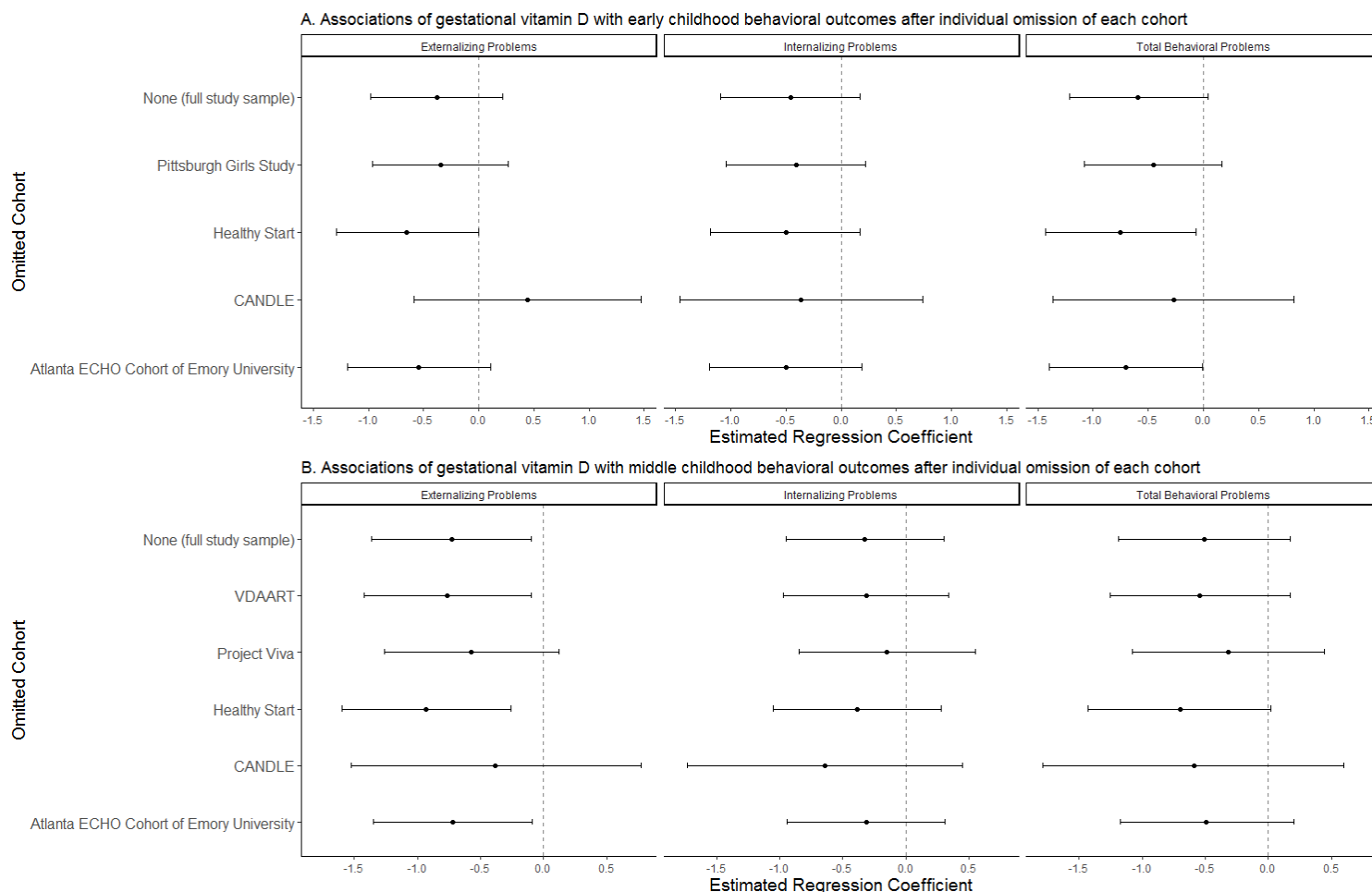
Supplemental Figure 1. Flowchart of ECHO Program participants included in the study.

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)



Supplemental Figure 2. Distribution of 25-hydroxyvitamin D concentrations in maternal and cord blood samples.

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)



**Supplemental Figure 3.** Sensitivity analysis: Associations of gestational vitamin D with (a) early childhood and (b) middle childhood behavioral problems among children in all cohorts and after omission of each cohort individually. Data presented as estimates with 95% confidence intervals for expected change in outcome T-scores per 10 ng/mL increase in 25-hydroxyvitamin D. Models included random intercepts accounting for within cohort correlation, and were adjusted for maternal age (continuous), child sex, and child age at assessment (continuous), maternal race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Other, Hispanic), maternal education (high school degree or lower, some college or associate degree, bachelor’s degree or higher), prenatal tobacco use (yes, no), prenatal alcohol use (yes, no), parity (continuous), pre-pregnancy BMI body mass index (continuous). CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)

**Supplemental Table 1.** Type and timing of biospecimen collection for 25-hydroxyvitamin D measurement across cohorts

Cohort	Biospecimen Type	Gestational weeks at time of collection		Prenatal period at time of collection, <i>n</i>				
		Mean (SD)	Range	T1	T2	T3	Birth	Missing
<b>Early Childhood Behavior Analysis</b>								
Healthy Start	cord blood serum	39.0 (1.35)	32.0 – 42.0	0	0	0	239	0
CANDLE	maternal plasma	22.9 (3.12)	14.9 – 39.0	0	1002	129	<5	<35
Pittsburgh Girls Study	maternal plasma	19.7 (9.32)	8.7 – 42.1	20	19	14	0	0
Atlanta ECHO Cohort of Emory University	maternal serum	11.4 (3.40)	2.6 – 31.3	208	<25	<5	0	0
<b>Middle Childhood Behavior Analysis</b>								
Healthy Start	cord blood serum	39.0 (1.40)	32.0 – 42.0	0	0	0	170	0
Project Viva	maternal plasma	27.7 (1.44)	24.4 - 35.7	<5	<65	216	0	0
CANDLE	maternal plasma	22.8 (3.08)	14.9 - 39.0	0	847	102	<5	<15
VDAART	maternal serum	14.6 (3.56)	9.6 - 33.1	<35	27	<5	0	0
Atlanta ECHO Cohort of Emory University	maternal serum	10.3 (2.55)	6.7 - 14.6	<10	<5	0	0	0

CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes; SD, standard deviation; T1, trimester 1 (1-12 weeks gestation); T2, trimester 2 (13-26 weeks gestation); T3, trimester 3 (27+ weeks gestation); VDAART, Vitamin D Antenatal Asthma Reduction Trial.

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)

**Supplemental Table 2.** Analytical details for 25-hydroxyvitamin D assessment across cohorts

<b>Cohort</b>	<b>Location</b>	<b>Dates of Pregnancy Data Collection</b>	<b>Laboratory</b>	<b>Laboratory Method</b>	<b>Quality Assurance/Control and Standardization Programs</b>
<b>Healthy Start</b>	Aurora, CO	2009-2014	University of Colorado Clinical and Translational Sciences Institute Core Laboratory	iSYS assay (ImmunoDiagnostic Systems)	Vitamin D Standardization Program, validated against liquid chromatography-tandem mass spectrometry methods
<b>Atlanta ECHO Cohort</b>	Atlanta, GA	2014-2019	Emory Vitamin D Research Laboratory	iSYS assay (ImmunoDiagnostic Systems)	Vitamin D external quality assessment scheme (DEQAS) and NIST Vitamin D Metabolites Quality Assurance Program (VitDQAP); assay detection range: 2-120 ng/mL
<b>Pittsburgh Girls Study</b>	Pittsburgh, PA	2018-2020	University of Michigan's Central Ligand Assay Satellite Services Laboratory	Human Total 25-OH Vitamin D IVD ELISA Kit (R&D Systems)	quality control samples provided with kit
<b>CANDLE</b>	Shelby County, TN	2006-2011	University of Tennessee Health Science Center Preventive Medicine Lab	commercial enzymatic immunoassay (ImmunoDiagnostic Systems)	College of American Pathology Quality Assessment Program for 25(OH)D assays; assay minimum detection range: 2 ng/mL; inter-assay variability <6% for laboratory assay controls; precision within 1 SD of the mean 25(OH)D concentration
<b>VDAART</b>	San Diego, CA; Boston, MA; St Louis, MO	2009-2011	Channing Division of Network Medicine	LIAISON chemiluminescent immunoassay (DiaSorin)	NIST level 1 SRM 972 for vitamin D in human serum included in each run; inter-assay CV: 11.2%; intra-assay CV: 8.1%
<b>Project Viva</b>	Boston, MA	1999-2002	Heartland Assays, Ames, IA	Automated chemiluminescence immunoassay and manual radioimmunoassay	NIST level 1 SRM

CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes; CV, coefficient of variation; ECHO, NIST, National Institute of Standards and Technology; SRM, Standard Reference Material; SD, standard deviation; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

**Supplemental Table 3.** Covariate data availability across cohorts in the analysis of early childhood behavioral outcomes<sup>1</sup>

<b>Covariate</b>	<b>Healthy Start</b>	<b>Atlanta ECHO Cohort</b>	<b>Pittsburg Girls Study</b>	<b>CANDLE</b>
Maternal age	239 (100%)	233 (100%)	53 (100%)	1163 (100%)
Parity	239 (100%)	233 (100%)	37 (69.81%)	1163 (100%)
Child age	239 (100%)	233 (100%)	53 (100%)	1163 (100%)
Child sex	239 (100%)	233 (100%)	53 (100%)	1163 (100%)
Maternal race & ethnicity	239 (100%)	233 (100%)	53 (100%)	1163 (100%)
Pre-pregnancy BMI	237 (99.16%)	205 (87.98%)	26 (49.06%)	1160 (99.74%)
Maternal education	239 (100%)	232 (99.57%)	53 (100%)	1163 (100%)
Prenatal alcohol use	239 (100%)	233 (100%)	15 (28.3%)	1163 (100%)
Prenatal tobacco use	239 (100%)	233 (100%)	18 (33.96%)	1162 (99.91%)

<sup>1</sup>Data represented as n (%). BMI, body mass index; CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes.

**Supplemental Table 4.** Covariate data availability across cohorts in analysis of middle childhood behavioral outcomes<sup>1</sup>

<b>Covariate</b>	<b>Healthy Start</b>	<b>Atlanta ECHO Cohort</b>	<b>CANDLE</b>	<b>VDAART</b>	<b>Project Viva</b>
Maternal age	170 (100%)	10 (100%)	961 (100%)	59 (100%)	280 (100%)
Parity	170 (100%)	10 (100%)	961 (100%)	59 (100%)	280 (100%)
Child age	170 (100%)	10 (100%)	961 (100%)	59 (100%)	280 (100%)
Child sex	170 (100%)	10 (100%)	961 (100%)	59 (100%)	280 (100%)
Maternal race & ethnicity	170 (100%)	10 (100%)	961 (100%)	59 (100%)	280 (100%)
Pre-pregnancy BMI	170 (100%)	9 (90%)	959 (99.79%)	0 (0%)	279 (99.64%)
Maternal education	170 (100%)	10 (100%)	961 (100%)	59 (100%)	279 (99.64%)
Prenatal alcohol use	170 (100%)	10 (100%)	961 (100%)	59 (100%)	277 (98.93%)
Prenatal tobacco use	170 (100%)	10 (100%)	960 (99.9%)	59 (100%)	274 (97.86%)

<sup>1</sup>Data represented as n (%). BMI, body mass index; CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

**Supplemental Table 5.** Concentration of 25(OH)D in maternal or cord blood samples according to timing of biospecimen collection

<b>Time of 25(OH)D measurement</b>	<b>Mean (standard deviation)</b>	<b>Median [Min, Max]</b>
Early Childhood Outcomes Analysis (n = 1688)		
First trimester	19.7 (8.91)	18.9 [6.0, 44.4]
Second trimester	21.8 (8.66)	21.2 [5.9, 68.4]
Third trimester	21.0 (7.59)	20.1 [9.5, 44.4]
Delivery	22.5 (8.17)	23.0 [7.0, 46.0]
Middle Childhood Outcomes Analysis (n = 1480)		
First trimester	26.2 (7.99)	26.1 [7.9, 42.5]
Second trimester	22.0 (8.88)	21.4 [5.7, 80.8]
Third trimester	19.6 (7.57)	18.6 [3.0, 44.2]
Delivery	23.8 (8.11)	25.0 [7.0, 46.0]

<sup>1</sup>Data represented as n (%). BMI, body mass index; CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; ECHO, Environmental influences on Child Health Outcomes; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

**Supplemental Table 6.** Sensitivity analysis: Relationships between second trimester 25(OH)D concentrations and behavioral problem T-scores in early and middle childhood in the ECHO program<sup>1</sup>

	<b>β (95% CI)</b>	<b>n</b>	<b>p<sup>2</sup></b>
<b>Early Childhood</b>			
		n = 1043	
Total Problems	-0.80 (-1.61, 0.00)		0.051
Externalizing	-0.74 (-1.49, 0.02)		0.056
Internalizing	-0.56 (-1.37, 0.25)		0.172
<b>Middle Childhood</b>			
		n = 935	
Total Problems	-0.71 (-1.58, 0.16)		0.111
Externalizing	-1.02 (-1.81, -0.24)		0.010
Internalizing	-0.29 (-1.10, 0.52)		0.478

<sup>1</sup>Estimates for expected change in outcome per 10ng/mL increase in 25(OH)D. Models included random intercepts accounting for within-cohort correlation, and were adjusted for maternal age (continuous), child sex, child age at assessment (continuous), maternal race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Other, Hispanic), maternal education (high school degree or lower, some college or associate degree, bachelor's degree or higher), prenatal tobacco use (yes, no), prenatal alcohol use (yes, no), parity (continuous), pre-pregnancy body mass index (continuous). ECHO, Environmental influences on Child Health Outcomes.

<sup>2</sup>P < 0.05 considered significant.



Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)

**Supplemental Table 7.** Sensitivity analysis: Estimated regression coefficients for associations of model terms with early childhood behavioral problem T-scores with added term for outcome instrument used

	Total problems		Externalizing		Internalizing	
	$\beta$ (95% CI)	p	$\beta$ (95% CI)	p	$\beta$ (95% CI)	p
<b>25(OH)D, 10 ng/mL</b>	-0.55 (-1.18, 0.08)	0.085	-0.40 (-1.00, 0.20)	0.186	-0.44 (-1.07, 0.19)	0.172
<b>Child Sex (male)</b>	-1.05 (-2.03, -0.06)	0.037	-1.67 (-2.61, -0.73)	<0.001	-0.09 (-1.07, 0.90)	0.865
<b>Child Age, years</b>	-0.83 (-1.48, -0.17)	0.013	-1.20 (-1.82, -0.57)	<0.001	0.13 (-0.52, 0.79)	0.691
<b>Maternal Age, years</b>	-0.11 (-0.22, 0.01)	0.078	-0.11 (-0.22, 0.00)	0.051	-0.09 (-0.21, 0.03)	0.138
<b>Maternal race/ethnicity</b>						
Non-Hispanic White	REF	REF	REF	REF	REF	REF
Non-Hispanic Black	-1.64 (-2.96, -0.33)	0.014	-1.68 (-2.94, -0.43)	0.009	-1.72 (-3.03, -0.40)	0.011
Non-Hispanic Other	-1.96 (-4.45, 0.53)	0.124	-1.58 (-3.97, 0.80)	0.192	-2.53 (-5.02, -0.03)	0.047
Hispanic	-2.29 (-4.88, 0.31)	0.084	-3.05 (-5.53, -0.57)	0.016	-2.23 (-4.83, 0.37)	0.093
<b>Maternal education</b>						
High school degree or lower	REF	REF	REF	REF	REF	REF
Some college or Associate degree	-1.34 (-2.65, -0.03)	0.044	-0.25 (-1.50, 1.00)	0.695	-1.86 (-3.17, -0.56)	0.005
Bachelor's degree and higher	-3.59 (-5.06, -2.11)	<0.001	-2.40 (-3.80, -0.99)	0.001	-4.06 (-5.54, -2.59)	<0.001
<b>Parity</b>	0.18 (-0.31, 0.67)	0.467	0.21 (-0.25, 0.67)	0.38	0.28 (-0.20, 0.77)	0.251
<b>Prenatal tobacco use (yes)</b>	2.89 (1.11, 4.66)	0.001	3.06 (1.45, 4.67)	<0.001	2.06 (0.24, 3.87)	0.026
<b>Pre-pregnancy BMI</b>	0.03 (-0.03, 0.10)	0.322	0.04 (-0.02, 0.11)	0.197	0.02 (-0.05, 0.09)	0.572
<b>Prenatal alcohol use (yes)</b>	1.95 (0.44, 3.47)	0.011	1.47 (0.08, 2.87)	0.038	1.48 (-0.03, 2.99)	0.055
<b>Outcome instrument (SDQ vs. CBCL)</b>	3.95 (1.02, 6.87)	0.008	-7.14 (-9.94, -4.34)	<0.001	5.00 (2.08, 7.91)	0.001

BMI, body mass index; CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; CBCL, Child Behavior Checklist; ECHO, Environmental influences on Child Health Outcomes; SDQ, Strengths and Difficulties Questionnaire; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

Greater Gestational Vitamin D Status is Associated with Reduced Childhood Behavioral Problems in the ECHO Program (Melissa M. Melough et al.)

**Supplemental Table 8.** Sensitivity analysis: Estimated regression coefficients for associations of model terms with middle childhood behavioral problem T-scores with added term for outcome instrument used

	Total problems		Externalizing		Internalizing	
	$\beta$ (95% CI)	p	$\beta$ (95% CI)	p	$\beta$ (95% CI)	p
<b>25(OH)D, 10 ng/mL</b>	-0.42 (-1.11, 0.28)	0.242	-0.61 (-1.25, 0.04)	0.065	-0.27 (-0.91, 0.37)	0.409
<b>Child Sex (male)</b>	-1.77 (-2.88, -0.66)	0.002	-1.84 (-2.86, -0.81)	<0.001	-1.18 (-2.20, -0.16)	0.023
<b>Child Age, years</b>	0.29 (-0.20, 0.78)	0.242	-0.07 (-0.52, 0.39)	0.777	0.42 (-0.03, 0.87)	0.067
<b>Maternal Age, years</b>	-0.04 (-0.17, 0.09)	0.537	-0.04 (-0.15, 0.08)	0.535	-0.03 (-0.15, 0.09)	0.616
<b>Maternal race/ethnicity</b>						
Non-Hispanic White	REF	REF	REF	REF	REF	REF
Non-Hispanic Black	-0.94 (-2.43, 0.55)	0.215	-0.07 (-1.44, 1.31)	0.923	-1.87 (-3.24, -0.50)	0.008
Non-Hispanic Other	0.46 (-1.98, 2.91)	0.709	0.69 (-1.57, 2.94)	0.551	0.61 (-1.63, 2.86)	0.592
Hispanic	-0.07 (-2.55, 2.42)	0.959	-0.11 (-2.40, 2.18)	0.924	0.15 (-2.13, 2.43)	0.898
<b>Maternal education</b>						
High school degree or lower	REF	REF	REF	REF	REF	REF
Some college or Associate degree	0.41 (-1.39, 2.22)	0.653	0.19 (-1.48, 1.85)	0.825	0.53 (-1.13, 2.18)	0.534
Bachelor's degree and higher	-0.90 (-2.81, 1.01)	0.358	-1.24 (-3.01, 0.52)	0.166	-0.26 (-2.01, 1.50)	0.774
<b>Parity</b>	-0.67 (-1.25, -0.09)	0.023	-0.25 (-0.78, 0.28)	0.355	-0.79 (-1.32, -0.25)	0.004
<b>Prenatal tobacco use (yes)</b>	2.25 (-0.02, 4.52)	0.052	2.09 (0.00, 4.18)	0.05	-0.07 (-2.16, 2.02)	0.947
<b>Pre-pregnancy BMI</b>	0.10 (0.02, 0.18)	0.015	0.06 (-0.02, 0.14)	0.117	0.06 (-0.01, 0.14)	0.105
<b>Prenatal alcohol use (yes)</b>	0.94 (-0.75, 2.63)	0.276	1.33 (-0.23, 2.89)	0.096	-0.18 (-1.74, 1.37)	0.819
<b>Outcome instrument (SDQ vs. CBCL)</b>	0.91 (-0.65, 2.46)	0.253	1.19 (-0.24, 2.63)	0.102	0.54 (-0.89, 1.97)	0.461

BMI, body mass index; CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; CBCL, Child Behavior Checklist; ECHO, Environmental influences on Child Health Outcomes; SDQ, Strengths and Difficulties Questionnaire; VDAART, Vitamin D Antenatal Asthma Reduction Trial.

**Supplemental Table 9.** Estimated risk difference of borderline or clinical CBCL scores for 25(OH)D exposure of  $\geq 20$  ng/mL or  $\geq 30$  ng/mL based on inverse probability of treatment weighting analysis<sup>1</sup>

	25(OH)D $\geq 20$ ng/mL		25(OH)D $\geq 30$ ng/mL	
	$\beta$ (95% CI)	p	$\beta$ (95% CI)	p
<b>Early Childhood Outcomes (n = 1688)</b>				
Total Problems (t-score $\geq 60$ )	-0.02 (-0.05, 0.02)	0.36	-0.02 (-0.07, 0.03)	0.41
Externalizing (t-score $\geq 60$ )	-0.01 (-0.03, 0.03)	0.67	-0.02 (-0.07, 0.03)	0.47
Internalizing (t-score $\geq 60$ )	-0.01 (-0.04, 0.03)	0.67	-0.02 (-0.07, 0.03)	0.47
<b>Middle Childhood Outcomes (n = 1480)</b>				
Total Problems (t-score $\geq 60$ )	0.01 (-0.03, 0.05)	0.55	-0.02 (-0.07, 0.03)	0.41
Externalizing (t-score $\geq 60$ )	0.01 (-0.03, 0.05)	0.58	-0.02 (-0.07, 0.03)	0.47
Internalizing (t-score $\geq 60$ )	-0.02 (-0.07, 0.02)	0.35	-0.02 (-0.08, 0.05)	0.62

<sup>1</sup>Measured confounders used to calculate the inverse-probability of treatment weight included maternal age (continuous), child sex, child age at assessment (continuous), maternal race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Other, Hispanic), maternal education (high school degree or lower, some college or associate degree, bachelor's degree or higher), prenatal tobacco use (yes, no), prenatal alcohol use (yes, no), parity (continuous), pre-pregnancy BMI (continuous). All confounders were modeled with an interaction term for an indicator of cohort to allow for the simulation of a randomized trial within cohorts. Weights in the 99<sup>th</sup> and 1<sup>st</sup> percentile were truncated to prevent over-influence of extreme weights. Weights were calculated using the *ipw* package in R version 4.1.3. Marginal structural models were fit using the *survey* package (R v 4.1.1). Estimates and standard errors were combined across imputed datasets using Rubin's rule, executed by the *mitools* package (R v 4.1.1). Effect estimates of linear marginal structural models were used to estimate the risk difference between the treated and untreated groups.