SUPPLEMENTAL MATERIALS

EARLY PREGNANCY PLASMA PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AND MATERNAL MIDLIFE ADIPOSITY

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Supplemental Table S1. Characteristics of Project Viva participants excluded from this study.

Characteristic	N = 1553
	Mean (SD) or n (%)
Age at enrollment (years)	31.6 (5.3)
Pre-pregnancy BMI (kg/m^2)	
< 25.0 kg/m^2	958 (62.9%)
25.0 to 29.9 kg/m^2	327 (21.1%)
≥ 30 kg/m^2	254 (16.3%)
Missing	14 (0.9%)
Race/Ethnicity	
White	996 (64.1%)
Black	271 (17.5%)
Hispanic	117 (7.5%)
Asian	92 (5.9%)
Other/More than 1 race	54 (3.5%)
Missing	22 (1.4%)
Parity	
0	744 (47.9%)
1	556 (35.8%)
≥ 2	253 (16.3%)
Married or cohabitating	
No	136 (8.8%)
Yes	1394 (89.8%)
Missing	23 (1.5%)
Smoking status	
Never smoked	1035 (66.6%)
Former smoker	287 (18.5%)
Smoked during pregnancy	211 (13.6%)
Missing	20 (1.3%)
Seafood consumption (servings/week)	0.2 (0.2)
Missing	285 (18.4%)

Abbreviations: BMI, body mass index; kg, kilogram; m, meter; SD, standard deviation.

Supplemental Table S2. Multivariable linear regression estimates[a] and 95% confidence intervals of the association between early pregnancy plasma PFAS concentrations[b] and maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[c] (N = 547) and DXA[d] (N = 433).

	Weight (kg)	Waist circumference (cm)	Trunk Fat Mass (kg)	Total Body Fat Mass (kg)
PFOS	3.8*** (1.6, 5.9)	0.7 (-0.7, 2.0)	0.5 (-0.2, 1.1)	1.0 (-0.1, 2.1)
PFOA	1.1 (-0.3, 2.4)	1.0 (-0.4, 2.4)	0.4 (-0.3, 1.1)	0.7 (-0.4, 1.8)
PFNA	-0.6 (-2.6, 1.4)	-1.1 (-2.4, -0.1)	-0.2 (-0.8, 0.4)	-0.3 (-1.3, 0.7)
PFHxS	0.0 (-1.4, 1.3)	-0.4 (-1.2, 0.4)	-0.3 (-0.7, 0.1)	-0.4 (-1.1, 0.2)
MeFOSAA	0.9 (-0.8, 2.5)	0.0 (-1.0, 0.9)	0.1 (-0.4, 0.5)	0.3 (-0.5, 1.1)
EtFOSAA	2.3** (0.9, 3.7)	0.7 (-0.2, 1.6)	0.4 (-0.1, 0.8)	0.6 (-0.1, 1.3)

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model), parity, marital status, smoking status, seafood consumption, and height.
[b] PFAS concentrations were log2-transformed.
[c] Anthropometric measures include weight and waist circumference.
[d] DXA measures include trunk fat mass and total body fat mass.
[**] p-value < 0.01 after accounting for multiple comparisons using the Benjamini-Hochberg false discovery rate correction.
[***] p-value < 0.001 after accounting for multiple comparisons using the Benjamini-Hochberg false discovery rate correction.

Abbreviations: cm, centimeters; DXA, dual-energy x-ray absorptiometry; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Table S3. Multivariable linear regression estimates[a] and 95% confidence intervals of the association between early pregnancy plasma PFAS concentrations[b] and maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[c] (N = 547) and DXA[d] (N = 433). Models are weighted with inverse probability of censoring weights.

	Weight (kg)	Waist circumference (cm)	Trunk Fat Mass (kg)	Total Body Fat Mass (kg)
PFOS	3.7** (1.4, 5.9)	0.7 (-0.7, 2.1)	0.4 (-0.2, 1.1)	1.0 (-0.1, 2.0)
PFOA	1.1 (-0.3, 2.5)	0.9 (-0.5, 2.3)	0.2 (-0.4, 0.9)	0.4 (-0.7, 1.5)
PFNA	-0.5 (-1.3, 1.2)	-1.3* (-2.5, 0.0)	-0.3 (-1.0, 0.3)	-0.5 (-1.5, 0.5)
PFHxS	-0.2 (-1.1, 0.7)	-0.4 (-1.2, 0.5)	-0.4 (-0.8, 0.0)	-0.5 (-1.2, 0.1)
MeFOSAA	0.6 (-0.5, 1.6)	-0.1 (-1.1, 0.9)	0.0 (-0.5, 0.5)	0.3 (-0.5, 1.1)
EtFOSAA	1.4** (0.5, 2.2)	0.9* (0.1, 1.8)	0.4* (0.0, 0.9)	0.8* (0.1, 1.5)

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model), parity, marital status, smoking status, seafood consumption, and height.
[b] PFAS concentrations were log2-transformed.
[c] Anthropometric measures include weight and waist circumference.
[d] DXA measures include trunk fat mass and total body fat mass.
[*] p-value < 0.05
[**] p-value < 0.01

Abbreviations: cm, centimeters; DXA, dual-energy x-ray absorptiometry; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Table S4. P-value of the interaction term between early pregnancy plasma PFAS concentrations[a] and having a pre-pregnancy body mass index ≥25 kg/m^2 in multivariable linear regression models[b] of the association between early pregnancy plasma PFAS concentrations and adiposity measured 17 – 20 years postpartum via anthropometric techniques[c] (N = 547) and DXA[d] (N = 433). Models are weighted with inverse probability of censoring weights.

	Weight	Waist circumference	Trunk Fat Mass	Total Body Fat Mass
PFOS	0.4	0.5	1.0	0.9
PFOA	0.4	0.3	0.9	0.8
PFNA	0.5	0.3	0.1	0.2
PFHxS	0.1	0.2	0.5	0.1
MeFOSAA	0.8	0.4	0.9	0.6
EtFOSAA	0.6	0.6	0.8	0.8

Notes:
[a] PFAS concentrations were log2-transformed.
[b] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model), parity, marital status, smoking status, seafood consumption, and height.
[c] Anthropometric measures include weight and waist circumference.
[d] DXA measures include trunk fat mass and total body fat mass.

Abbreviations: cm, centimeters; DXA, dual-energy x-ray absorptiometry; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Table S5. BKMR estimates[a] and 95% confidence intervals of the associations of single plasma PFAS concentrations[b] in early pregnancy with maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[c] (N = 547) and DXA[d] (N = 433). Estimates can be interpreted as the mean difference in adiposity measure when a plasma PFAS concentration increases from its 25th percentile to its 75th percentile, holding all other PFAS components at their 50th percentile concentration.

	Weight (kg)	Waist circumference (cm)	Trunk Fat Mass (kg)	Total Body Fat Mass (kg)
PFOS	7.7 (4.0, 11.5)	1.2 (-1.1, 3.6)	1.2 (0.0, 2.3)	3.0 (0.8, 5.2)
PFOA	1.1 (-1.8, 3.9)	1.4 (-0.6, 3.5)	0.7 (-0.4, 1.7)	0.6 (-1.2, 2.4)
PFNA	-6.0 (-8.5, -3.5)	-1.8 (-3.2, -0.3)	-0.8 (-1.5, -0.1)	-1.4 (-2.7, -0.1)
PFHxS	-2.0 (-4.0, -0.1)	-1.0 (-2.2, 0.3)	-0.8 (-1.4, -0.1)	-1.4 (-2.6, -0.2)
MeFOSAA	-0.6 (-2.6, 1.5)	-0.9 (-2.4, 0.6)	-0.3 (-1.1, 0.4)	-0.1 (-1.5, 1.2)
EtFOSAA	1.5 (-0.5, 3.5)	1.0 (-0.6, 2.5)	0.6 (-0.2, 1.4)	1.2 (-0.3, 2.6)

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model), parity, marital status, smoking status, seafood consumption, and height.
[b] PFAS concentrations were log2-transformed.
[c] Anthropometric measures include weight and waist circumference.
[d] DXA measures include trunk fat mass and total body fat mass.

Abbreviations: BKMR, Bayesian Kernel Machine Regression; cm, centimeters; DXA, dual-energy x-ray absorptiometry; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Table S6. Quantile g-computation estimates[a] and 95% CIs of the associations and individual PFAS weights for the overall effect of the early pregnancy plasma PFAS mixture on maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[b] (N = 547) and DXA[c] (N = 433).

	Weight (kg)	Waist circumference (cm)	Trunk Fat Mass (cm)	Total Body Fat Mass (kg)
Mixture Effect[d]				
ψ (95% CI)	1.64 (0.64, 3.92)	0.07 (-1.82, 1.97)	0.13 (-0.44, 0.70)	0.34 (-0.61, 1.29)
p-value	0.16	0.94	0.65	0.83
Weights[e]				
PFOS	+ 0.62	+ 0.60	+ 0.82	+ 0.95
PFOA	+ 0.17	+ 0.26	+ 0.09	− 0.03
PFNA	− 0.73	− 0.68	− 0.52	− 0.57
PFHxS	− 0.25	− 0.23	− 0.34	− 0.38
MeFOSAA	− 0.02	− 0.10	− 0.14	− 0.02
EtFOSAA	+ 0.21	+ 0.14	+ 0.09	+ 0.05

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model), parity, marital status, smoking status, seafood consumption, and height.
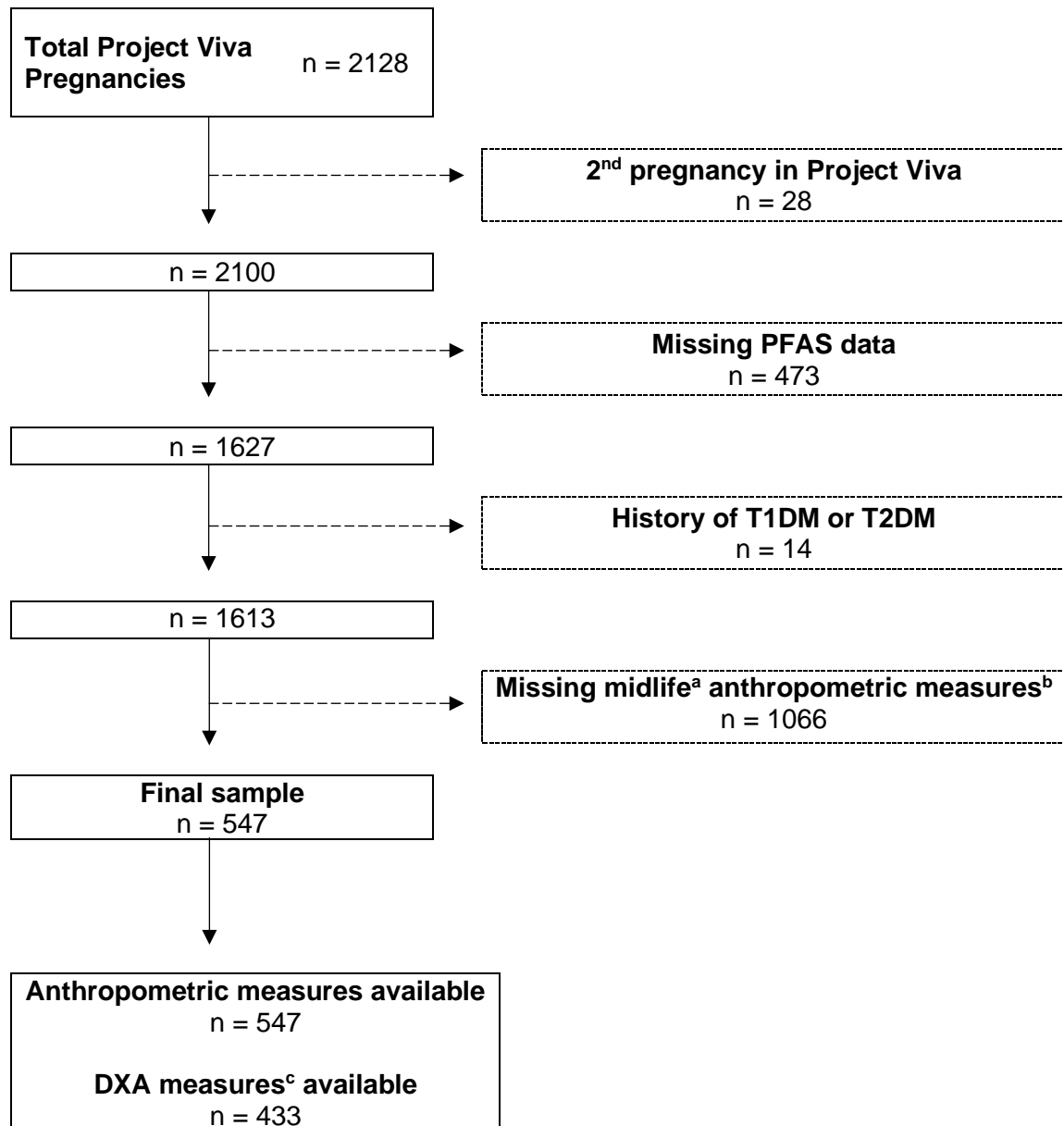[b] Anthropometric measures include weight and waist circumference.
[c] DXA measures include trunk fat mass and total body fat mass.
[d] Estimates represent the mean change in adiposity measure per quartile increase in the PFAS mixture index.
[e] Individual weights represent the relative contribution of each PFAS to the partial positive or negative mixture effect. Weights are constrained to sum to 1 in each direction.

Abbreviations: CI, confidence interval; cm, centimeters; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Figure S1. Study participant flow chart.

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┌────────────────────────────────┐
│ Total Project Viva   n = 2128  │
│ Pregnancies                    │
└────────────────────────────────┘
          │         ┌─────────────────────────────────────┐
          ┆ ─ ─ ─ ▶ │ 2ⁿᵈ pregnancy in Project Viva       │
          ▼         │ n = 28                              │
┌────────────────────┐          └─────────────────────────────────────┘
│ n = 2100           │
└────────────────────┘
          │         ┌─────────────────────────────────────┐
          ┆ ─ ─ ─ ▶ │ Missing PFAS data                   │
          ▼         │ n = 473                             │
┌────────────────────┐          └─────────────────────────────────────┘
│ n = 1627           │
└────────────────────┘
          │         ┌─────────────────────────────────────┐
          ┆ ─ ─ ─ ▶ │ History of T1DM or T2DM             │
          ▼         │ n = 14                              │
┌────────────────────┐          └─────────────────────────────────────┘
│ n = 1613           │
└────────────────────┘
          │         ┌──────────────────────────────────────────────────┐
          ┆ ─ ─ ─ ▶ │ Missing midlifeᵃ anthropometric measuresᵇ       │
          ▼         │ n = 1066                                         │
┌────────────────────┐          └──────────────────────────────────────────────────┘
│ Final sample       │
│ n = 547            │
└────────────────────┘
          │
          ▼
┌────────────────────────────────┐
│ Anthropometric measures        │
│ available                      │
│ n = 547                        │
│                                │
│ DXA measuresᶜ available        │
│ n = 433                        │
└────────────────────────────────┘
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Notes:
[a] Midlife is defined as 15 to 20 years after the index pregnancy.
[b] Anthropometric measures include weight and waist circumference.
[c] DXA measures include trunk fat mass and total body fat mass.

Abbreviations: PFAS, per- and polyfluoroalkyl substances; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

Supplemental Figure S2. Directed acyclic graph of the association between early pregnancy PFAS (exposure), maternal midlife adiposity (outcome), and potential confounding covariates of the exposure-outcome association.
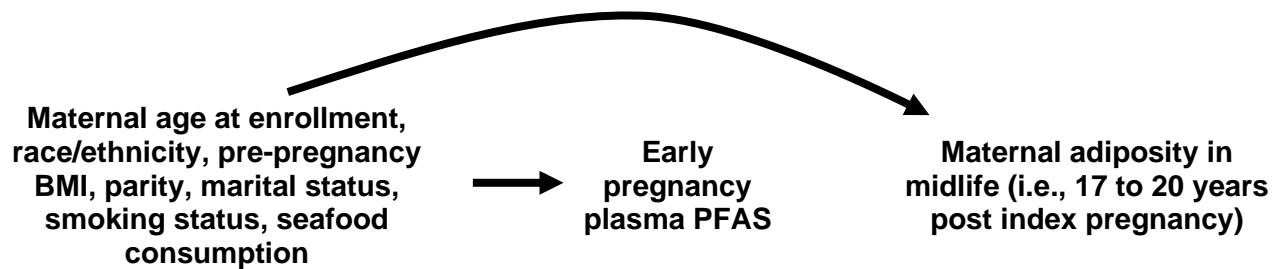
**Note**: This graph assumes the association between PFAS and maternal adiposity in midlife is null.

Abbreviations: BMI, body mass index; PFAS, per- and polyfluoroalkyl substances.

Supplemental Figure S3. Spearman rank correlation coefficients among early pregnancy plasma PFAS concentrations (N = 547).
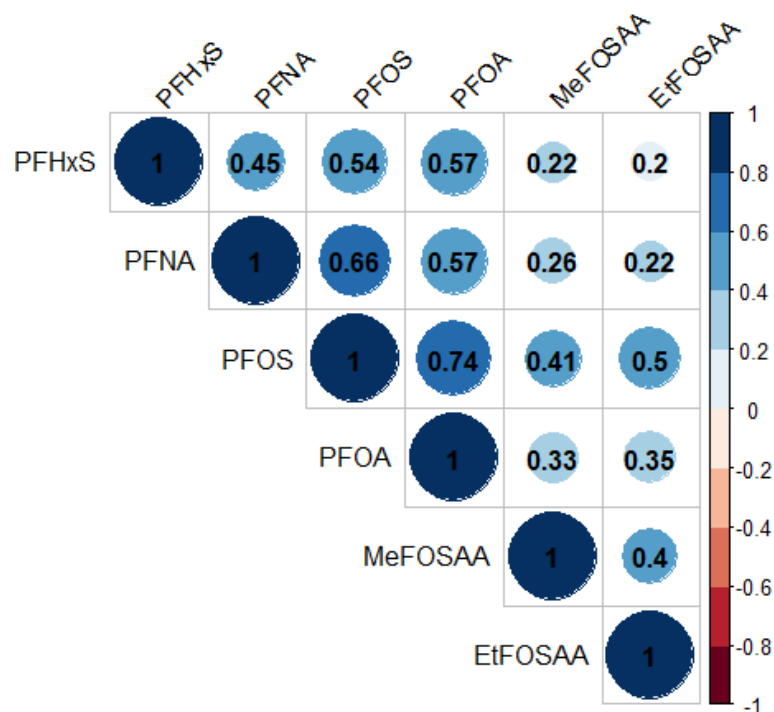
Abbreviations: EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate

Supplemental Figure S4. BKMR estimated[a] associations between early pregnancy plasma concentrations of individual PFAS and maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[b] (N = 547) and DXA[c] (N = 433), fixing other PFAS in the mixture at their 50th percentile. Black lines indicate the predictor-response function, grey areas represent 95% CIs, and red dashed lines are placed at the 0 value of the parameter.
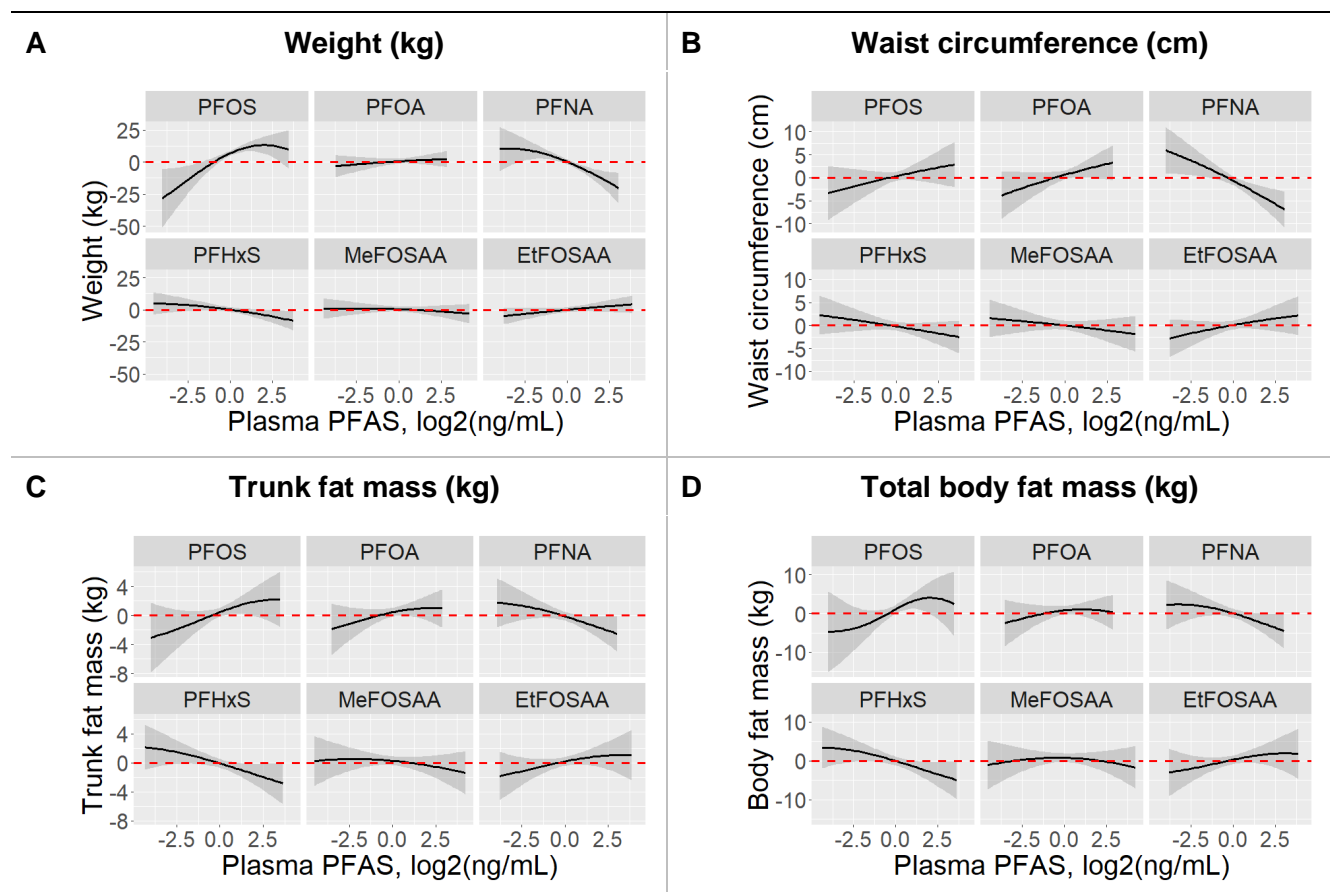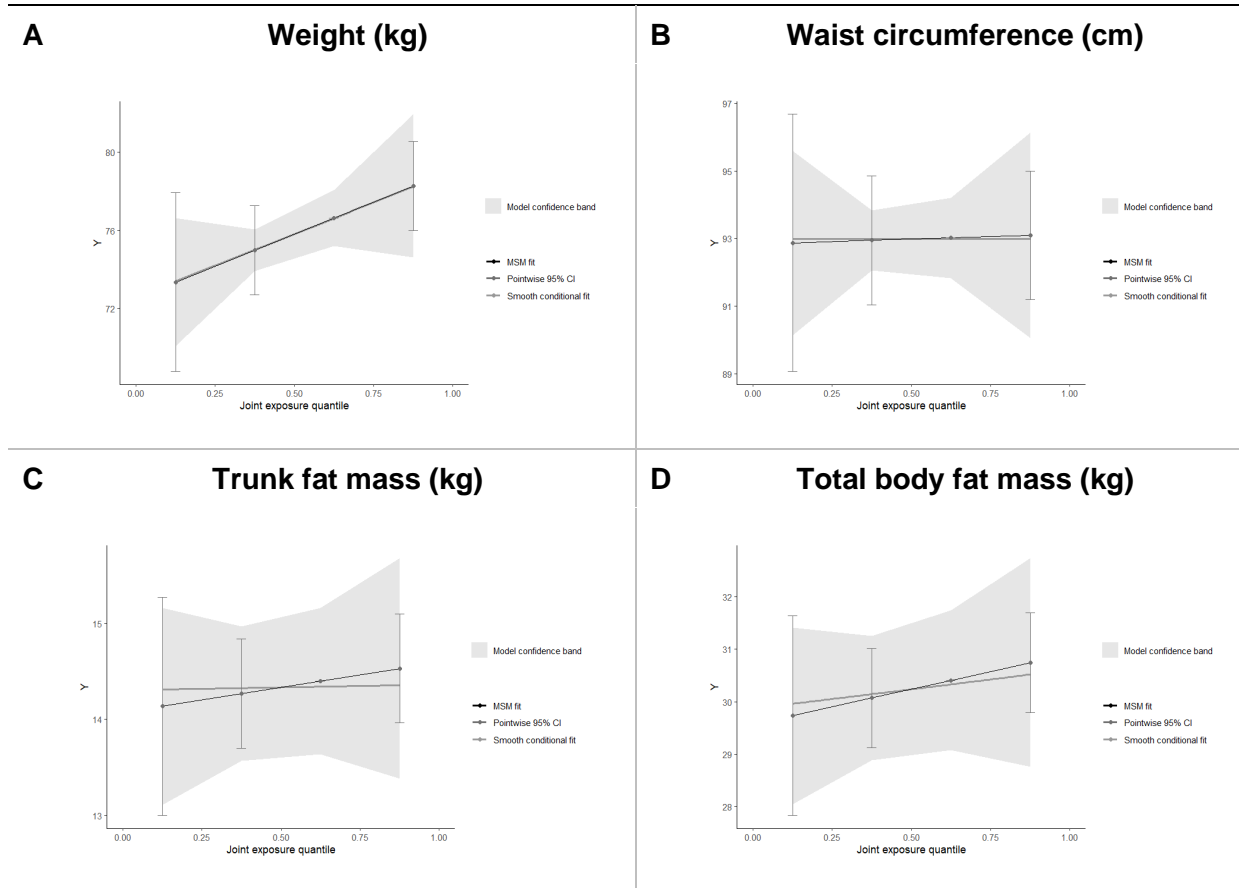

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model [A]), parity, marital status, smoking status, seafood consumption, and height.
[b] Anthropometric measures include weight and waist circumference.
[c] DXA measures include trunk fat mass and total body fat mass.

Abbreviations: CI, credible intervals; cm, centimeters; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; mL, milliliters; ng, nanograms; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate

Supplemental Figure S5. Quantile g-computation estimated[a] overall associations, and 95% CIs, of concentrations of early pregnancy plasma PFAS mixture and maternal adiposity measured 17 – 20 years postpartum via anthropometric techniques[b] (N = 547) and DXA[c] (N = 433). The plot illustrates the change in mean adiposity measure per quartile increase in the PFAS mixture index.

Notes:
[a] Adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index (except for the weight model [A]), parity, marital status, smoking status, seafood consumption, and height.
[b] Plasma PFAS concentrations were log2-transformed.
[c] Anthropometric measures include weight and waist circumference.
[d] DXA measures include trunk fat mass and total body fat mass.

Abbreviations: CI, credible intervals; cm, centimeters; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilograms; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
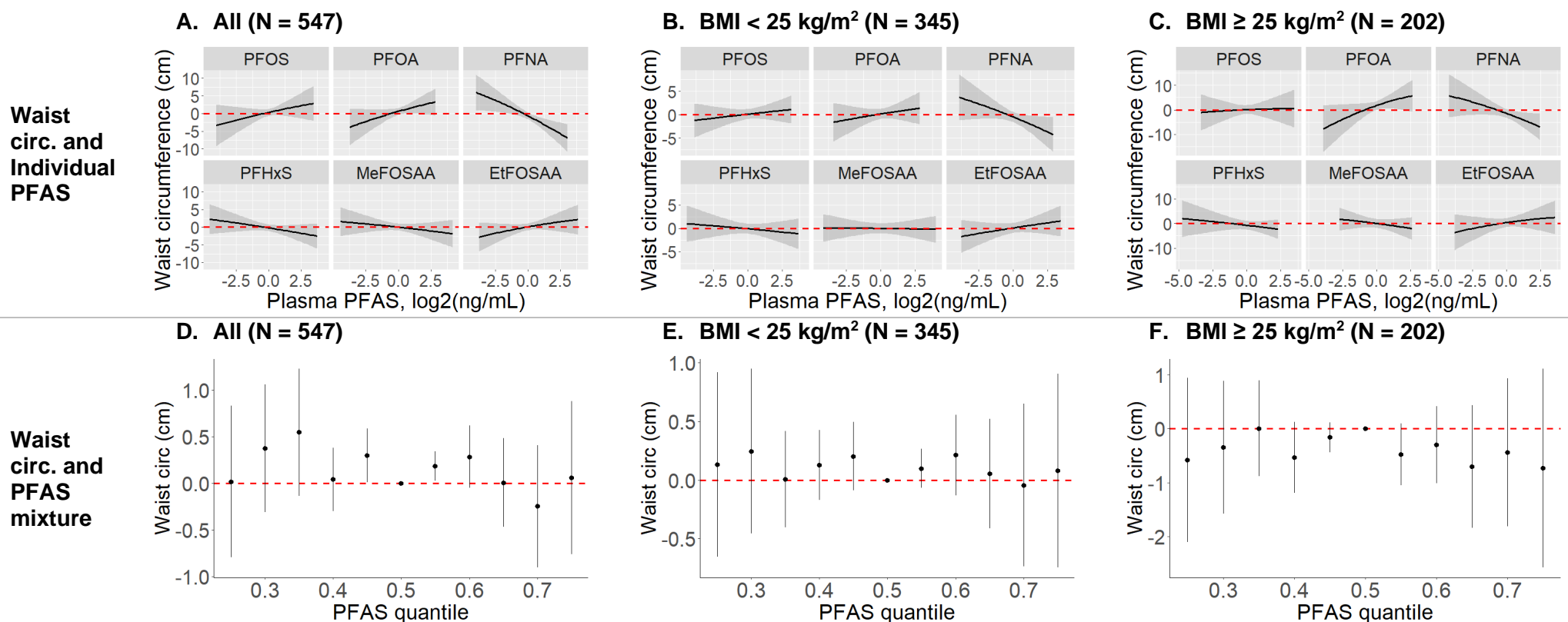
Supplemental Figure S6. Associations between early pregnancy plasma concentrations of individual PFAS and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR, fixing other PFAS in the mixture at their 50th percentile (Supplemental Figure S3.7A – S3.7C). Additionally, overall association of concentrations of early pregnancy plasma PFAS mixture at various percentiles, versus the 50th percentile, and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR (Supplemental Figure S3.7D – S3.7F). Associations are shown for the entire study population as well as stratified by pre-pregnancy BMI.
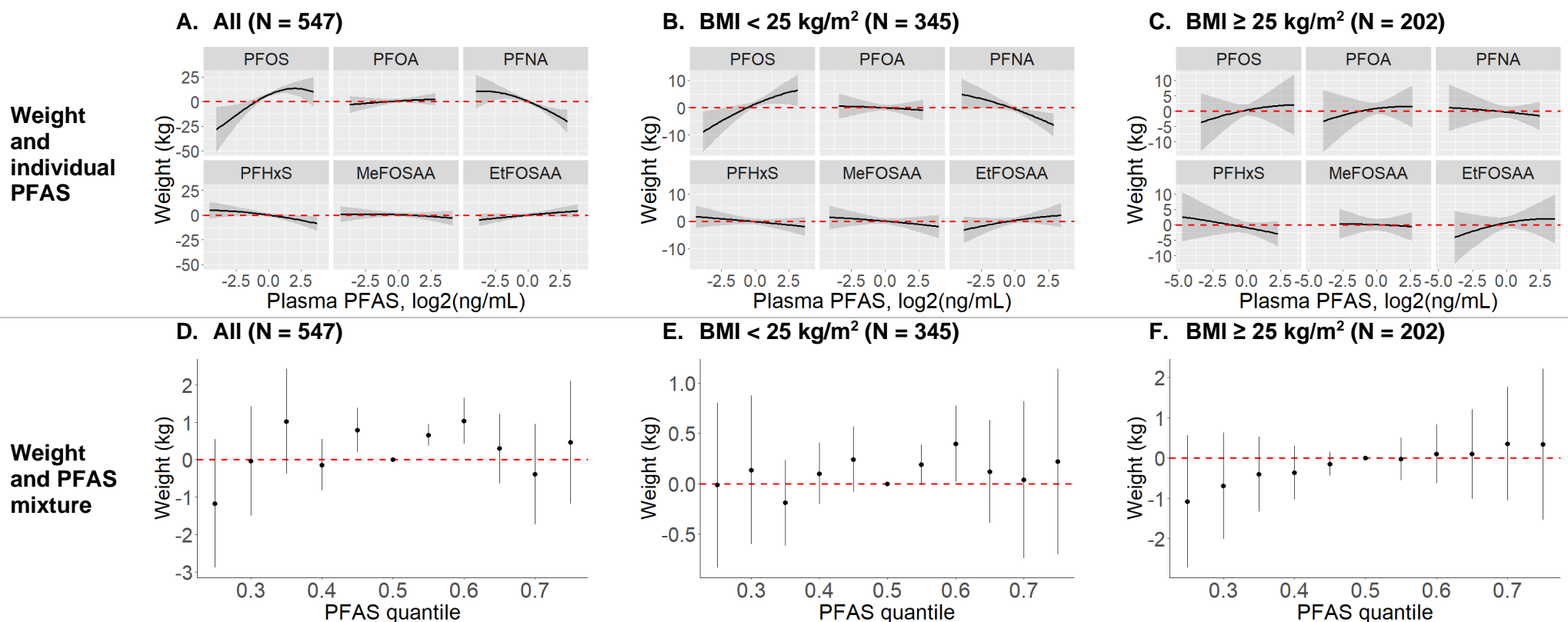
Note: Models adjusted for maternal age, race and ethnicity, parity, marital status, smoking status, seafood consumption, and height.

Abbreviations: BKMR, Bayesian Kernel Machine Regression; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilogram; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; mL, milliliter; ng, nanogram; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
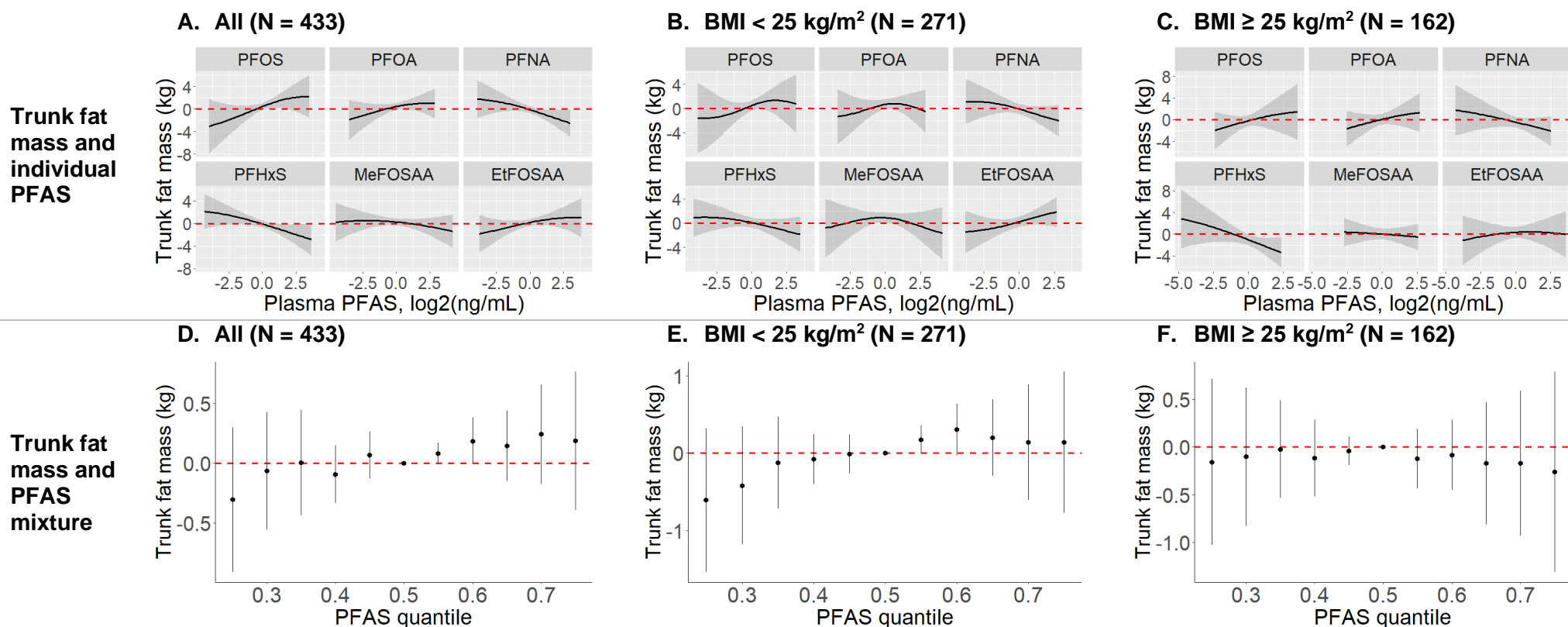
Supplemental Figure S7. Associations between early pregnancy plasma concentrations of individual PFAS and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR, fixing other PFAS in the mixture at their 50th percentile (Supplemental Figure S3.7A – S3.7C). Additionally, overall association of concentrations of early pregnancy plasma PFAS mixture at various percentiles, versus the 50th percentile, and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR (Supplemental Figure S3.7D – S3.7F). Associations are shown for the entire study population as well as stratified by pre-pregnancy BMI.

Note: Models adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index, parity, marital status, smoking status, seafood consumption, and height.

Abbreviations: BKMR, Bayesian Kernel Machine Regression; circ, circumference; cm, centimeter; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; mL, milliliter; ng, nanogram; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
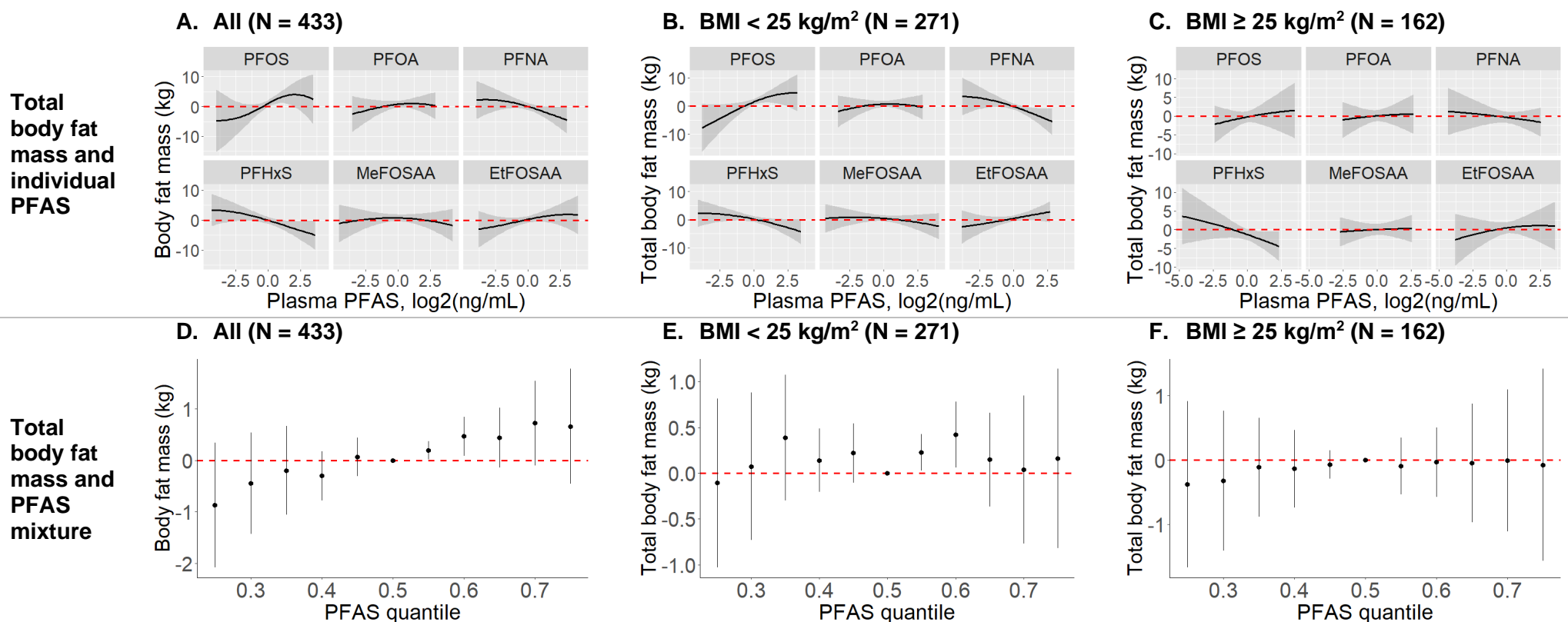
Supplemental Figure S8. Associations between early pregnancy plasma concentrations of individual PFAS and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR, fixing other PFAS in the mixture at their 50th percentile (Supplemental Figure S3.7A – S3.7C). Additionally, overall association of concentrations of early pregnancy plasma PFAS mixture at various percentiles, versus the 50th percentile, and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR (Supplemental Figure S3.7D – S3.7F). Associations are shown for the entire study population as well as stratified by pre-pregnancy BMI.

Note: Models adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index, parity, marital status, smoking status, seafood consumption, and height.

Abbreviations: BKMR, Bayesian Kernel Machine Regression; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilogram; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; mL, milliliter; ng, nanogram; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Supplemental Figure S9. Associations between early pregnancy plasma concentrations of individual PFAS and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR, fixing other PFAS in the mixture at their 50th percentile (Supplemental Figure S3.7A – S3.7C). Additionally, overall association of concentrations of early pregnancy plasma PFAS mixture at various percentiles, versus the 50th percentile, and midlife adiposity measured 17 – 20 years postpartum estimated using BKMR (Supplemental Figure S3.7D – S3.7F). Associations are shown for the entire study population as well as stratified by pre-pregnancy.

Note: Models adjusted for maternal age, race and ethnicity, pre-pregnancy body mass index, parity, marital status, smoking status, seafood consumption, and height.

Abbreviations: BKMR, Bayesian Kernel Machine Regression; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; kg, kilogram; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; mL, milliliter; ng, nanogram; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.