Supplemental Materials

Prenatal Maternal Phthalate Exposures and Trajectories of Childhood Adiposity from Four to Twelve Years

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Supplemental Table S1. Limits of detection of the 15 phthalate metabolites (µg/L).

Class	Parent Phthalate	Phthalate Metabolite	Limit of Detection (µg/L)	% Below the LOD
High Molecular Weight	Di(2-ethylhexyl) phthalate (DEHP)	MEHP	0.8	7
		MEOHP	0.2	0
		MEHHP	0.4	0
		MECPP	0.4	0
	Di(2-ethylhexyl) terephthalate (DEHTP)	MECPTP	0.2	3.5
	Di-isononyl phthalate (DiNP)	MONP	0.4	18.9
		MCOP	0.3	0.6
	Di-isodecyl phthalate (DiDP)	MCNP	0.2	5.1
	Di-n-octyl phthalate (DOP)	MCPP*	0.4	14
	Benzylbutyl phthalate (BBzP)	MBzP	0.3	3.8
Low Molecular Weight	Di-iso-butyl phthalate (DiBP)	MHiBP	0.4	3.1
		MiBP	0.8	2.5
	Di-n-butyl phthalate (DBP)	MBP	0.4	0
		MHBP	0.4	3.4
	Diethyl phthalate (DEP)	MEP	1.2	0

*Minor metabolite of DBP and non-specific metabolite of DOP and several other high molecular weight phthalates

Supplemental Table S2. Comparison of analysis population and excluded cohort population characteristics.

	Included in Analysis (N = 514)		Excluded from Analysis (N = 272)	
Maternal Characteristics	N	Mean ± SD or %	N	Mean ± SD or %
Age at Enrollment (years)	514	27.7 ± 5.7	272	27.8 ± 5.3
Pre-Pregnancy BMI (kg/m2)	514	26.4 ± 4.1	272	26.1 ± 4.2
Education				
More than High School	126	24.5	64	23.5
High School	180	35	94	34.6
Less than High School	208	40.5	114	41.9
Socioeconomic Status				
High	52	10.1	36	13.2
Medium	195	37.9	96	35.3
Low	267	51.9	140	51.5
Parity				
Multi-parous	320	62.3	173	63.6
Primi-parous	194	37.7	99	36.4
Child Sex				
Female	258	50.2	121	44.5
Male	256	49.8	151	55.5
Birthweight (kg)	514	3.1 ± 0.42	272	3.1 ± 0.5
Gestational Age at Birth	514	38.4 ± 1.6	272	38.4 ± 1.6

Supplemental Table S3. Comparison of analysis population and original cohort population geometric mean 2nd and 3rd trimester phthalate metabolites (µg/L) and metabolite sums (µg/L of primary metabolite) adjusted for specific gravity in the PROGRESS birth cohort.

	Included Analysis Population (N = 514)						Excluded Cohort Population (N = 272)					
Phthalate	**% < LOD**	**GM**	**SD**	**Med**	**25th**	**75th**	**GM**	**SD**	**Med**	**25th**	**75th**	**P**
ΣDEHP[a]	7.0	95.0	2.2	98.8	58.4	157	104	1.8	103	68.8	147	0.11
MEHP	7.0	5.5	2.5	5.6	3.2	10.1	6.2	2.1	6.3	3.9	9.2	0.04
MEOHP	0	21.0	2.3	22.2	12.4	36.8	22.8	2.0	22.6	15.1	34.0	0.14
MEHHP	0	19.8	2.3	20.7	11.9	33.7	21.9	1.9	21.8	14.9	31.5	0.08
MECPP	0	44.7	2.1	45.3	27.7	74.3	48.3	1.8	47.4	32.2	68.0	0.14
ΣDiBP[b]	3.7	12.5	2.2	13.1	7.6	20.9	14.9	2.1	14.7	9.3	23.8	<0.001
MHiBP	3.1	3.4	2.2	3.5	2.0	6.0	4.1	2.2	4.0	2.4	6.4	<0.01
MiBP	2.5	9.2	2.2	9.6	5.6	15.7	11.0	2.1	10.7	6.8	17.2	<0.01
ΣDiNP[c]	18.9	6.1	2.1	6.2	3.8	10.2	6.6	1.9	6.5	4.6	9.4	0.21
MONP	18.9	1.4	2.3	1.4	0.9	2.5	1.6	2.0	1.5	1.1	2.3	0.11
MCOP	0.6	4.6	2.1	4.5	2.8	7.6	4.9	1.9	4.8	3.3	6.9	0.24
ΣDBP[d]	3.4	86.0	2.3	91.9	49.8	150	105	2.2	97.8	63.7	177	<0.01
MBP	0	79.3	2.3	84.8	45.7	137	96.4	2.2	90.6	58.3	164	<0.01
MHBP	3.4	6.9	2.5	7.4	3.9	13.0	8.7	2.3	8.7	5.0	14.9	<0.01
MBzP	3.8	5.0	2.8	5.2	2.4	9.9	6.7	2.3	6.4	4.0	11.3	<0.01
MECPTP	3.5	2.1	2.5	2.1	1.2	3.5	2.0	2.3	2.0	1.2	3.1	0.80
MCNP	5.1	1.0	1.9	1.0	0.7	1.4	1.1	1.7	1.1	0.7	1.5	0.04
MCPP	14.0	1.4	2.1	1.5	0.9	2.3	1.7	1.9	1.7	1.2	2.6	<0.01
MEP	0.0	144	3.2	139	66.1	309	159	2.9	152	72.0	318	0.25

Note: LOD: Limit of detection; GM: Geometric Mean; SD: Geometric Standard Deviation; Med.: Median; 25th: 25th Percentile; 75th: 75th Percentile; P: P value

[a]DEHP molar sum expressed as MECPP

[b]DiBP molar sum expressed as MiBP

[c]DiNP molar sum expressed as MCOP

[d]DBP molar sum expressed as MBP

Supplemental Table S4. Latent class growth model diagnostics for centered and non-centered trajectory classes.

	Relative Entropy	Posterior Probability	Odds of Correct Classification
Non-centered Trajectories	0.746		
Low-Stable		0.9	9.2
High-High		0.91	38
Low-High		0.82	10.9
Centered Trajectories	0.927		
Stable		0.927	19
High Increasing		0.977	140
Slow Increasing		0.981	87

Supplemental Table S5. Overlap between centered and non-centered trajectory classes.

		Centered Trajectory Class		
		Stable	High Increasing	Slow Increasing
Non-Centered Trajectory Class	Low-Stable	204	2	54
	High-High	5	84	18
	Low-High	12	32	103

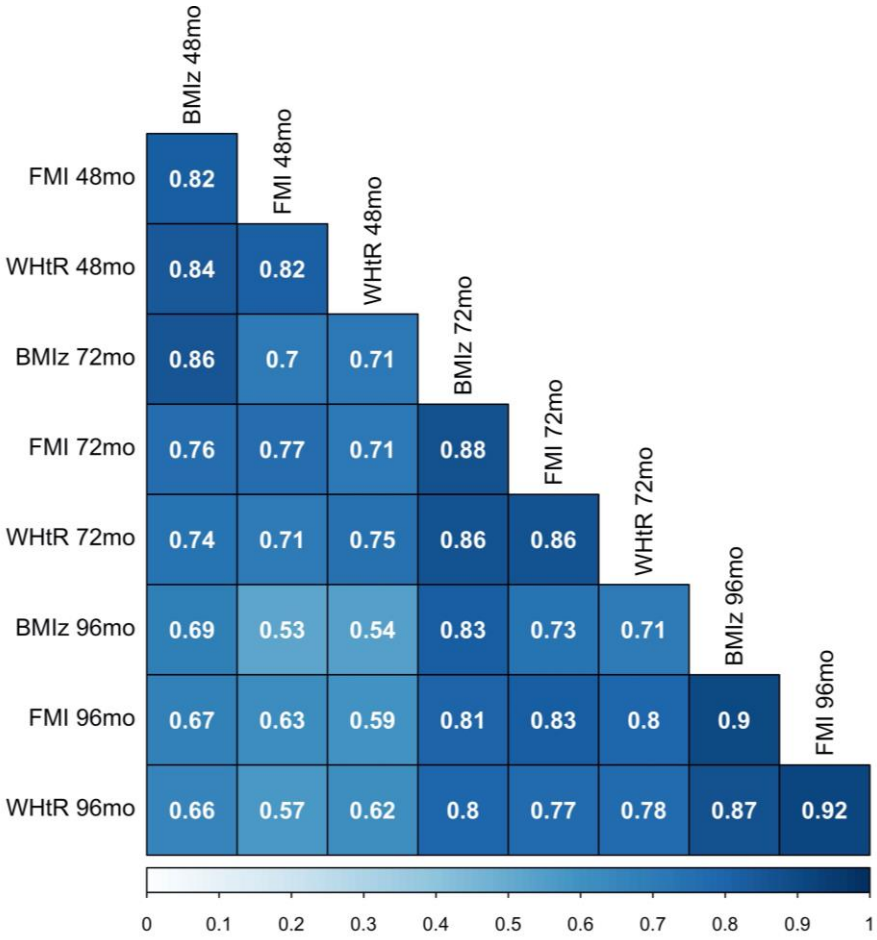
Supplemental Table S6. Numbers of male and female children in each trajectory class.

		Female	Male
Non-centered trajectory class	Low-Stable	121	134
	Low-High	77	79
	High-High	60	43
Centered trajectory class	Stable	118	103
	High Increasing	59	56
	Slow Increasing	81	97

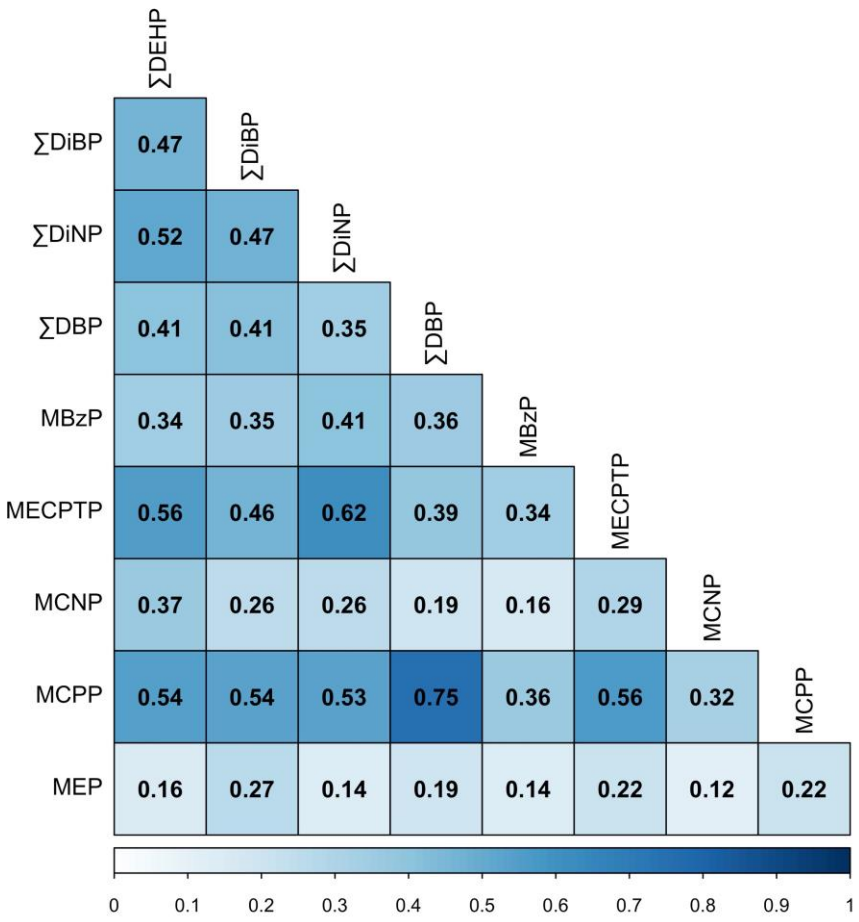
Supplemental Table S7. Associations of the total phthalates mixture with overall adiposity and adiposity trajectories in male and female children as measured by quantile g-computation (N = 514).

Model Type	Outcome	Sex	Rate Ratio / Effect Estimate (95%CI)	P Value
Non-Centered Trajectories	Low Stable	Female	1 (Ref)	
		Male	1 (Ref)	
	High-High	Female	1.13 (0.85, 1.49)	0.40
		Male	1.06 (0.74, 1.51)	0.76
	Low-High	Female	0.87 (0.66, 1.16)	0.34
		Male	1.07 (0.84, 1.37)	0.59
Centered Trajectories	Stable	Female	1 (Ref)	
		Male	1 (Ref)	
	High Increasing	Female	1.06 (0.8, 1.42)	0.67
		Male	1.13 (0.87, 1.48)	0.36
	Slow Increasing	Female	0.93 (0.73, 1.2)	0.59
		Male	1.03 (0.87, 1.23)	0.71
Overall Adiposity	BMI	Female	1.06 (0.89, 1.26)	0.51
		Male	1 (0.84, 1.19)	0.98
	FMI	Female	1.14 (0.8, 1.62)	0.46
		Male	1.07 (0.83, 1.38)	0.6

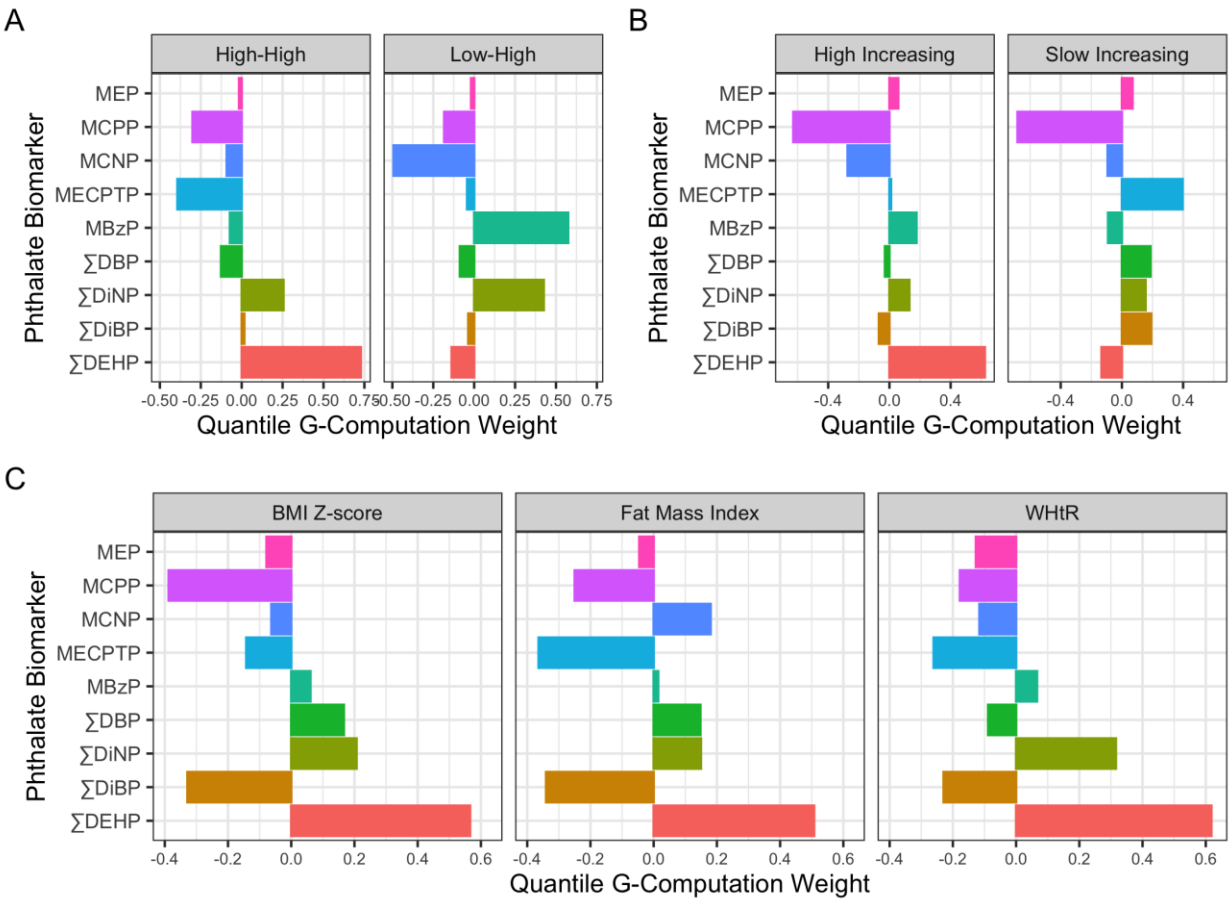
	WHtR	Female	1 (0.99, 1.01)	0.98
		Male	1 (0.99, 1.01)	0.96



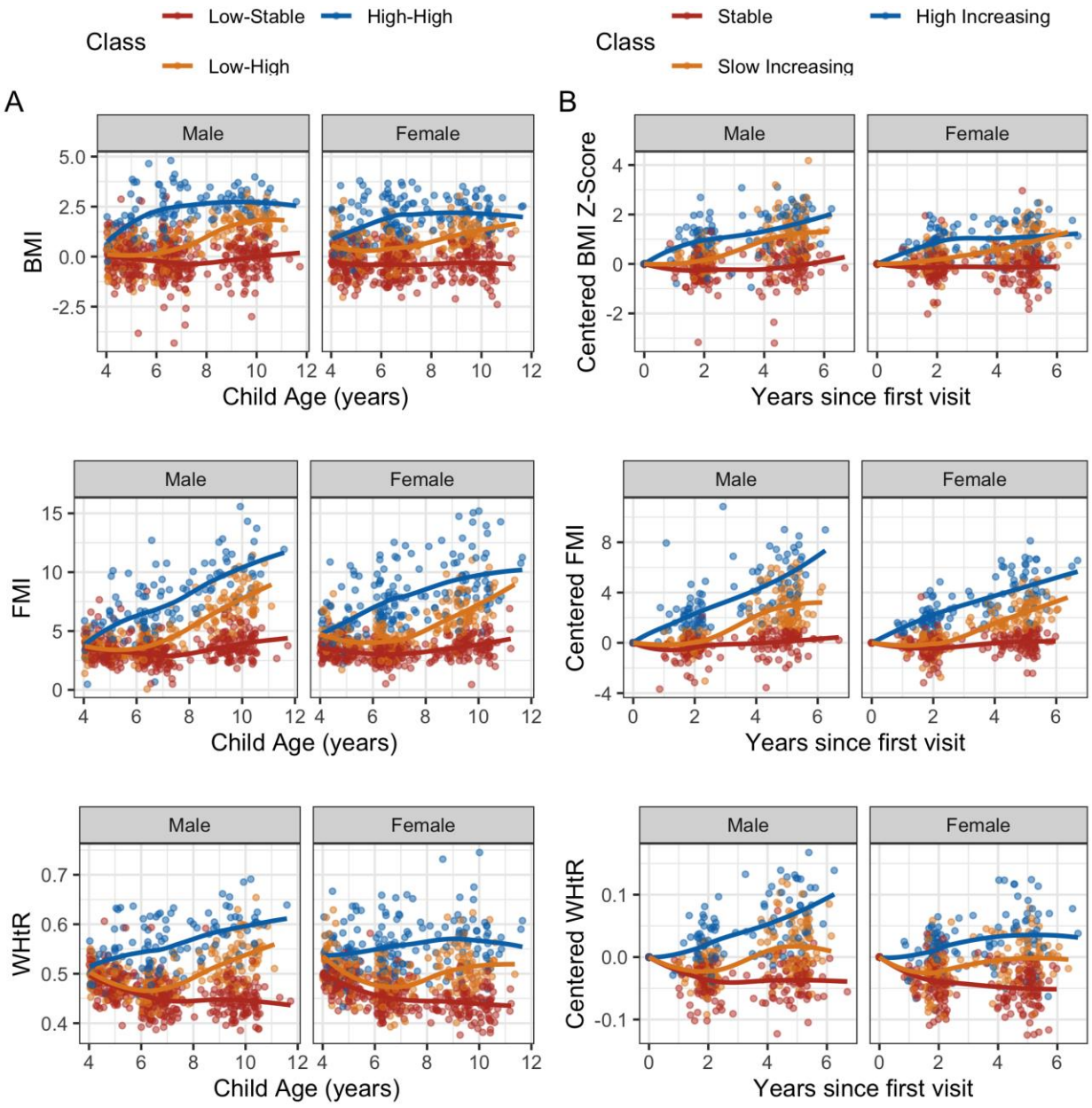
Supplemental Figure S1. Correlation plots between adiposity outcomes used in the present analysis. Gradient of cell indicates the strength of the Pearson correlation and the rho value is labeled in each cell.



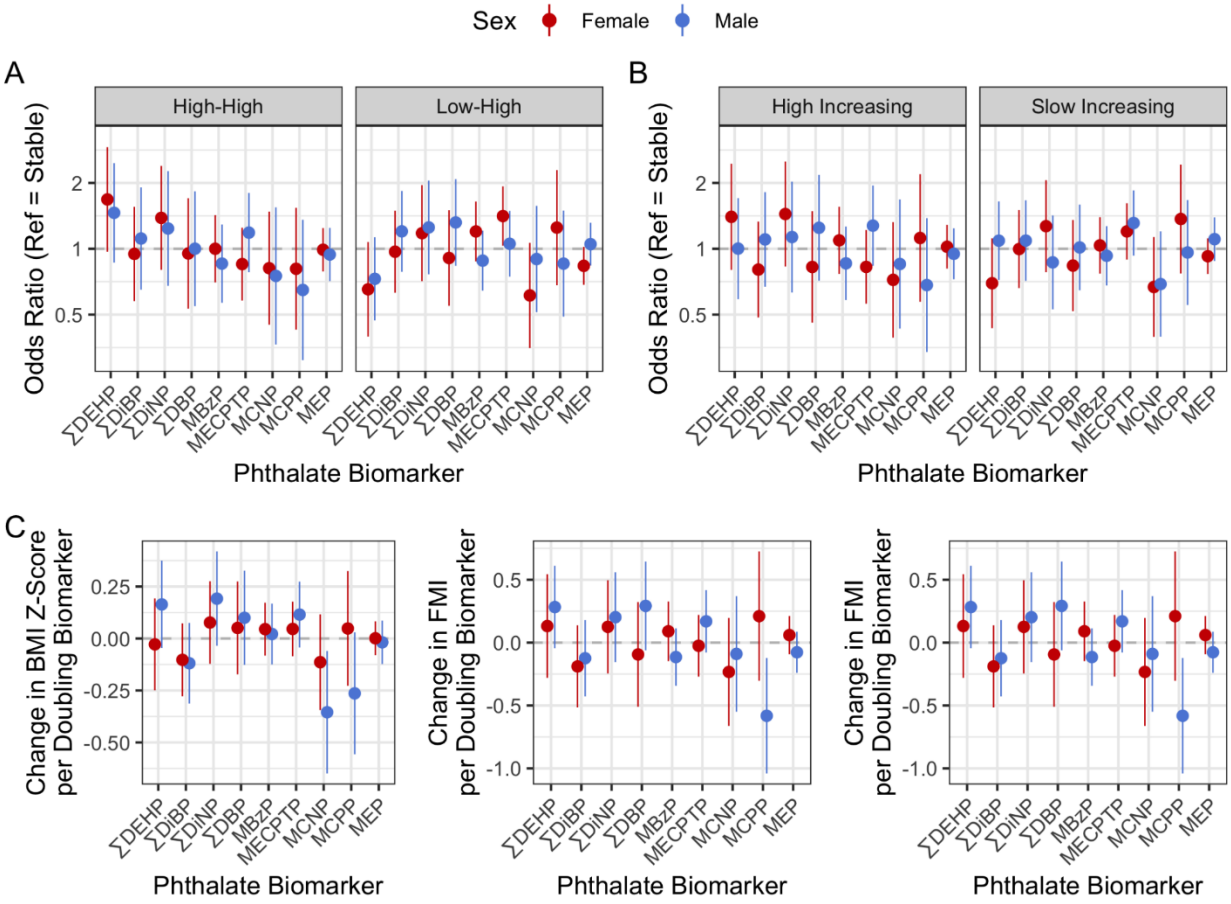
Supplemental Figure S2. Correlation plots between mean prenatal phthalate urinary biomarkers. Gradient of cell indicates the strength of the Pearson correlation and the rho value is labeled in each cell.



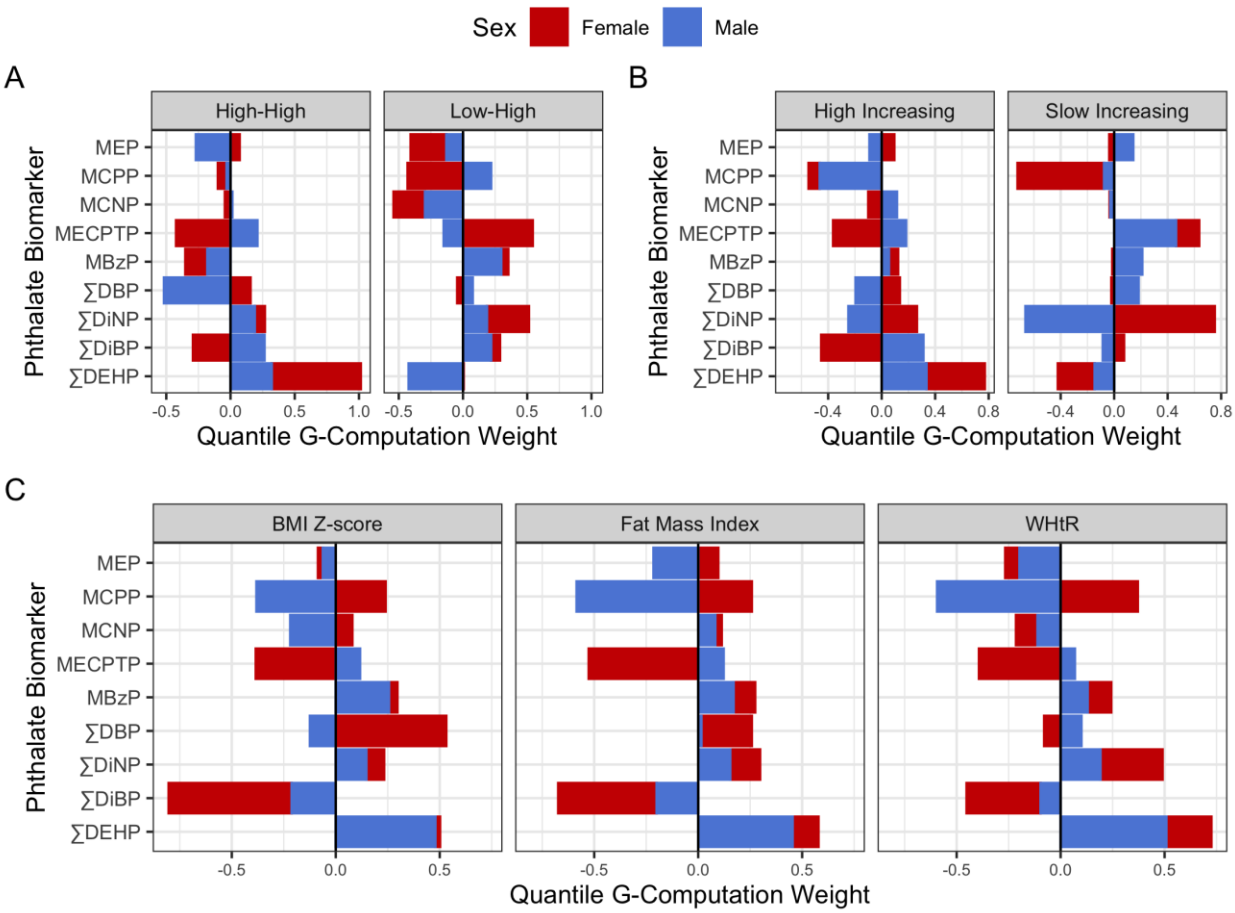
Supplemental Figure S3. Weights for associations of the total phthalates biomarker mixture with A) non-centered adiposity trajectories, B) centered adiposity trajectories, and C) longitudinal BMI Z-score, FMI and WHtR as measured by quantile g-computation (N = 514).



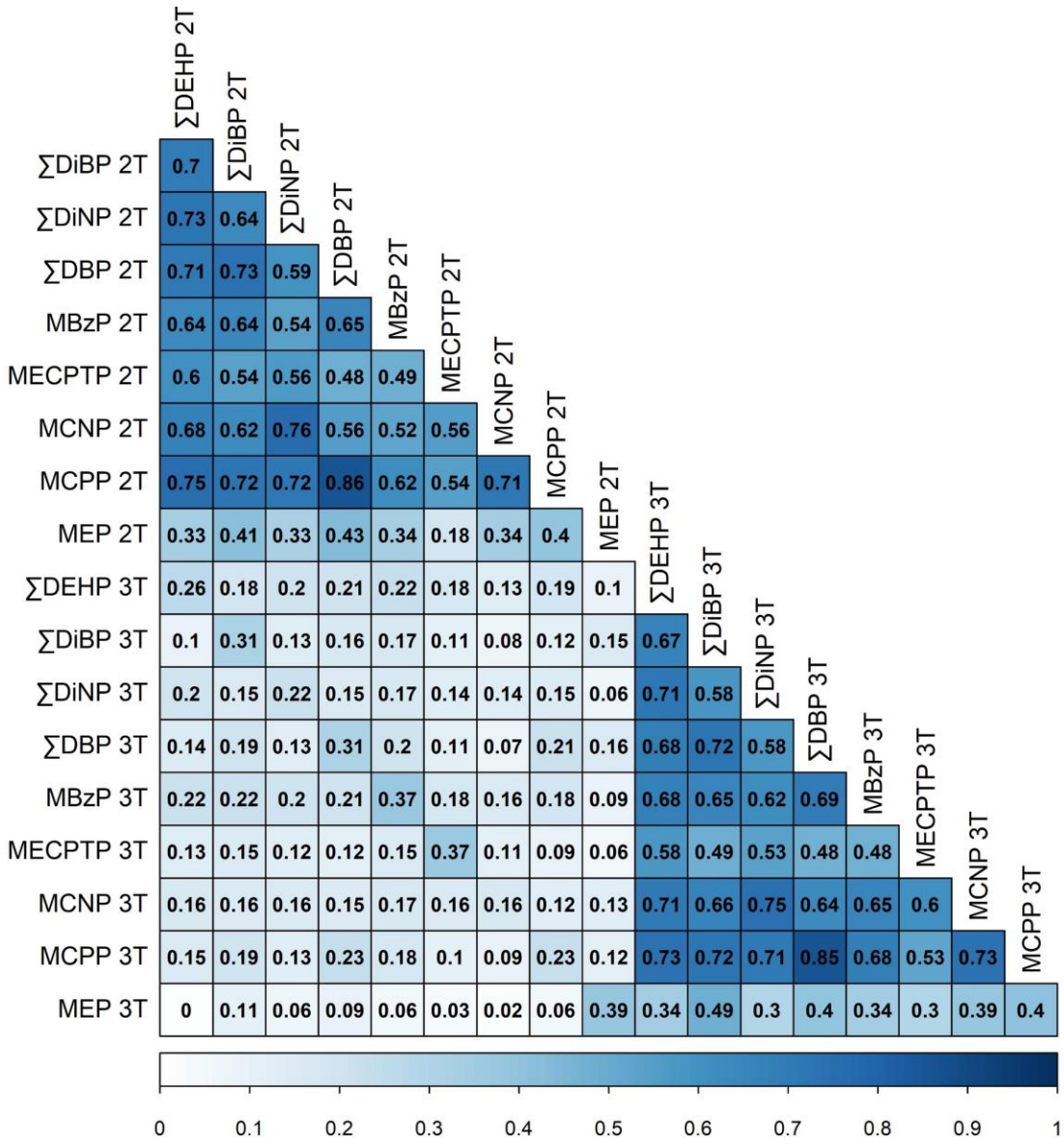
Supplemental Figure S4. Adiposity trajectory classes for male and female children identified by latent class growth modeling for A) non-centered BMI z-score, FMI and WHtR, and B) centered BMI-zscore, FMI, and WHtR. Colors indicate class membership (red: "low-stable"/"stable", blue: "high-high"/"high-increasing" and orange: "low-high"/"slow increasing") and smooth loess curves describe the trend of each trajectory. LCGM models for non-centered adiposity outcomes included linear and quadratic fixed effects for child age at follow-up, random intercepts for age and subject and link functions of 3 quantile splines on each adiposity outcome. LCGM models for centered adiposity outcomes included: linear and quadratic terms for age centered at the four-year visit, random intercepts for subject, 4 equidistant splines on BMI z-score and FMI, and a linear link function on WHtR.



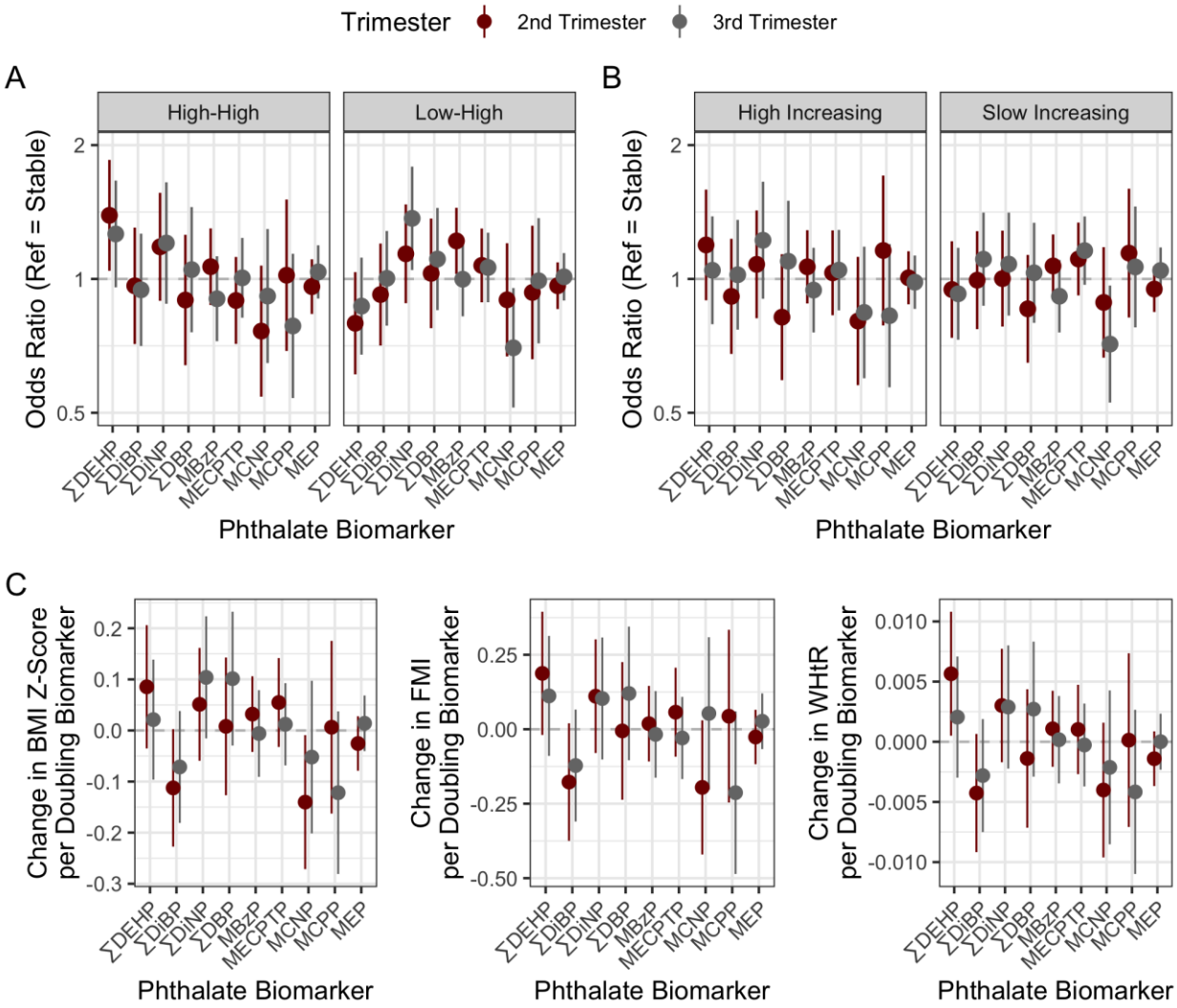
Supplemental Figure S5. Associations of sex-specific 2nd and 3rd trimester geometric mean urinary phthalate metabolites with A) non-centered adiposity trajectory classes, B) centered adiposity trajectory classes, and C) overall BMI z-score, fat mass index (FMI) and WHtR (waist-to-height ratio), using multinomial logistic regression with the "low-stable" and "stable" classes as the reference groups (A and B, respectively), or linear mixed-effects models (C). Models were stratified by child sex and color corresponds to sex-specific effect estimates (blue = male and red = female). Logistic regression model estimates are presented as odds ratios per doubling of metabolite concentrations with 95% confidence intervals (CI's). Colors represent the comparison classes (blue: "high-high" or "high-stable"; yellow: "low-high" or "high increasing". Linear mixed-effects models are presented as the change in outcome per doubling of phthalate biomarker with 95% CI's. All models included all 9 phthalate biomarkers and were adjusted for maternal age, maternal pre-pregnancy BMI, parity, socioeconomic status, education, and date and phthalate measurement. Mixed-effects models were additionally adjusted for linear and quadratic terms for child age at visit.



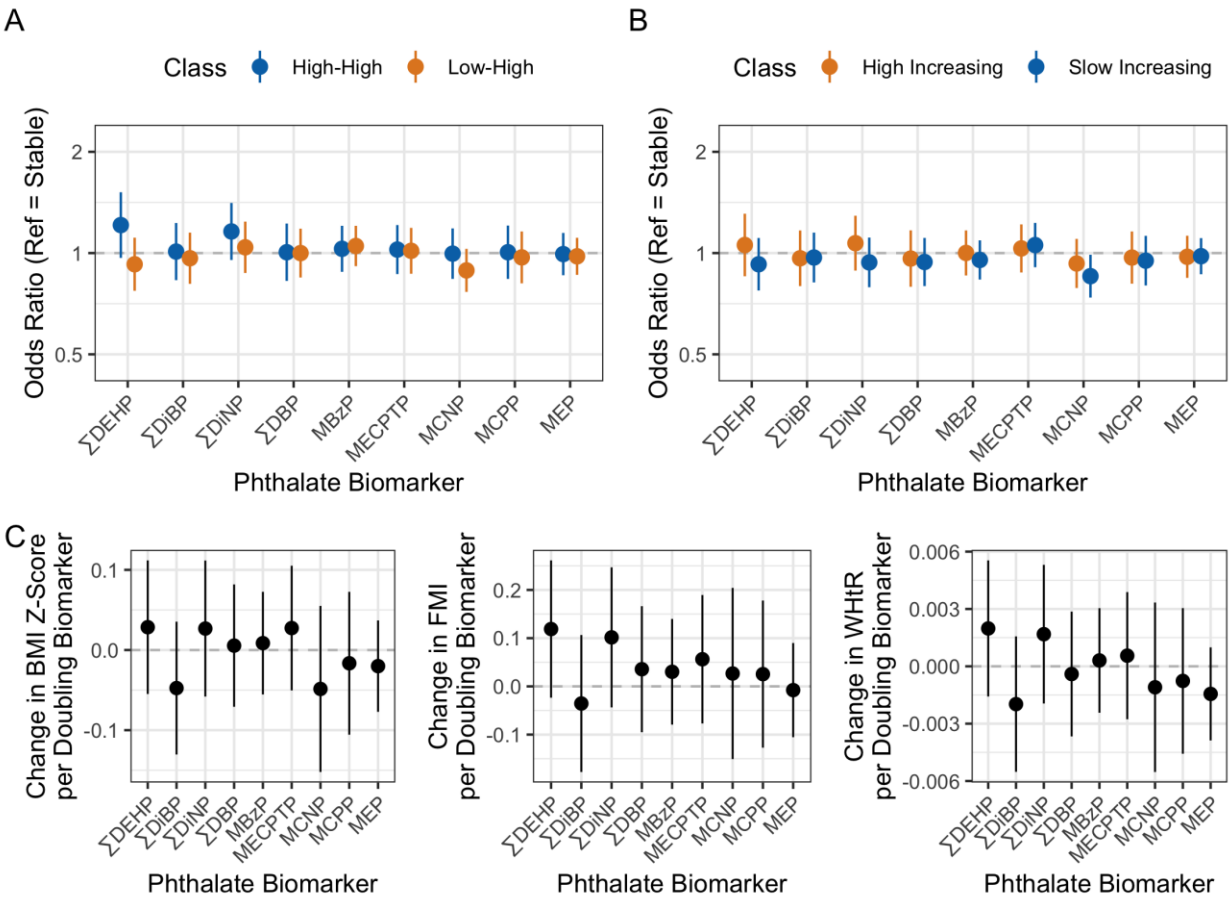
Supplemental Figure S6. Weights for associations of the total phthalates biomarker mixture with A) non-centered adiposity trajectories, B) centered adiposity trajectories, and C) longitudinal BMI Z-score, FMI and WHtR in females (red) and males (blue) as measured by quantile g-computation (N = 514).



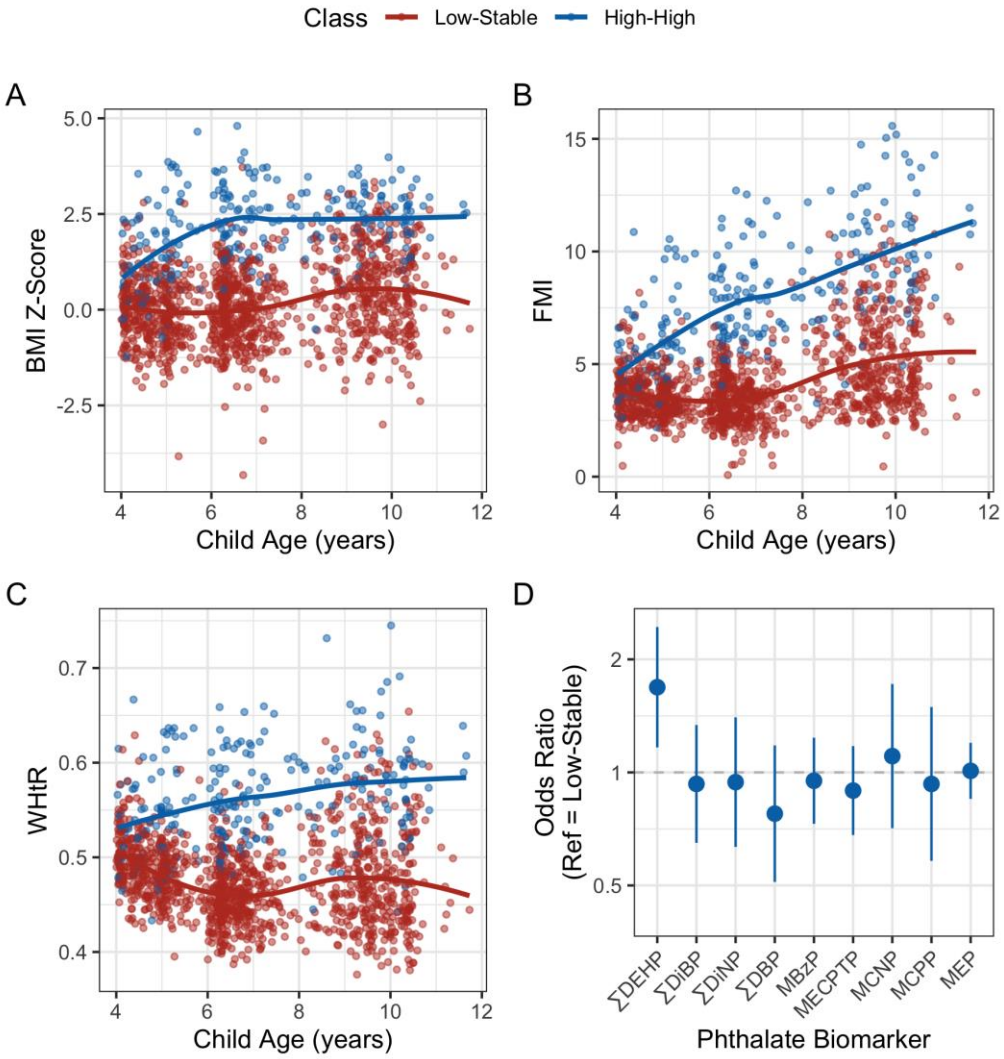
Supplemental Figure S7. Correlation plots between 2nd (2T) and 3rd (3T) phthalate biomarkers used in the present analysis. Gradient of cell indicates the strength of the Pearson correlation and the rho value is labeled in each cell.



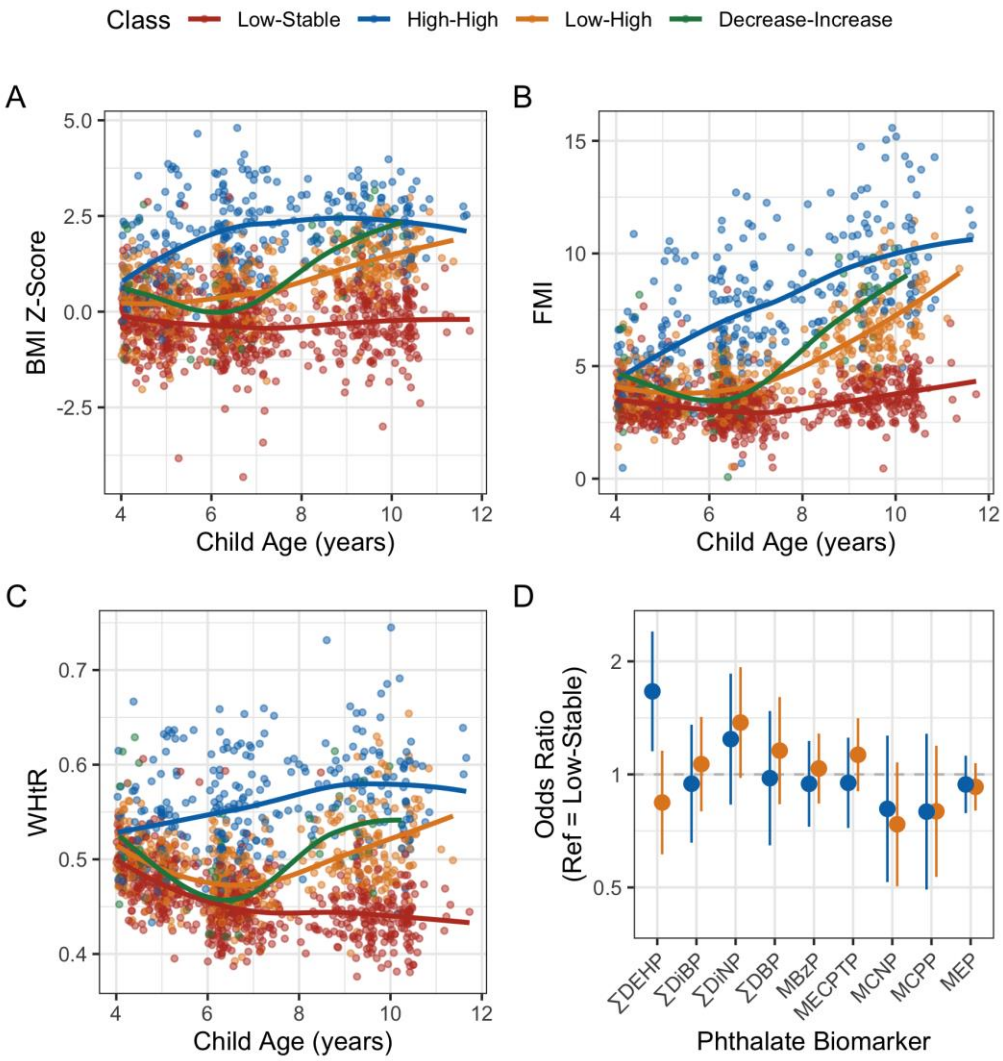
Supplemental Figure S8. Associations of 2nd (maroon) and 3rd trimester (gray) urinary phthalate metabolites with A) non-centered adiposity trajectory classes, B) centered adiposity trajectory classes, and C) overall BMI z-score, fat mass index (FMI) and WHtR (waist-to-height ratio), using multinomial logistic regression with the "low-stable" and "stable" classes as the reference groups (A and B, respectively), or linear mixed-effects models (C). Logistic regression model estimates are presented as odds ratios per doubling of metabolite concentrations with 95% confidence intervals (CI's). Linear mixed-effects models are presented as the change in outcome per doubling of phthalate biomarker with 95% CI's. All models included all 9 phthalate biomarkers and were adjusted for maternal age, maternal pre-pregnancy BMI, parity, socioeconomic status, education, child sex, and date and phthalate measurement. Mixed-effects models were additionally adjusted for linear and quadratic terms for child age at visit.



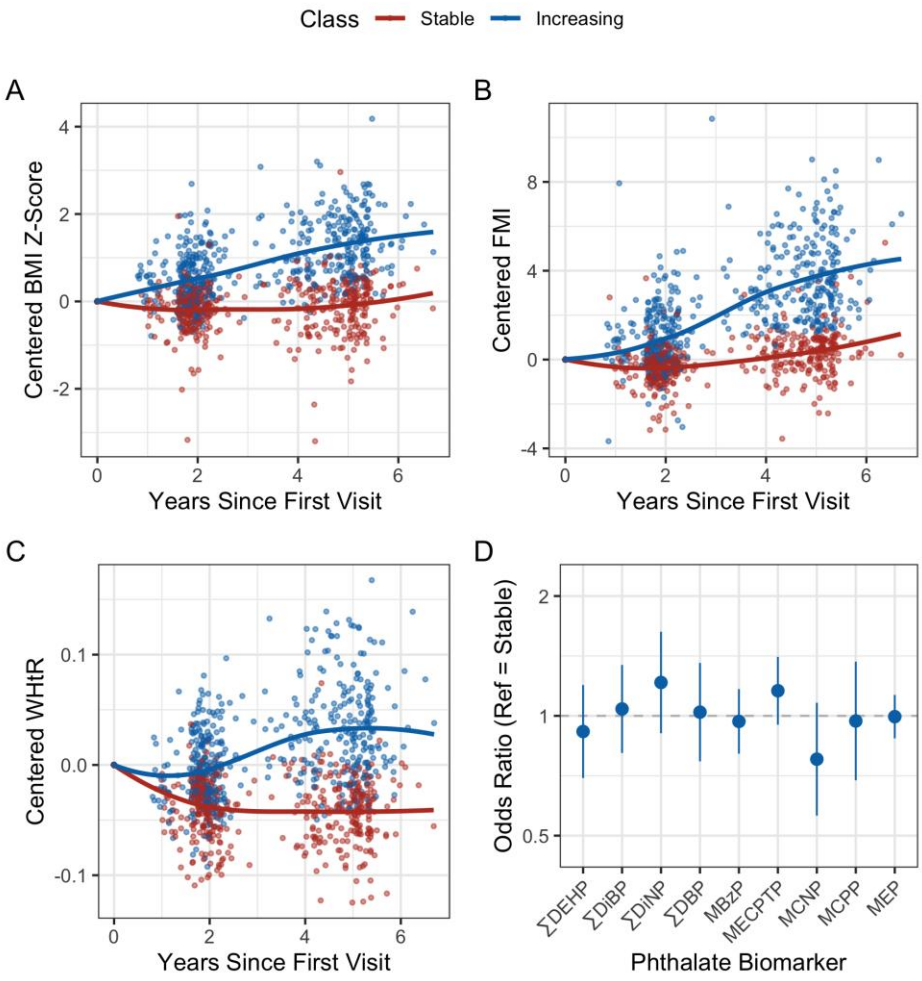
Supplemental Figure S9. Associations of 2nd and 3rd trimester geometric mean urinary phthalate metabolites with A) non-centered adiposity trajectory classes, B) centered adiposity trajectory classes, and C) overall BMI z-score, fat mass index (FMI) and WHtR (waist-to-height ratio), using multinomial logistic regression with the "low-stable" and "stable" classes as the reference groups (A and B, respectively), or linear mixed-effects models (C). Each phthalate was specified in a separate model. Logistic regression model estimates are presented as odds ratios per doubling of metabolite concentrations with 95% confidence intervals (CI's). Colors represent the comparison classes (blue: "high-high" or "high-stable"; yellow: "low-high" or "high increasing". Linear mixed-effects models are presented as the change in outcome per doubling of phthalate biomarker with 95% CI's. All models were adjusted for maternal age, maternal pre-pregnancy BMI, parity, socioeconomic status, education, child sex, and date and phthalate measurement. Mixed-effects models were additionally adjusted for linear and quadratic terms for child age at visit.



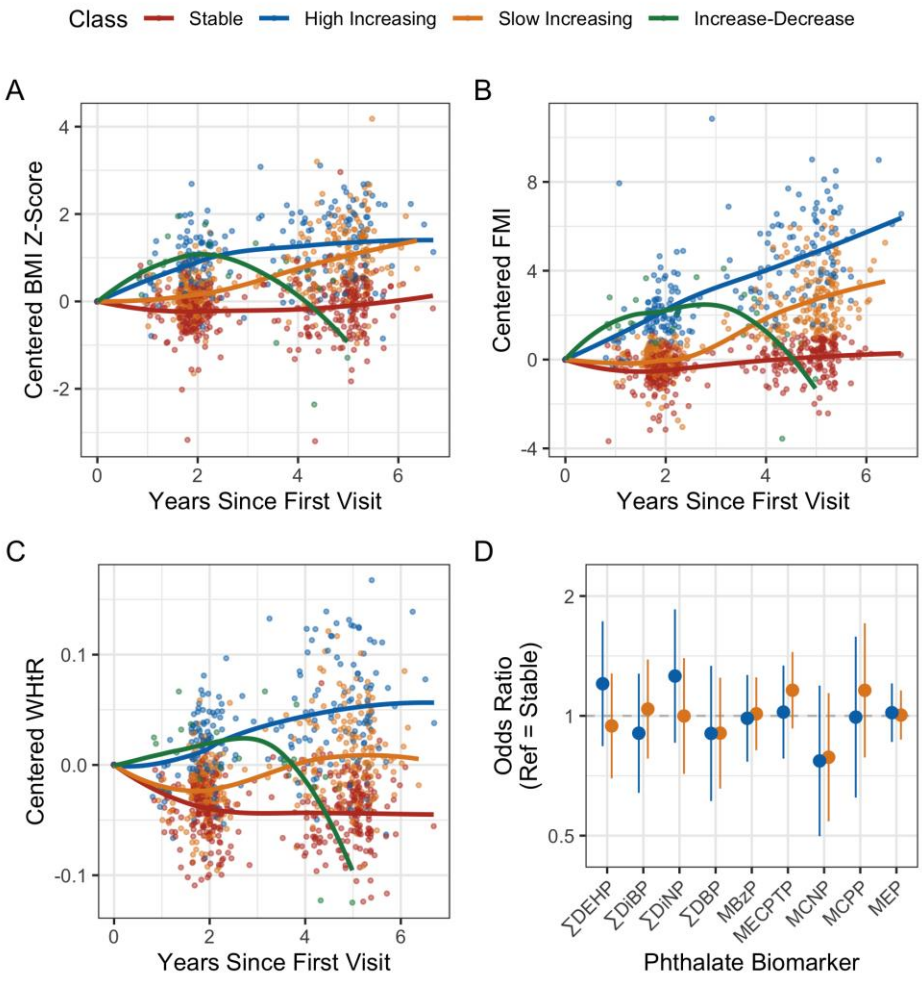
Supplemental Figure S10. Two-class adiposity trajectories identified by latent class growth modeling for A) non-centered BMI z-score, B) FMI and C) WHtR. D) Associations of 2nd and 3rd trimester geometric mean urinary phthalate metabolites with membership in the "high-high" class in comparison to the "low-stable" class using logistic regression. Colors indicate class membership (red: "low-stable", blue: "high-high") and smooth loess curves describe the trend of each trajectory.



Supplemental Figure S11. Four-class adiposity trajectories identified by latent class growth modeling for A) non-centered BMI z-score, B) FMI and C) WHtR. D) Associations of geometric mean urinary phthalate metabolites with membership in the "high-high" class and the "low-high" class in comparison to the "low-stable" class using multinomial logistic regression. Colors indicate class membership (red: "low-stable", blue: "high-high", yellow: "low-high", green: "decrease-increase") and smooth loess curves describe the trend of each trajectory.



Supplemental Figure S12. Two-class adiposity trajectories identified by latent class growth modeling for A) centered BMI z-score, B) centered FMI and C) centered WHtR. D) Associations of geometric mean urinary phthalate metabolites with membership in the "increasing" class in comparison to the "stable" class using logistic regression. Colors indicate class membership (red: "stable", blue: "increasing") and smooth loess curves describe the trend of each trajectory.



Supplemental Figure S13. Four-class adiposity trajectories identified by latent class growth modeling for A) centered BMI z-score, B) centered FMI and C) centered WHtR. D) Associations of geometric mean urinary phthalate metabolites with membership in the "high increasing" class and the "slow increasing" class in comparison to the "stable" class using multinomial logistic regression. Colors indicate class membership (red: "stable", blue: "high increasing", yellow: "slow increasing", green: "increase-decrease") and smooth loess curves describe the trend of each trajectory.