

Supplementary Materials

Iron status from diet and serum in relation to biological aging acceleration: A NHANES cross-sectional study

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Manuscript received 12 February 2026. Initial review completed 26 June 2026. Revision accepted 20 August 2026.

doi: 10.6133/apjcn.202610_35(5).0009

Supplementary Table 1. Linear and non-linear association between dietary iron intake and biological aging in all participants (n = 13284)[†]

Outcome	Linear β (95% CI); p	RCS					
		p -overall	p -nonlinear	Median exposure	Lowest point $x(\beta)$	Highest point $x(\beta)$	Zero crossing x
PA deviation	-0.05 (-0.08, -0.02); <0.01	0.02	0.30	12.95	27.45 (-0.06)	5.82 (0.06)	9.26; 12.95; 13.50
GoldBA deviation	-0.11 (-0.16, -0.07); < 0.001	< 0.001	< 0.001	12.95	27.45 (-0.09)	5.82 (0.20)	11.03; 12.95; 13.76
LightBA deviation	-0.02 (-0.05, 0.01); 0.11	0.13	0.18	12.95	27.45 (0.00)	5.82 (0.07)	12.95; 14.67; 24.07

[†]Models adjusted for sex (men/women), ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), education levels (less than high school graduate, high school graduate to some college, college graduate or higher), poverty income ratio, BMI (kg/m²), cigarette smoking (non-smoker, former smoker, current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity levels (low, moderate, and vigorous), energy intake (kcal/day), the presence of chronic diseases (hypertension, diabetes, dyslipidemia, chronic respiratory disease, hepatopathy, cardiovascular disease, stroke, arthritis, and thyroid disease) (yes and not), and alternative healthy eating index (AHEI).

Supplementary Table 2. Nonlinear associations between iron exposure and biological aging stratified by age group

Exposure and outcome	<i>p-int</i> ^a	< 65 yrs						≥ 65 yrs					
		n	<i>p-ov</i> [†]	<i>p-nl</i> [†]	Min x (β)	Max x (β)	ZeroCross x	n	<i>p-ov</i>	<i>p-nl</i>	Min x (β)	Max x (β)	ZeroCross x
Total iron intake													
GoldBA deviation	0.46	9987	< 0.001	< 0.001	28.19 (-0.10)	13.72 (0.00)	NA	3297	<0.001	<0.001	31.08 (-0.14)	16.35 (0.03)	7.61;18.76
LightBA deviation	0.12	9987	0.670	0.664	16.95 (0.00)	30.76 (0.01)	20.80	3297	0.090	0.050	12.51 (-0.01)	12.51 (-0.01)	11.18
PA deviation	0.42	9987	< 0.001	0.001	22.75 (-0.02)	22.75 (-0.02)	33.97	3297	0.040	0.050	28.61 (-0.07)	15.72 (0.01)	9.51;17.19
Dietary iron intake													
GoldBA deviation	0.9	8468	0.010	0.030	27.3 (-0.05)	5.77 (0.16)	NA	2635	<0.001	<0.01	10.21	-0.07 (14.47)	
LightBA deviation	< 0.01	8468	0.930	0.840	14.07 (0.00)	14.07 (0.00)	15.41	2635	0.060	0.130	13.74	0 (20.54)	0.03 (14.65)
PA deviation	< 0.01	8468	0.230	0.470	27.3 (-0.05)	5.77 (0.07)	NA	2635	0.040	0.380	11.48	0 (13.67)	
Supplemental iron intake													
GoldBA deviation	0.49	1519	< 0.001	0.410	1 (-0.18)	65 (0.33)	NA	662	0.020	0.030	1.00 (-0.35)	65 (0.28)	NA
LightBA deviation	0.78	1519	0.460	0.220	44.52 (0.04)	44.52 (0.04)	NA	662	0.380	0.460	1.00 (-0.10)	65 (0.09)	NA
PA deviation	0.15	1519	0.040	0.090	1.00 (-0.16)	65 (0.16)	NA	662	0.020	0.070	1.00 (-0.25)	65 (0.22)	NA
Serum iron													
GoldBA deviation	0.96	9952	< 0.001	< 0.001	21.64 (-0.05)	14.98 (0.00)	11.60;15.40	3290	<0.001	<0.001	12.78 (-0.01)	14.62 (0.00)	11.82;14.65
LightBA deviation	< 0.01	9952	< 0.001	< 0.001	21.98 (-0.06)	21.98 (-0.06)	NA	3290	<0.001	0.170	26.10 (-0.23)	6.4 (0.36)	NA
PA deviation	0.001	9952	< 0.001	< 0.001	15.77 (-0.02)	10.03 (0.15)	17.29	3290	<0.001	0.520	26.10 (-0.16)	6.4 (0.16)	NA

[†]p-int, p-interaction; p-ov, p-overall; p-nl, p-nonlinear.

Supplementary Table 3. Nonlinear associations between iron exposure and biological aging stratified by sex

Exposure and outcome	<i>p-int</i> ^a	Men						Women					
		n	<i>p-ov</i> [†]	<i>p-nl</i> [†]	Min x (β)	Max x (β)	ZeroCross x	n	<i>p-ov</i>	<i>p-nl</i>	Min x (β)	Max x (β)	ZeroCross x
Total iron intake													
GoldBA deviation	0.26	6503	< 0.01	0.001	31.7 (-0.13)	14.85 (0)	9.89;15.28	6781	< 0.001	< 0.001	27.68 (-0.08)	15.24 (0)	9.66;16.10
LightBA deviation	0.23	6503	0.130	0.090	13.44 (0)	13.44 (0)	12.69	6781	0.210	0.300	16.67 (0)	31.03 (0.01)	19.73
PA deviation	< 0.001	6503	0.030	0.010	28.08 (-0.04)	28.08 (-0.04)	NA	6781	< 0.001	< 0.001	21.36 (-0.01)	21.36 (-0.01)	27.13
Dietary iron intake													
GoldBA deviation	< 0.01	5759	< 0.01	0.100	27.3 (-0.06)	5.77 (0.18)	NA	5344	< 0.01	< 0.01	10.94 (-0.01)	13.14 (0)	9.83;13.34
LightBA deviation	0.34	5759	0.080	0.040	13.44 (0)	24.14 (0.05)	13.95	5344	0.490	0.850	27.3 (-0.06)	5.77 (0.05)	NA
PA deviation	0.001	5759	< 0.01	0.180	27.3 (-0.08)	5.77 (0.16)	NA	5344	0.060	0.170	11.27 (0)	14.32 (0)	10.17;15.87
Supplemental iron intake													
GoldBA deviation	0.02	744	< 0.01	0.260	1 (-0.15)	65 (0.22)	NA	1437	< 0.001	0.120	1 (-0.26)	65 (0.36)	NA
LightBA deviation	0.64	744	0.010	0.470	1 (-0.12)	65 (0.18)	NA	1437	0.200	0.080	41.86 (0.05)	41.86 (0.05)	NA
PA deviation	0.05	744	0.020	0.360	1 (-0.12)	65 (0.17)	NA	1437	< 0.01	0.020	1 (-0.26)	65 (0.24)	NA
Serum iron													
GoldBA deviation	< 0.001	6472	< 0.001	0.001	24.78 (-0.08)	14.63 (0)	11.69;14.67	6770	< 0.001	< 0.001	20.39 (-0.06)	14.69 (0)	11.84;14.80
LightBA deviation	0.05	6472	< 0.001	< 0.001	26.1 (-0.09)	6.4 (0.45)	NA	6770	< 0.001	< 0.001	20.45 (-0.09)	20.45 (-0.09)	NA
PA deviation	< 0.001	6472	< 0.001	< 0.001	15.96 (-0.01)	22.19 (0.01)	17.90	6770	< 0.01	0.001	16.44 (-0.02)	10.72 (0.11)	6.81;19.42

[†]p-int, p-interaction; p-ov, p-overall; p-nl, p-nonlinear.

Supplementary Table 4. Nonlinear associations between iron exposure and biological aging stratified by women's menopausal status

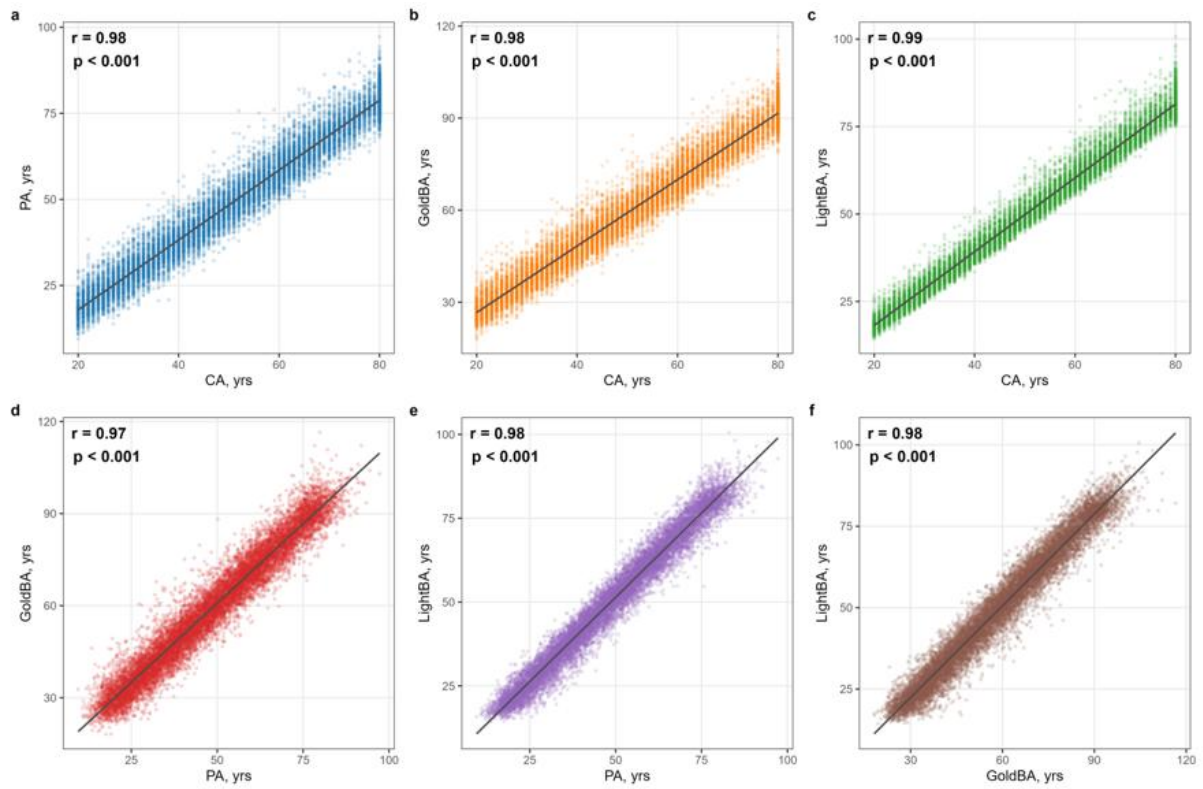
Exposure and outcome	<i>p-int</i> ^a	Pre-menopausal women						Post-menopausal women					
		n	<i>p-ov</i> [†]	<i>p-nl</i> [†]	Min x (β)	Max x (β)	ZeroCross x	n	<i>p-ov</i>	<i>p-nl</i>	Min x (β)	Max x (β)	ZeroCross x
Total iron intake													
GoldBA deviation	0.89	3668	< 0.001	<0.01	26.72 (-0.07)	15.26 (0)	9.40;16.15; 39.08	3113	< 0.01	<0.01	30.14 (-0.12)	15.23 (0)	9.65;16.08
LightBA deviation	0.03	3668	0.090	0.580	16.49 (-0.01)	7.74 (0.05)	19.19	3113	0.270	0.190	30.67 (-0.04)	15.22 (0)	10.54;16.05
PA deviation	0.1	3668	0.010	0.040	17.18 (-0.01)	17.18 (-0.01)	21.23	3113	< 0.01	<0.01	27.94 (-0.04)	16.16 (0.01)	9.25;18.28
Dietary iron intake													
GoldBA deviation	0.06	0.040	0.150	10.37	-0.02 (13.4)			2432	0.050	0.120	19.25 (-0.02)	19.25 (-0.02)	26.41
LightBA deviation	0.36	0.320	0.210	20.02	-0.08 (12.21)	0 (11.34)		2432	0.050	0.370	13.61 (0)	17.36 (0.01)	14.36;19.68
PA deviation	0.14	0.370	0.650	24.33	-0.1 (12.94)	0 (8.19)		2432	0.070	0.090	12.45 (0)	20.82 (0.09)	11.94
Supplemental iron intake													
GoldBA deviation	0.35	756	< 0.001	0.320	1 (-0.24)	65 (0.4)	NA	681	0.020	0.090	1 (-0.29)	65 (0.24)	NA
LightBA deviation	0.11	756	0.026	0.610	9.67 (0)	9.67 (0)	NA	681	0.130	0.380	1 (-0.14)	65 (0.14)	NA
PA deviation	0.79	756	0.107	0.090	1 (-0.24)	65 (0.19)	NA	681	< 0.01	0.050	1 (-0.3)	65 (0.3)	NA
Serum iron													
GoldBA deviation	0.61	3658	< 0.001	< 0.001	20.45 (-0.05)	14.79 (0)	11.69;15.02	3112	< 0.001	< 0.001	21.12 (-0.1)	14.65 (0)	11.43;14.72
LightBA deviation	< 0.001	3658	< 0.01	0.020	17.5 (-0.02)	17.5 (-0.02)	23.75	3112	< 0.001	0.010	22.63 (-0.21)	22.63 (-0.21)	NA
PA deviation	< 0.01	3658	< 0.001	0.00057	15.3 (-0.01)	10.59 (0.15)	6.60;16.10	3112	0.064	0.130	20.79 (-0.15)	11.79 (0.02)	NA

[†]p-int, p-interaction; p-ov, p-overall; p-nl, p-nonlinear.

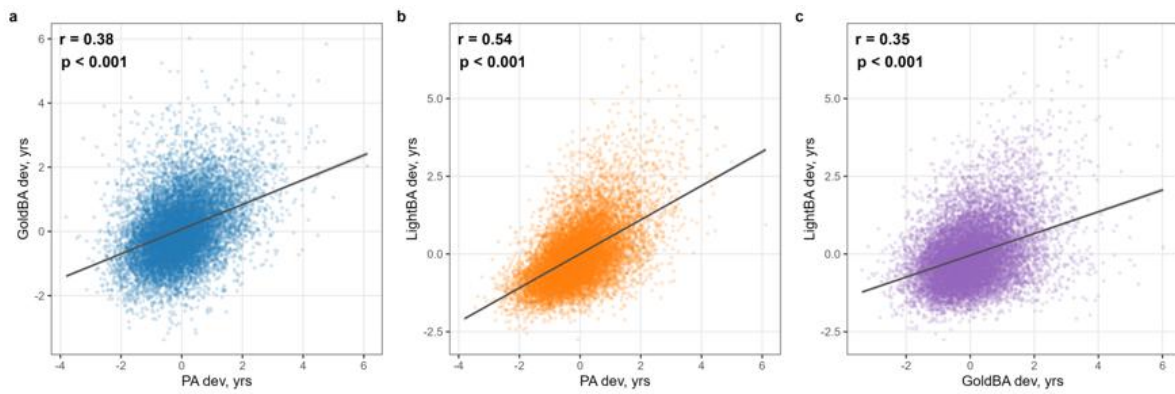
Supplementary Table 5. Nonlinear associations between iron exposure and biological aging stratified by the presence of chronic disease

Exposure and outcome	<i>p-int</i> ^a	Without chronic disease						With at least one chronic disease					
		n	<i>p-ov</i> [†]	<i>p-nl</i> [†]	Min x (β)	Max x (β)	ZeroCross x	n	<i>p-ov</i>	<i>p-nl</i>	Min x (β)	Max x (β)	ZeroCross x
Total iron intake													
GoldBA deviation	0.6	9449	< 0.001	< 0.001	28.52 (-0.12)	14.23 (0)	10.59;14.02	3835	< 0.01	<0.01	29.36 (-0.1)	15.65 (0.01)	8.56;17.02
LightBA deviation	0.16	9449	0.260	0.310	16.19 (0)	30.16 (0.02)	18.34	3835	0.250	0.160	26.04 (-0.03)	26.04 (-0.03)	39.39
PA deviation	0.02	9449	< 0.01	0.020	21.29 (-0.02)	21.29 (-0.02)	32.25	3835	0.030	0.020	27.27 (-0.07)	14.28 (0)	11.52;14.14
Dietary iron intake													
GoldBA deviation	0.95	0.020	0.110	27.30	-0.06 (5.77)			3126	0.001	0.030	10.02 (-0.06)	14.09 (0.01)	7.90;15.44
LightBA deviation	0.44	0.910	0.840	14.46	0 (6.27)	0.03 (16.61)		3126	0.680	0.670	14.17 (0)	25.64 (0.03)	15.67
PA deviation	0.24	0.140	0.890	27.30	-0.07 (5.77)			3126	0.250	0.710	14.99 (0)	16.22 (0)	NA
Supplemental iron intake													
GoldBA deviation	0.44	1472	< 0.001	0.210	1 (-0.22)	65 (0.38)	NA	709	0.010	0.150	1 (-0.27)	65 (0.27)	NA
LightBA deviation	0.06	1472	0.360	0.150	38.22 (0.03)	38.22 (0.03)	NA	709	0.030	0.160	1 (-0.23)	65 (0.24)	NA
PA deviation	0.06	1472	0.030	0.070	1 (-0.16)	65 (0.16)	NA	709	< 0.001	0.020	1 (-0.36)	65 (0.34)	NA
Serum iron													
GoldBA deviation	< 0.001	9418	< 0.001	< 0.001	22.34 (-0.04)	15.43 (0.01)	10.85;16.42	3824	< 0.001	< 0.001	22.32 (-0.18)	22.32 (-0.18)	NA
LightBA deviation	< 0.001	9418	< 0.001	<0.01	24.85 (-0.08)	24.85 (-0.08)	NA	3824	< 0.001	< 0.001	22.89 (-0.14)	22.89 (-0.14)	NA
PA deviation	< 0.01	9418	< 0.001	< 0.001	15.94 (-0.02)	10.32 (0.13)	17.78	3824	< 0.001	<0.01	19.49 (-0.07)	19.49 (-0.07)	NA

[†]p-int, p-interaction; p-ov, p-overall; p-nl, p-nonlinear.

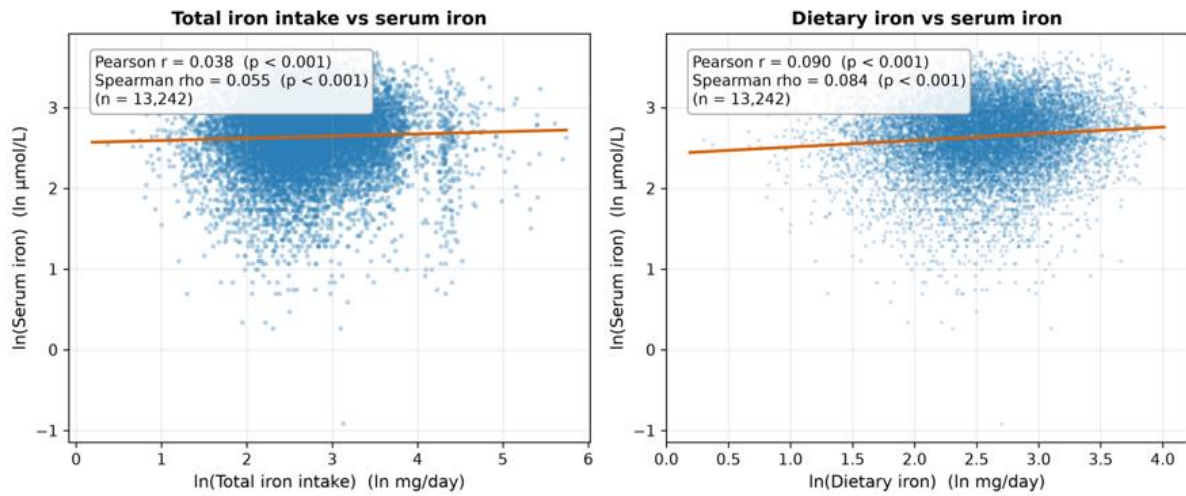


Supplementary Figure 1. Inter-correlations between CA, PA, GoldBA, and LightBA



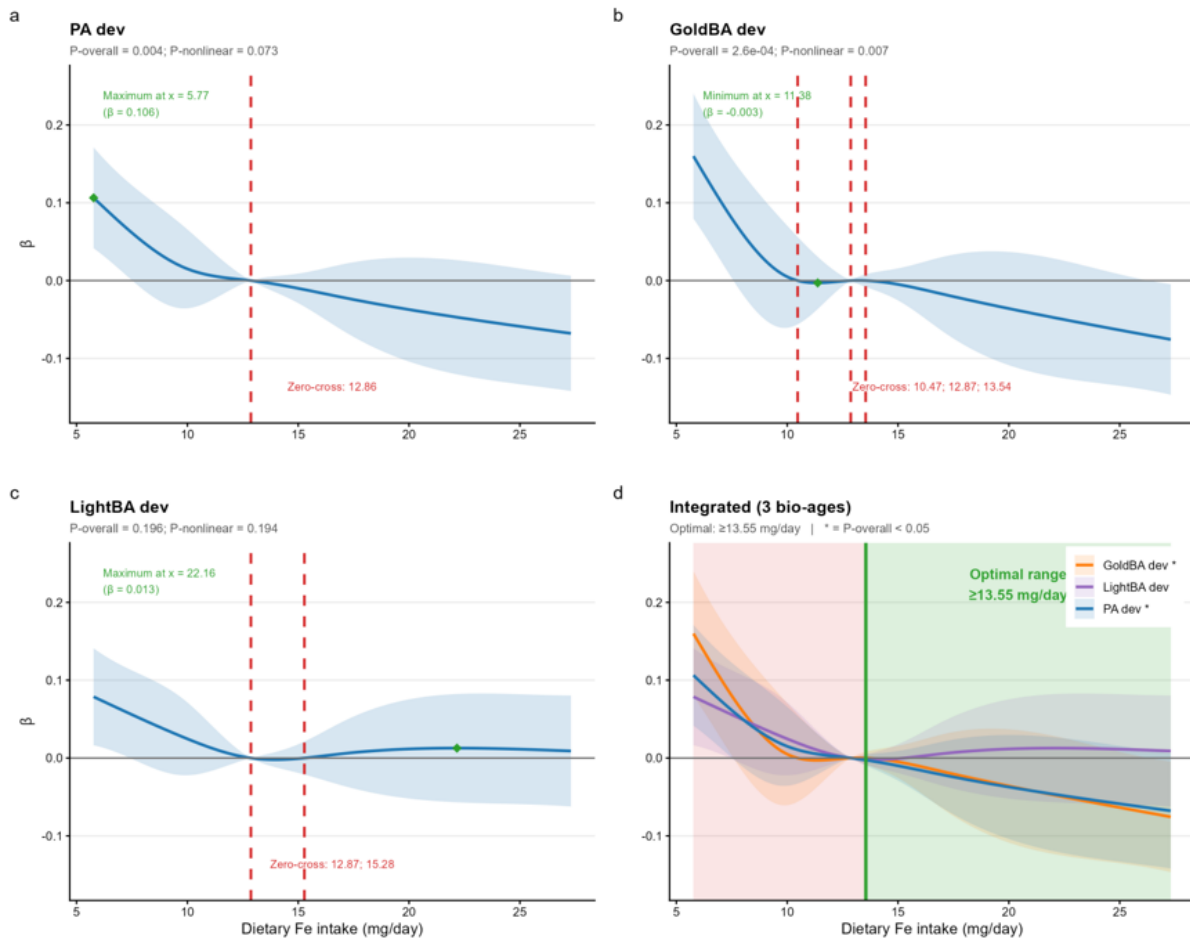
Supplementary Figure 2. Inter-correlations between deviations of PA, GoldBA, and LightBA

Association between iron intake and serum iron (NHANES)

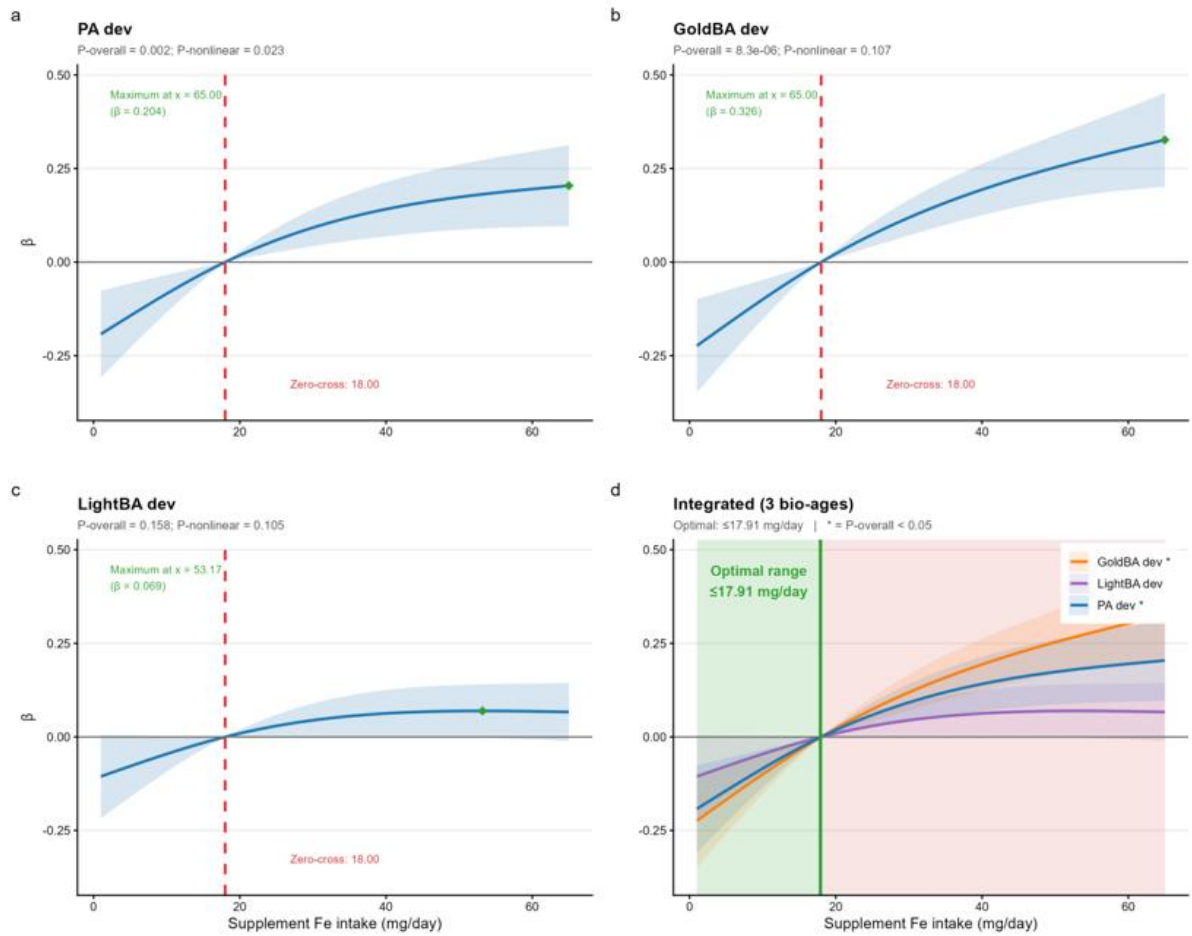


Supplementary Figure 3. Correlations between total and dietary iron intake and serum iron levels

Dietary Fe intake vs biological age deviation [No supplement]



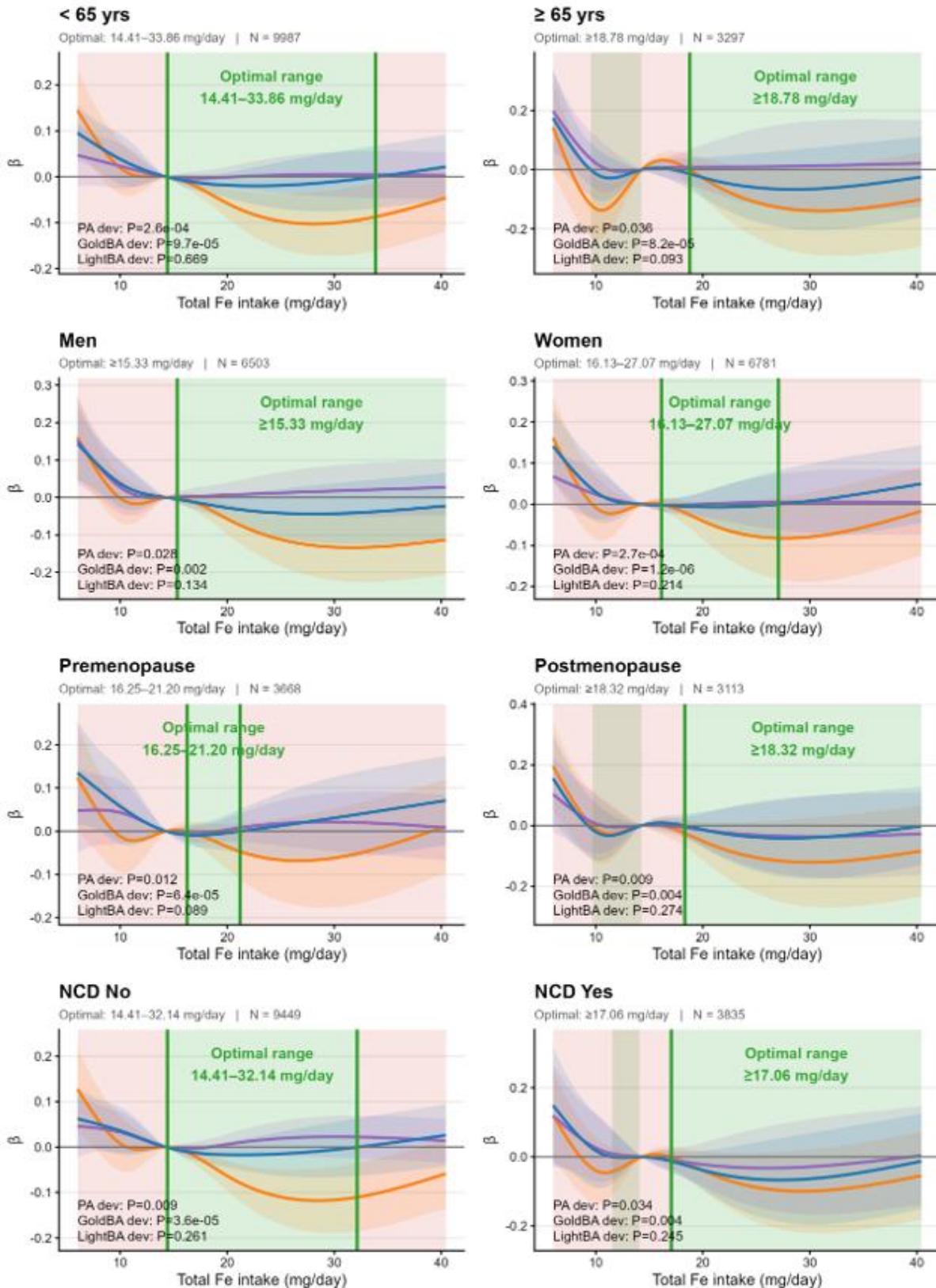
Supplementary Figure 4. The restricted cubic spline (RCS) regression and optimal range of dietary iron intake over deviations of PA, GoldBA, and LightBA in the NHANES participants ($n = 13284$). Models adjusted for sex, ethnicity, education levels, poverty income ratio, BMI (kg/m^2), cigarette smoking, alcohol consumption, physical activity levels, the presence of chronic diseases, energy intake, AHEI, and supplemental iron intake



Supplementary Figure 5. The RCS regression and optimal range of supplemental iron intake over deviations of PA, GlodBA, and LightBA in the NHANES participants taking iron supplements (n = 2,181). Models adjusted for for sex, ethnicity, education levels, poverty income ratio, BMI (kg/m2), cigarette smoking, alcohol consumption, physical activity levels , the presence of chronic diseases, energy intake, AHEI, and dietary iron intake

Total Fe intake - Optimal range by subgroup (integrated 3 bio-ages)

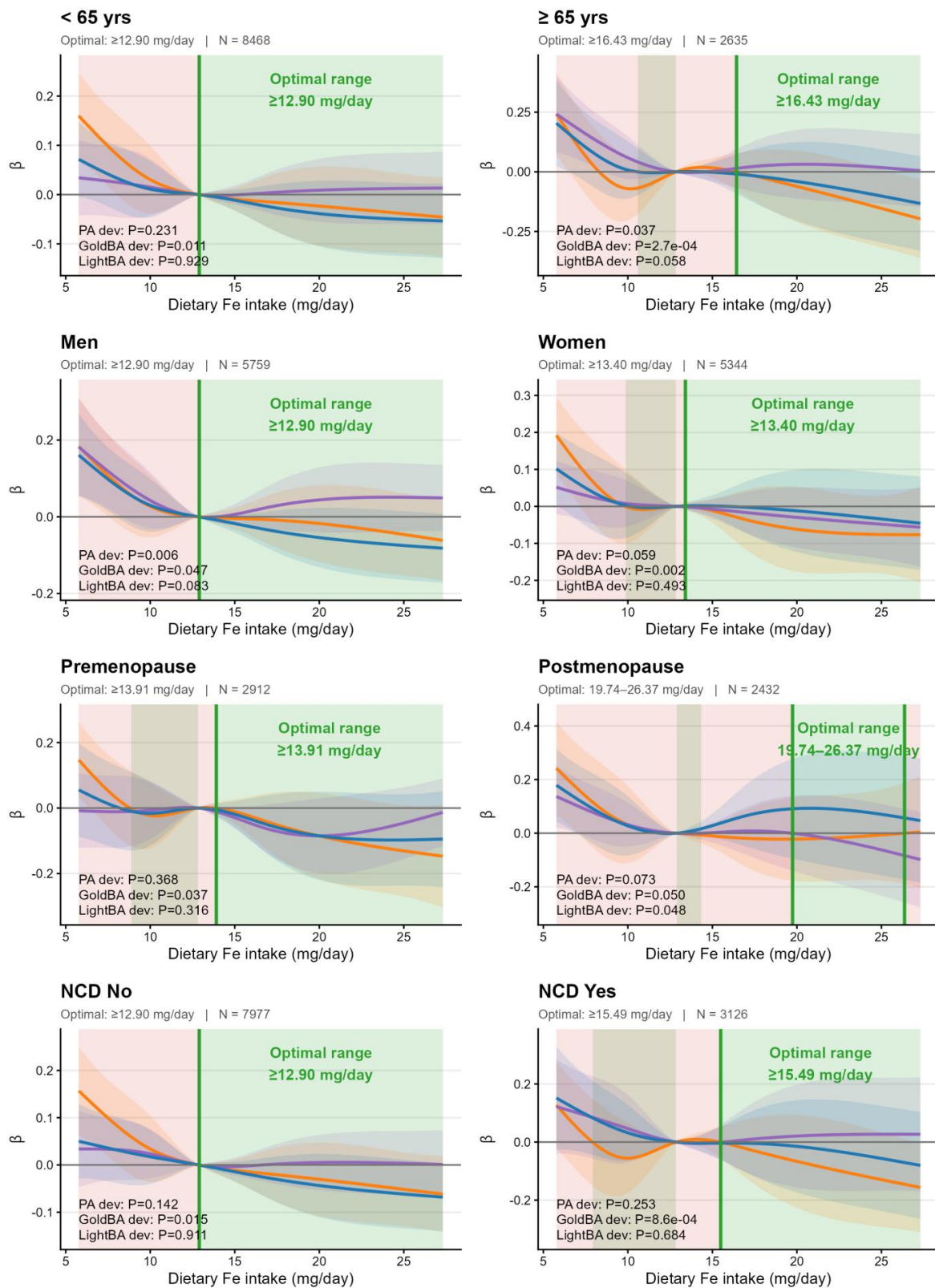
Restricted cubic spline, Model 4, beta centered at overall median exposure. Green = optimal range (all P-overall-significant outcomes have $\beta < 0$); red = outside



Supplementary Figure 6. The optimal ranges of total iron intake in subpopulations of the NHANES participants

Dietary Fe intake - Optimal range by subgroup (integrated 3 bio-ages)

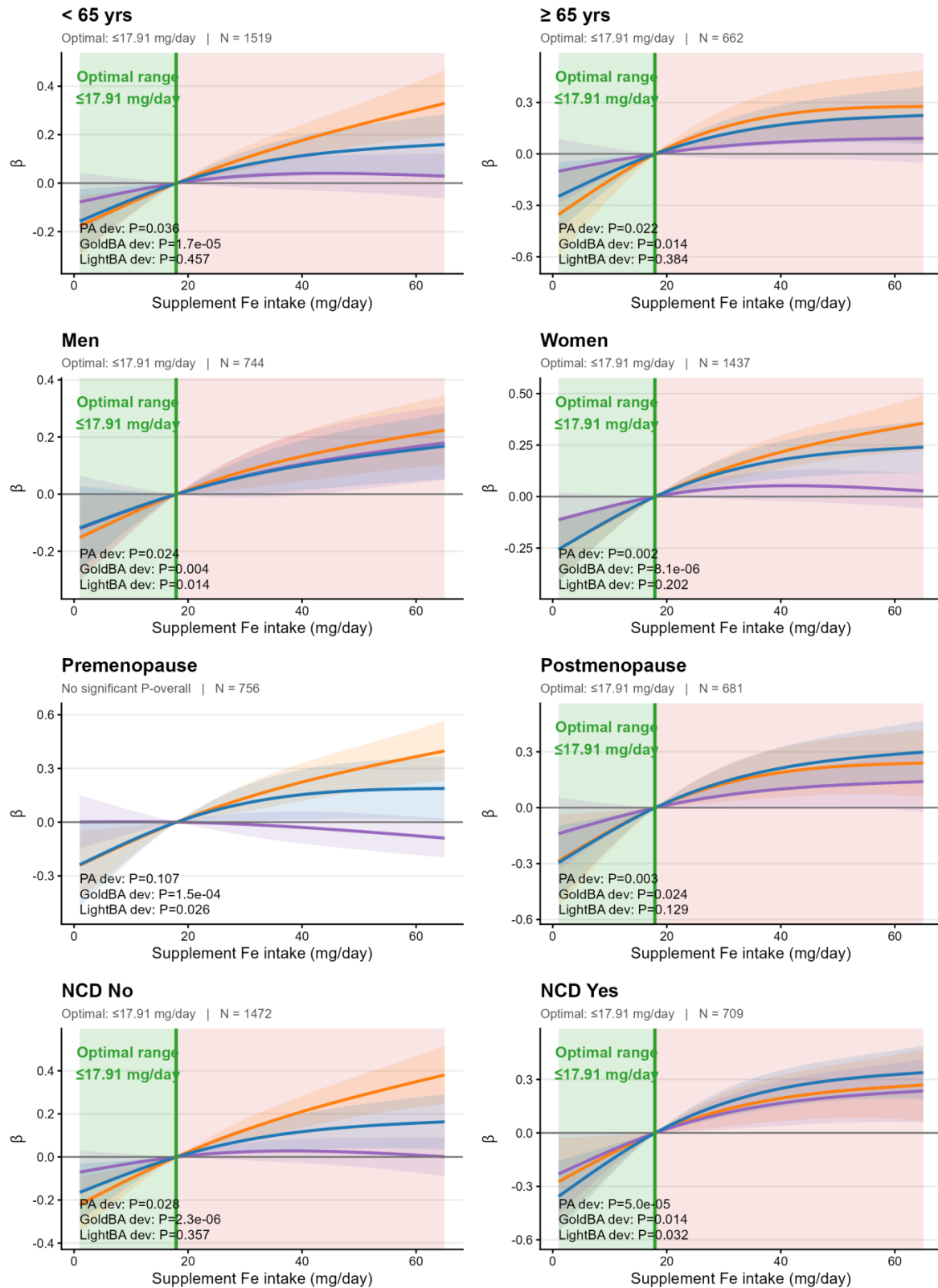
Restricted cubic spline, Model 4. beta centered at overall median exposure. Green = optimal range (all P-overall-significant outcomes have beta<0); red = outside



Supplementary Figure 7. The optimal ranges of dietary iron intake in subpopulations of the NHANES participants

Supplement Fe intake - Optimal range by subgroup (integrated 3 bio-ages)

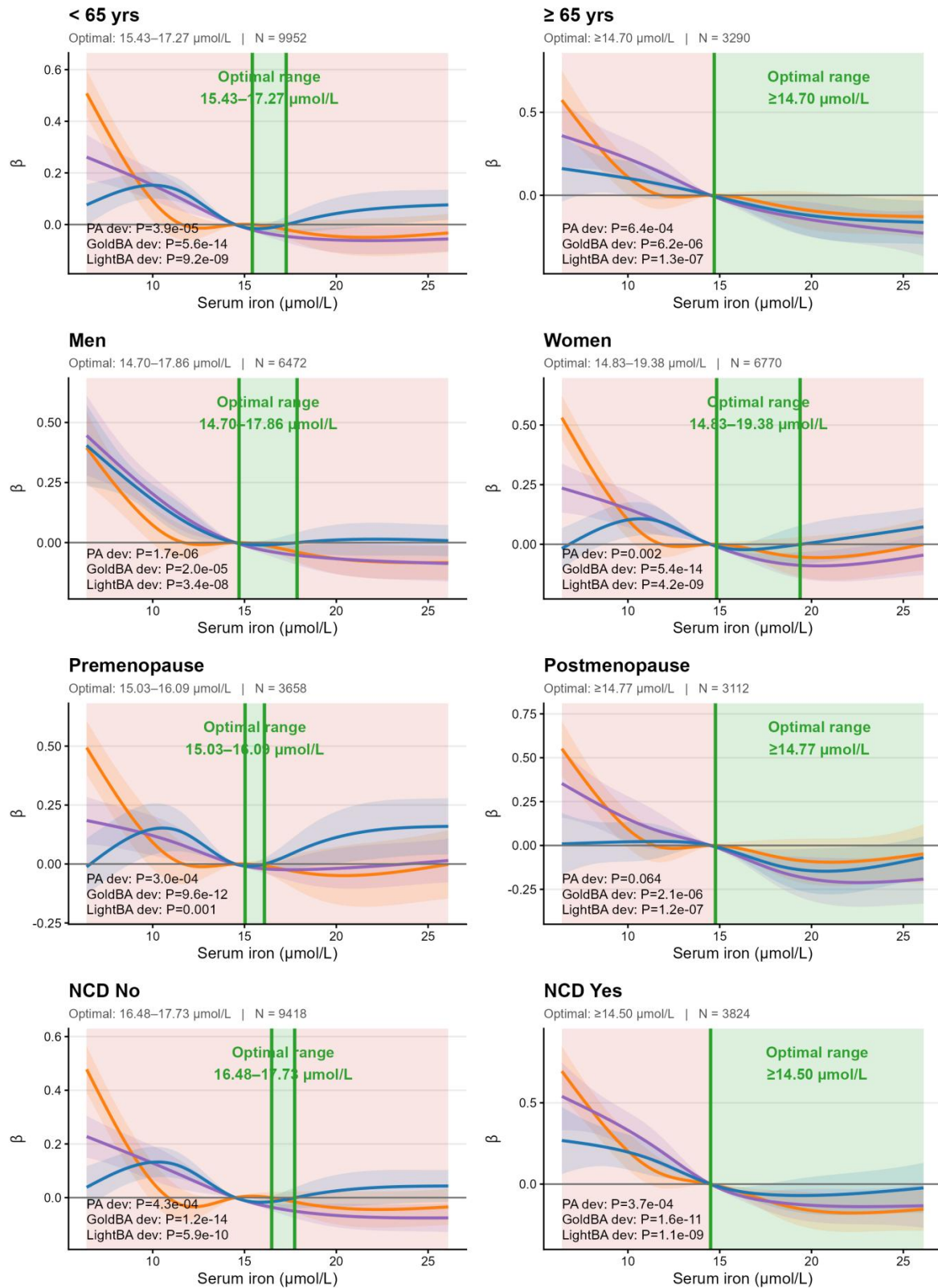
Restricted cubic spline, Model 4. beta centered at overall median exposure. Green = optimal range (all P-overall-significant outcomes have beta<0); red = outside



Supplementary Figure 8. The optimal ranges of supplemental iron intake in subpopulations of the NHANES participants

Serum iron - Optimal range by subgroup (integrated 3 bio-ages)

Restricted cubic spline, Model 4. beta centered at overall median exposure. Green = optimal range (all P-overall-significant outcomes have beta<0); red = outside



Supplementary Figure 9. The optimal ranges of serum iron concentrations in subpopulations of the NHANES participants