Supplementary materials for

"Patterns and determinants of exhaled nitric oxide trajectories in schoolchildren over a 7-year period"

Contents

eMethods: Additional details on the methods

Measurement of Exhaled Nitric Oxide

In years 5 and 6 five 50mL/s eNO samples were collected per subject and F_ENO_{50} was calculated as the mean of the 2-3 most reproducible plateau estimates within 10%. In years 8 and 10 three 50mL/s eNO samples were collected per subject as part of a multiple flow protocol and F_ENO_{50} was calculated as the mean of 2-3 plateau estimates within 15%.

Statistical analysis

The intra-class correlation of repeated measurements of F_ENO_{50} was calculated using the ratio of between-participant variance to the total variance from sex-specific linear mixed effects models of $\log F_ENO_{50}$ with only a random intercept for participant.

Model fit was assessed by the Bayesian Information Criterion (BIC) [1]. Generalized Additive Mixed Models (GAMMs) were estimated using restricted maximum likelihood except when reporting model fit according to the BIC [1] when we refit models using maximum likelihood.

To determine whether there was evidence for a joint smooth with age and year 3 BMI percentile (a continuous variable), we first fit models using a tensor product interaction, an ANOVA-like decomposition of the joint smooth. Given evidence of an interaction ($p<0.05$) in either sex, we then refit using the standard tensor product smooth which allows for more flexibility in the smooth surface. For categorical variables (race/ethnicity and year 3 ever reported allergic rhinitis), we fit a model with a single common smooth surface for age in addition to "difference" surfaces for age for each level of the categorical variable using the "by" variable approach in gamm().This parameterization of the model permitted testing whether the smooth for a given group was

different from that of the overall population smooth and allows for potentially only one complex smooth surface to be estimated [2]. We imposed a first order penalty (rather than a second order penalty) on the difference smooths for identifiability, encouraging shrinkage to the mean.

Table E1: Distribution of demographic characteristics in full study population and three subsets used in the analyses. N (%), unless otherwise specified.

	Full Study Population	Participants Not Missing Year 3 BMI	Participants Not Missing Year 3 F_ENO_{50}	Participants Not Missing Year 3 or Year 10 F_ENO_{50}
Participants	1791	1717	1429[a]	681[b]
Total observations	7185	6989	-	-
Observations per participant, median (IQR)	4 (2)	4 (2)	-	-
1	214 (11.9%)	195 (10.9%)		
2	163 (9.1%)	144 (8.0%)		
3	236 (13.2%)	223 (12.5%)		
4	344 (19.2%)	329 (18.4%)		
5	443 (24.7%)	435 (24.3%)		
6	391 (21.8%)	391 (21.8%)		
Sex				
Male	851 (47.5%)	809 (47.1%)	672 (47.0%)	318 (46.7%)
Female	940 (52.5%)	908 (52.9%)	757 (53.0%)	363 (53.3%)
Race				
Hispanic	1072 (59.9%)	1022 (59.5%)	835 (58.4%)	380 (55.8%)
White/non-Hispanic	525 (29.3%)	507 (29.5%)	445 (31.3%)	229 (33.6%)
Black	39 (2.2%)	39 (2.3%)	26 (1.8%)	10 (1.5%)
Asian	75 (4.2%)	73 (4.3%)	58 (4.1%)	25 (3.7%)
Other	71 (4.0%)	69 (4.0%)	60 (4.2%)	35 (5.1%)
Missing	9 (0.5%)	7 (0.4%)	5 (0.3%)	2 (0.3%)
Year 3 covariates				
F_ENO_{50}, ppb, median (IQR)	9.7 (7.6)	9.7 (7.6)	9.7 (7.6)	9.7 (7.5)
Missing*	362 (20.2%)	288 (16.8%)	0	0
BMI Percentile, median (IQR)	72.7 (48.7)	72.7 (48.7)	72.7 (48.7)	73.1 (47.4)
Missing*	362 (20.2%)	288 (16.8%)	0	0
Ever reported allergic rhinitis				
No	847 (47.3%)	847 (49.3%)	847 (59.3%)	418 (61.4%)
Yes	399 (22.3%)	399 (22.3%)	399 (27.9%)	178 (26.1%)
Missing*	545 (30.4%)	471 (27.4%)	183 (12.8%)	85 (12.5%)
Related tables/figures	Table E2, Figures 1-2	Tables 2-3, Figure 3	Tables 1a-1b [column 1]	Tables 1a-1b [column 2]

* Counts both participants without a year 3 visit and participants with a year 3 visit but who were missing this variable.

[a] Only year 3 data used.

[b] Only year 3 and year 10 data used.

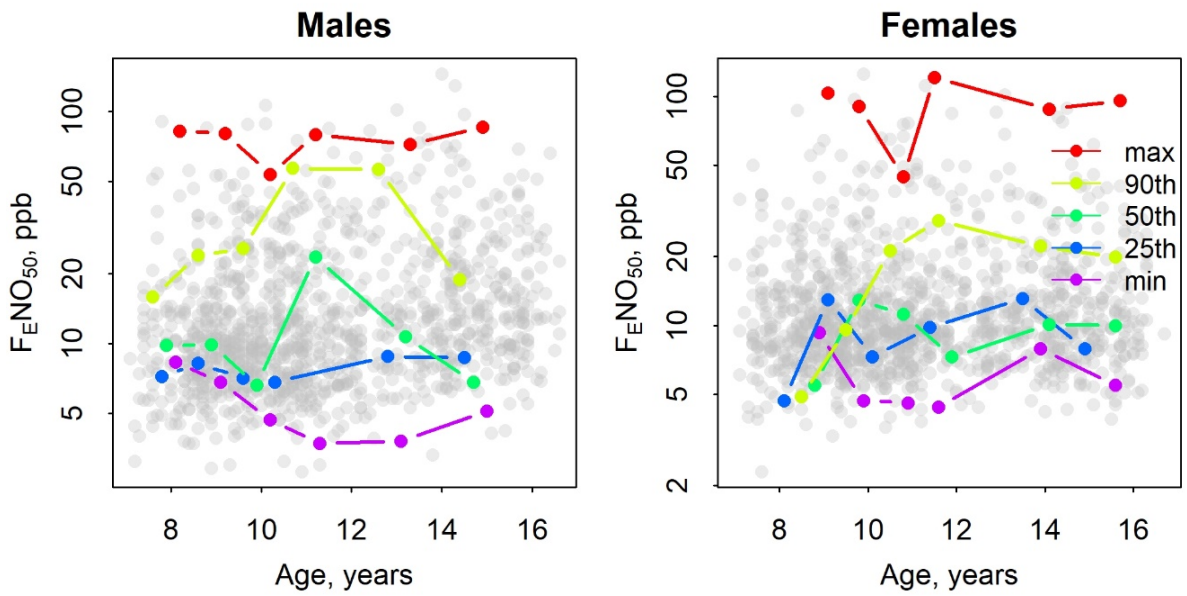

Figure E1. Selected F_ENO_{50} trajectories for participants with 6 measurements and whose median detrended residuals of logF_ENO_{50} on age were at selected sex-specific percentiles (min, 25th, 50th, 90th and max).

Table E2. Summary of fitted Generalized Additive Mixed Models presented in Figure 1 that model longitudinal logF_ENO_{50} trajectories as smooth functions of: time-varying age (centered at age 8) with a random intercept and slope on age at the participant-level, time-varying height (centered at 130 cm) with a random intercept and slope on height, and two different parameterizations of a joint smooth of age and height with a random intercept and slope on age.

		Males (N=851)			Females (N=940)		
Model	**Smooth term**	**EDF***	**p-value**	**BIC†**	**EDF***	**p-value**	**BIC†**
Age only	s(age)	5.7	<0.0001	5539	5.1	<0.0001	5688
Height only	s(height)	2.2	<0.0001	5544	3.8	<0.0001	5679
Age and height	te(age, height)	8.8	<0.0001	5554	7.9	<0.0001	5701
	ti(age)	1.0	0.08	5566	1.0	0.93	5713
	ti(height)	1.0	0.33		1.0	0.05	
	ti(age,height)	6.5	0.001		4.7	0.0002	

* Effective degrees of freedom (EDF) for the smooth term; a value of 1 indicates a linear effect, higher values indicate a higher degree of non-linearity

† Bayesian Information Criterion (BIC)

Table E3. Bayesian Information Criteria (BIC) for fitted Generalized Additive Mixed Models presented in Table 3 that model longitudinal log fractional exhaled nitric oxide ($logF_ENO_{50}$) trajectories as smooth functions of time-varying age and a time-constant covariate plus a participant-level random intercept and random slope on age. Model fit is measured by the BIC.

	BIC	
Model	**Males (N=809)**	**Females (N=908)**
Age only	5338	5580
Age and year 3 BMI%		
Using a tensor product interaction for BMI%	5358	5601
Using a tensor product smooth for BMI%	5369	5616
Age and race/ethnicity	5367	5616
Age and ever reported allergic rhinitis at year 3	5367	5608
Full model		
Using a tensor product interaction for BMI%	5368	5615
Using a tensor product smooth for BMI%	5379	5630

Table E4. Predicted geometric mean F_ENO_{50} (and 95% CI) at ages 8 and 15, as well as the percent difference from age 8 to age 15, from the sex-specific model with only a smooth function of age and for specific covariate combinations from the sex-specific "final" models including a joint smooth of age and year 3 BMI percentile and adjustments for race/ethnicity and allergic rhinitis status at year 3.

Sex	Model	Year 3 child characteristics (BMI%, Race/Ethnicity, Allergic rhinitis)	Age 8, Geo. Mean	Age 15, Geo. Mean	Percent Difference
Male	s(age) only model		10.6 (10.1, 11.2)	16.5 (15.4,17.6)	54.9 (44.8, 65.8)
	Final model	15%, Hispanic, No	10.5 (9.3, 11.9)	18.9 (16.5, 21.7)	80.0 (58.5, 104.4)
		15%, White/non-Hispanic, No	9.5 (8.4, 10.8)	17.1 (14.9, 19.7)	80.0 (58.5, 104.4)
		15%, Other, No	12.0 (10.1, 14.2)	21.6 (18.0, 25.8)	80.0 (58.5, 104.4)
		15%, Hispanic, Yes	12.2 (10.7, 13.9)	21.9 (19.0, 25.4)	80.0 (58.5, 104.4)
		15%, White/non-Hispanic, Yes	11.0 (9.7, 12.6)	19.8 (17.2, 22.9)	80.0 (58.5, 104.4)
		15%, Other, Yes	13.9 (11.7, 16.5)	25.0 (20.8, 30.0)	80.0 (58.5, 104.4)
		95%, Hispanic, No	10.4 (9.5, 11.2)	15.0 (13.7, 16.5)	45.1 (33.5, 57.7)
		95%, White/non-Hispanic, No	9.4 (8.5, 10.3)	13.6 (12.2, 15.1)	45.1 (33.5, 57.7)
		95%, Other, No	11.8 (10.2, 13.7)	17.1 (14.7, 20.0)	45.1 (33.5, 57.7)
		95%, Hispanic, Yes	12.0 (10.9, 13.3)	17.4 (15.6, 19.4)	45.1 (33.5, 57.7)
		95%, White/non-Hispanic, Yes	10.8 (9.7, 12.1)	15.7 (14.0, 17.7)	45.1 (33.5, 57.7)
		95%, Other, Yes	13.6 (11.7, 15.9)	19.8 (16.9, 23.3)	45.1 (33.5, 57.7)
Female	s(age) only model		10.7 (10.2, 11.2)	12.6 (11.9, 13.4)	17.8 (11.0, 25.0)
	Final model	15%, Hispanic, No	10.1 (9.1, 11.1)	13.2 (11.7, 14.9)	31.0 (17.3, 46.3)
		15%, White/non-Hispanic, No	9.4 (8.5, 10.4)	12.3 (10.9, 14.0)	31.0 (17.3, 46.3)
		15%, Other, No	10.6 (9.3, 12.0)	13.9 (12.0, 16.1)	31.0 (17.3, 46.3)
		15%, Hispanic, Yes	11.7 (10.4, 13.0)	15.3 (13.4, 17.4)	31.0 (17.3, 46.3)
		15%, White/non-Hispanic, Yes	10.9 (9.7, 12.2)	14.3 (12.5, 16.3)	31.0 (17.3, 46.3)
		15%, Other, Yes	12.3 (10.7, 14.0)	16.1 (13.8, 18.7)	31.0 (17.3, 46.3)
		95%, Hispanic, No	10.8 (10.0, 11.6)	12.2 (11.2, 13.4)	13.7 (4.9, 23.3)
		95%, White/non-Hispanic, No	10.1 (9.2, 11.0)	11.4 (10.3, 12.7)	13.7 (4.9, 23.3)
		95%, Other, No	11.3 (10.1, 12.8)	12.9 (11.3, 14.7)	13.7 (4.9, 23.3)
		95%, Hispanic, Yes	12.5 (11.4, 13.7)	14.2 (12.8, 15.7)	13.7 (4.9, 23.3)
		95%, White/non-Hispanic, Yes	11.6 (10.5, 12.9)	13.2 (11.8, 14.9)	13.7 (4.9, 23.3)
		95%, Other, Yes	13.1 (11.6, 14.9)	14.9 (13.0, 17.1)	13.7 (4.9, 23.3)

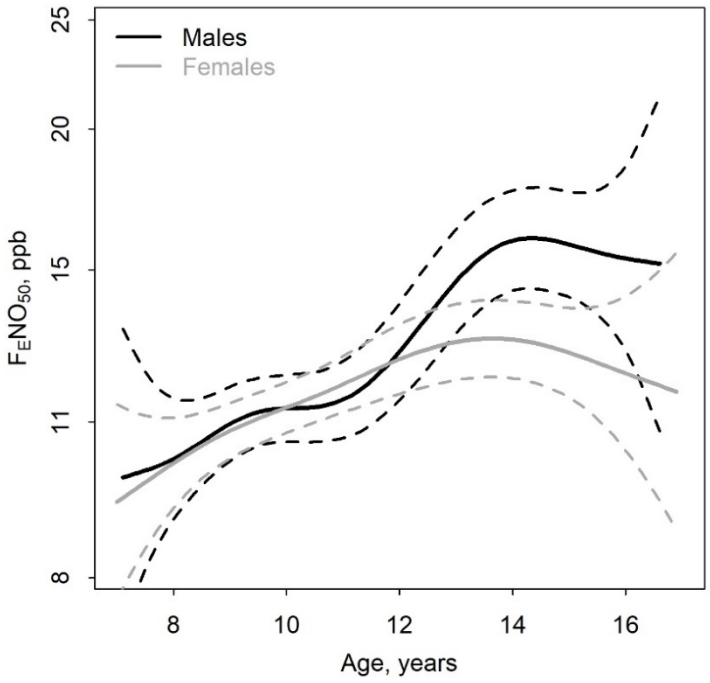

Figure E2. Population average sex-specific trajectories of F_ENO_{50} (solid lines) and 95% Confidence Intervals (dashed lines) in subset of participants with data on age at peak height velocity (N=288 males, N=323 females) modeled as a smooth function of age only, for males (black) or females (grey). Analyses performed using logF_ENO_{50} with results here presented using back-transformed values on an axis scaled using natural log.

Table E5. Predicted geometric mean F_ENO_{50} (and 95% CI) at selected ages, from the sex-specific model with a joint smooth of age and year 3 BMI percentile by age at peak height velocity (dichotomized based on sex-specific median) and adjustments for race/ethnicity and allergic rhinitis status at year 3.

	Age at Peak Height Velocity			
	Early		Late	
Age (yrs)	**Male**	**Female**	**Male**	**Female**
9	10.3 (8.9, 11.8)	10.2 (8.9, 11.6)	10.1 (8.8, 11.5)	10.3 (9.2, 11.5)
10	11.2 (9.8, 12.9)	10.7 (9.4, 12.2)	10.6 (9.3, 12.1)	10.9 (9.7, 12.1)
12	13.4 (11.6, 15.4)	11.7 (10.1, 13.5)	11.9 (10.4, 13.7)	11.8 (10.4, 13.3)
14	15.9 (13.6, 18.6)	11.9 (10.2, 13.9)	13.6 (11.8, 15.7)	13.6 (11.9, 15.4)
16	18.9 (15.8, 22.7)	11.8 (9.7, 14.2)	14.6 (12.3, 17.4)	12.1 (10.2, 14.3)

Table E6. Summary of fitted Generalized Additive Mixed Models that model longitudinal logF_ENO_{50} trajectories as smooth functions of time-varying **height** and a time-constant covariate. Model fit is measured by the Bayesian Information Criterion (BIC).

		Males (N=809)				Females (N=908)			
Model	**Parameter***	**Regression coefficient ****	**EDF†**	**p-value**	**BIC**	**Regression coefficient ****	**EDF†**	**p-value**	**BIC**
Height only	s(height)		2.0	<0.0001	5339		3.7	<0.0001	5571
Height and year 3 BMI %	te(height, bmi)		5.5	<0.0001	5350		6.2	<0.0001	5595
	ti(height)		1.9	<0.0001	5366		3.4	<0.0001	5610
	ti(bmi)		1.0	0.0008			1.0	0.89	
	ti(height, bmi)		1.0	0.03			1.0	0.78	
Height and race/ ethnicity‡	Intercept	2.55		<0.0001	5370	2.50		<0.0001	5607
	White/non-Hispanic	-0.08		0.08		-0.07		0.11	
	Other	0.15		0.04		0.06		0.33	
	s(height)		1.3	<0.0001			3.5	<0.0001	
	s(height:Hispanic)		<0.1	0.31			1.2	0.15	
	s(height:White/non-Hisp)		1.4	0.18			<0.1	0.99	
	s(height:Other)		<0.1	0.72			<0.1	0.36	
Height and ever reported allergic rhinitis at year 3	Intercept	2.50		<0.0001	5366	2.45		<0.0001	5596
	Allergic Rhinitis	0.15		0.001		0.15		0.0002	
	Missing	-0.01		0.92		-0.03		0.63	
	s(height)		1.0	<0.0001			3.7	<0.0001	
	s(height:None)		2.5	0.04			<0.1	0.99	
	s(height:AllergicRhinitis)		<0.1	0.61			<0.1	0.91	
	s(height: Missing)		1.5	0.12			<0.1	0.56	
Full model	Intercept	2.53		<0.0001	5360	2.47		<0.0001	5606
	White/non-Hispanic	-0.11		0.01		-0.08		0.05	
	Other	0.12		0.10		0.04		0.45	
	Allergic Rhinitis	0.15		0.001		0.16		0.0002	
	Missing	-0.01		0.87		-0.04		0.52	
	te(height, bmi)		5.0	<0.0001			6.3	<0.0001	
	Intercept	2.53		<0.0001	5376	2.47		<0.0001	5621
	White/non-Hispanic	-0.11		0.01		-0.08		0.05	
	Other	0.12		0.10		0.04		0.46	
	Allergic Rhinitis	0.14		0.001		0.16		0.0002	
	Missing	-0.01		0.87		-0.04		0.52	
	ti(height)		1.9	<0.0001			3.4	<0.0001	
	ti(bmi)		1.0	0.0005			1.0	0.87	
	ti(height, bmi)		1.2	0.03			1.0	0.71	

* Smooth terms definitions: s(): penalized regression spline; ti():tensor product interaction; te():tensor product smooth.

** For categorical variables; on logF_ENO_{50} scale

† Effective degrees of freedom (EDF) for the smooth term; a value of 1 indicates a linear effect, higher values indicate a higher degree of non-linearity

‡ k=5 specified in the s(age, by=race, m=1, k=5) term for improved model convergence

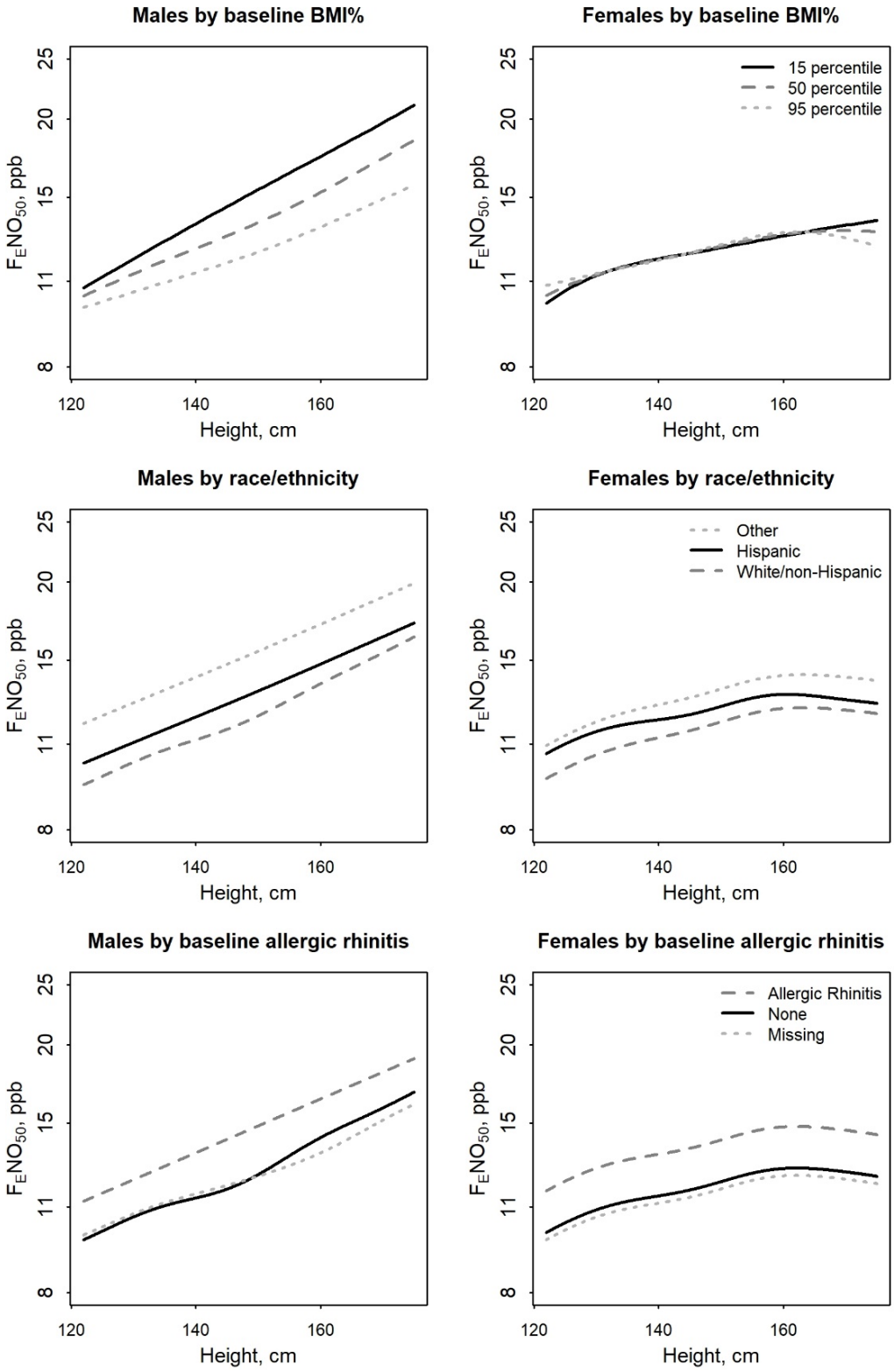

Figure E3. Modeled sex-specific trajectory of F_ENO_{50} as a joint smooth of **height** and year 3 BMI percentile or as smooths of **height** that vary by race/ethnicity or ever reported allergic rhinitis at year 3.

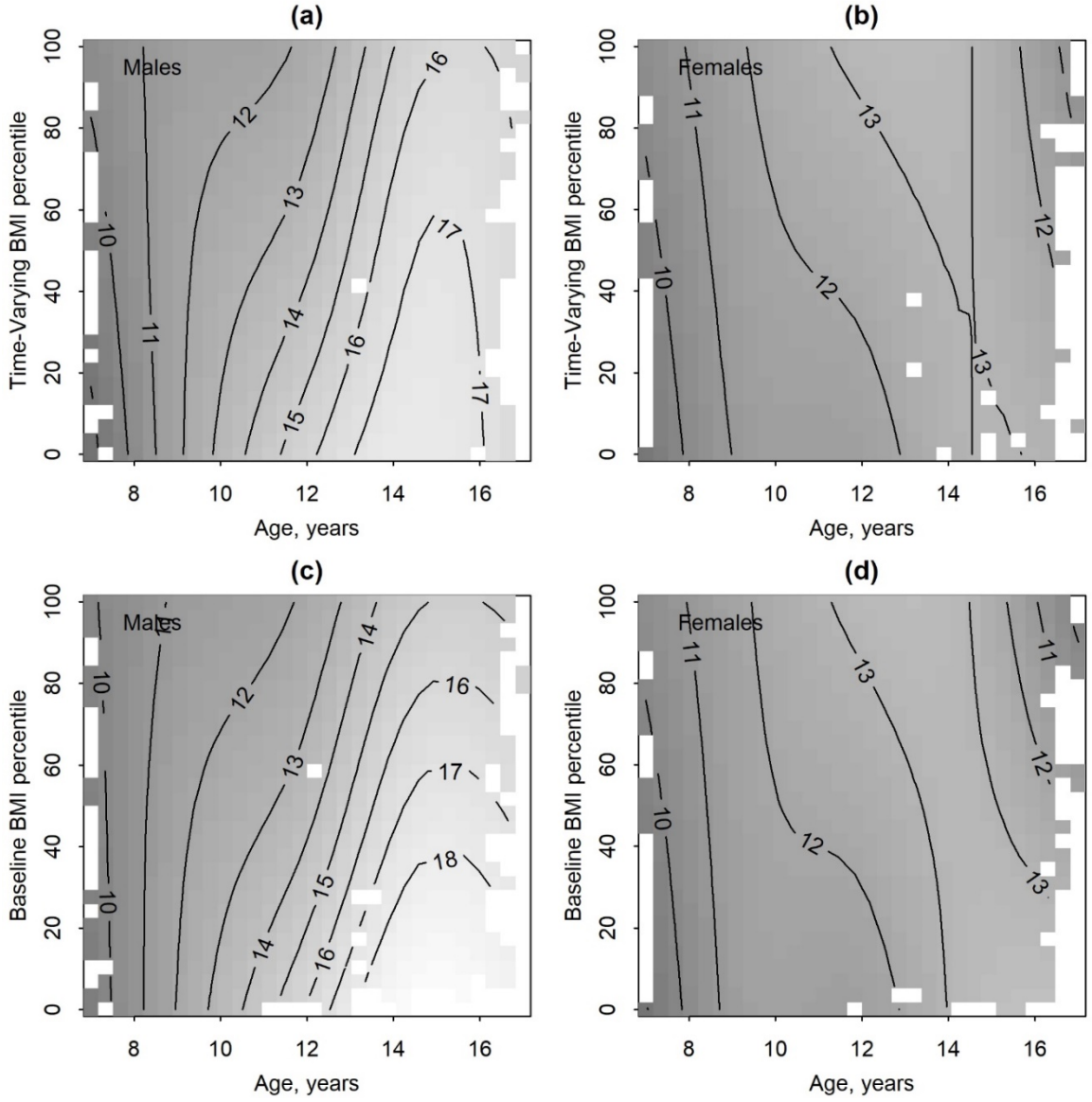

Figure E4. Modeled sex-specific trajectory of F_ENO_{50} as a joint smooth of age and time-varying BMI percentile for males (a) or females (b) (N=851 males, N=940 females), or a joint smooth of age and year 3 BMI percentile for males (c) or females (d) (N=809 males, N=908 females). The contour lines labels refer to predicted geometric mean F_ENO_{50} in ppb. Analyses performed using $\log F_ENO_{50}$ with results here presented using back-transformed values on an axis scaled using natural log.

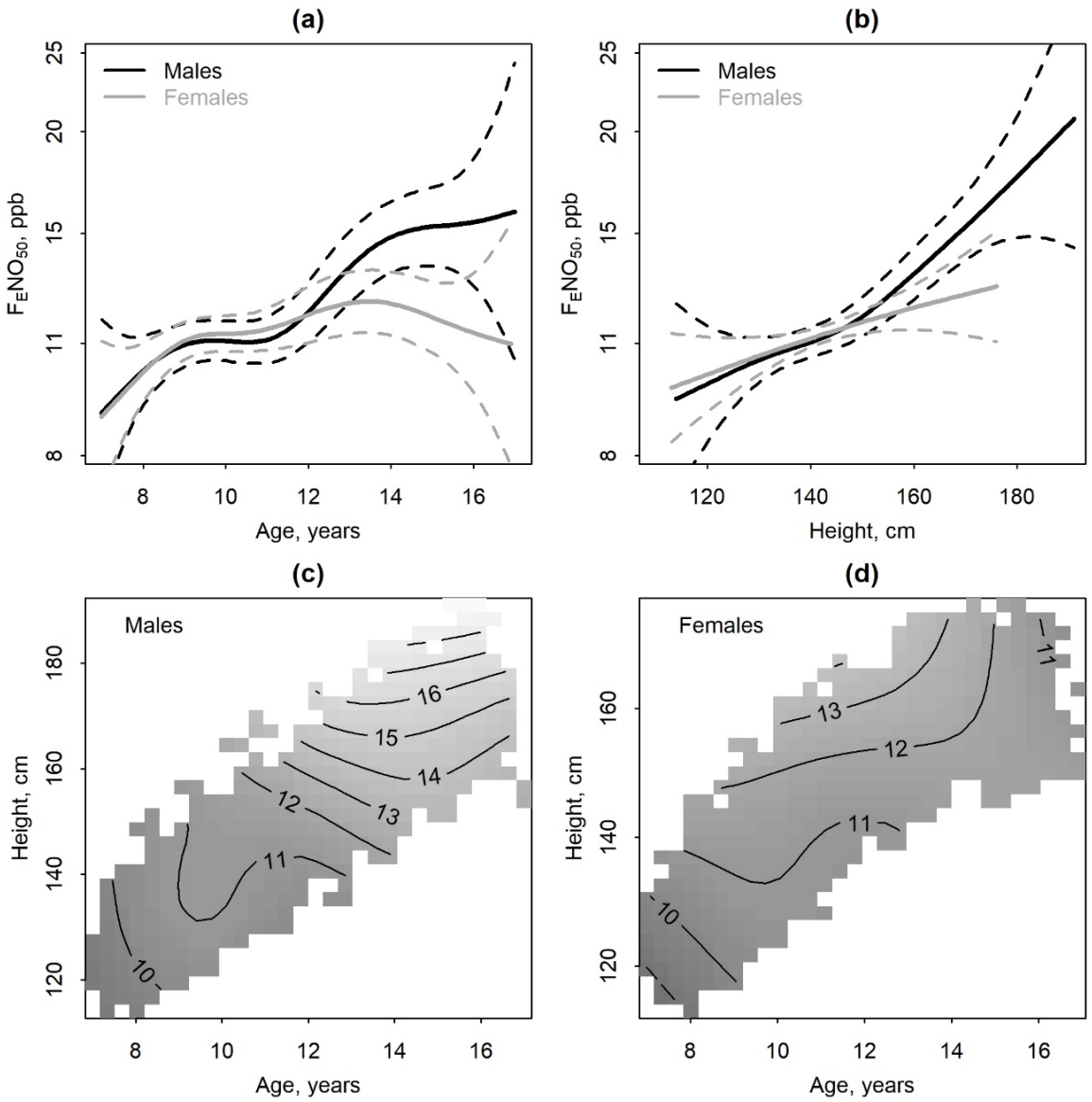

Figure E5. Modeled sex-specific trajectory of F_ENO_{50} as a: (a) smooth function of age only*, (b) a smooth function of height only*, or a joint smooth of age and height for males (c) or females (d) among participants who never reported allergic rhinitis through year 10 of the study (N=512 males, N=550 females).

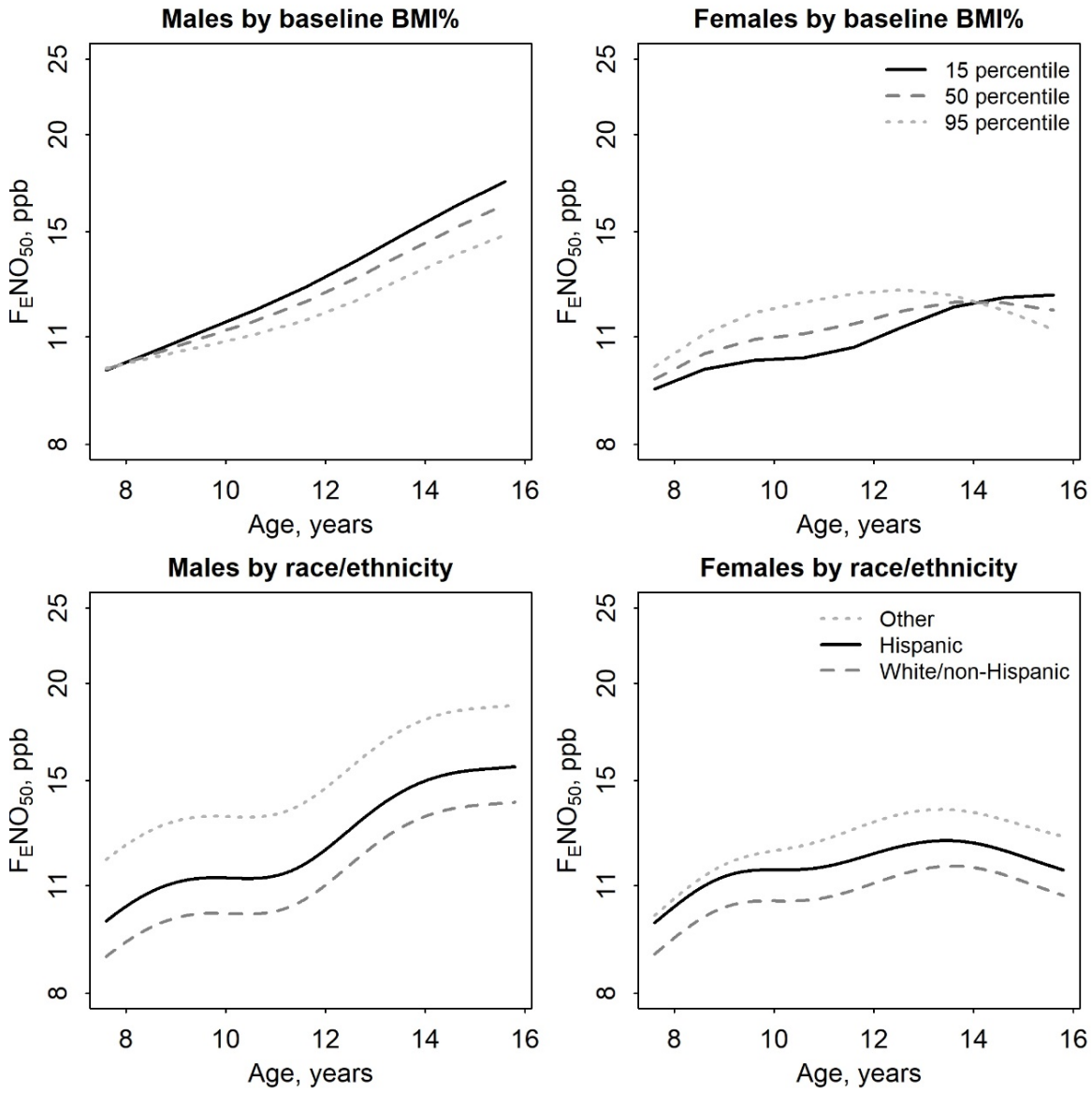

Figure E6. Modeled sex-specific trajectory of F_ENO_{50} as a joint smooth of age and year 3 BMI percentile or as smooths of age that vary by race/ethnicity among participants who never reported allergic rhinitis through year 10 of the study and without missing year 3 BMI percentile (N=485 males, N=527 females).

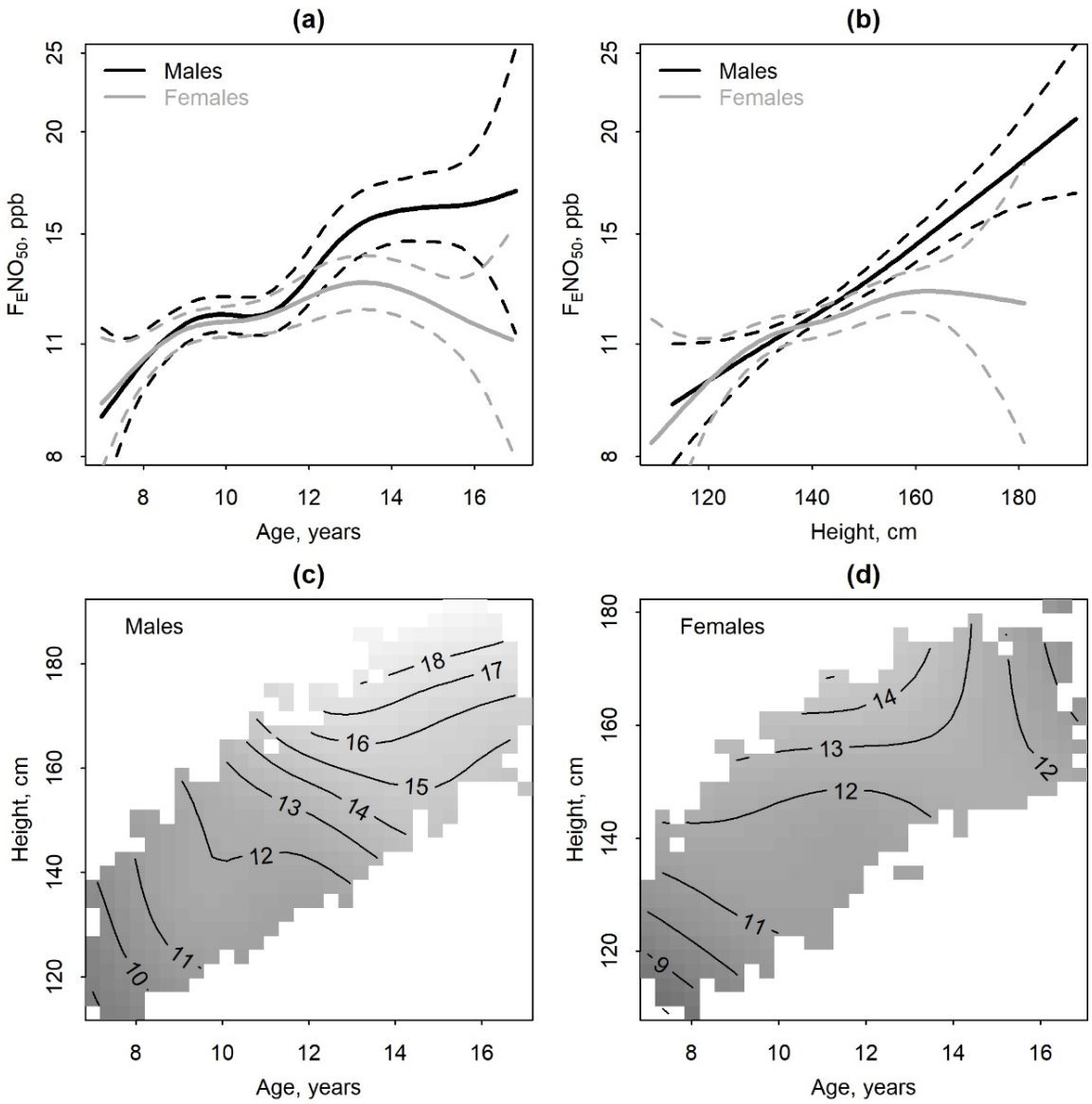

Figure E7. Modeled sex-specific trajectory of F_ENO_{50} as a: (a) smooth function of age only*, (b) a smooth function of height only*, or a joint smooth of age and height for males (c) or females (d) among participants who never reported wheeze or whistling in the chest through year 10 of the study (N=735 males, N=791 females).

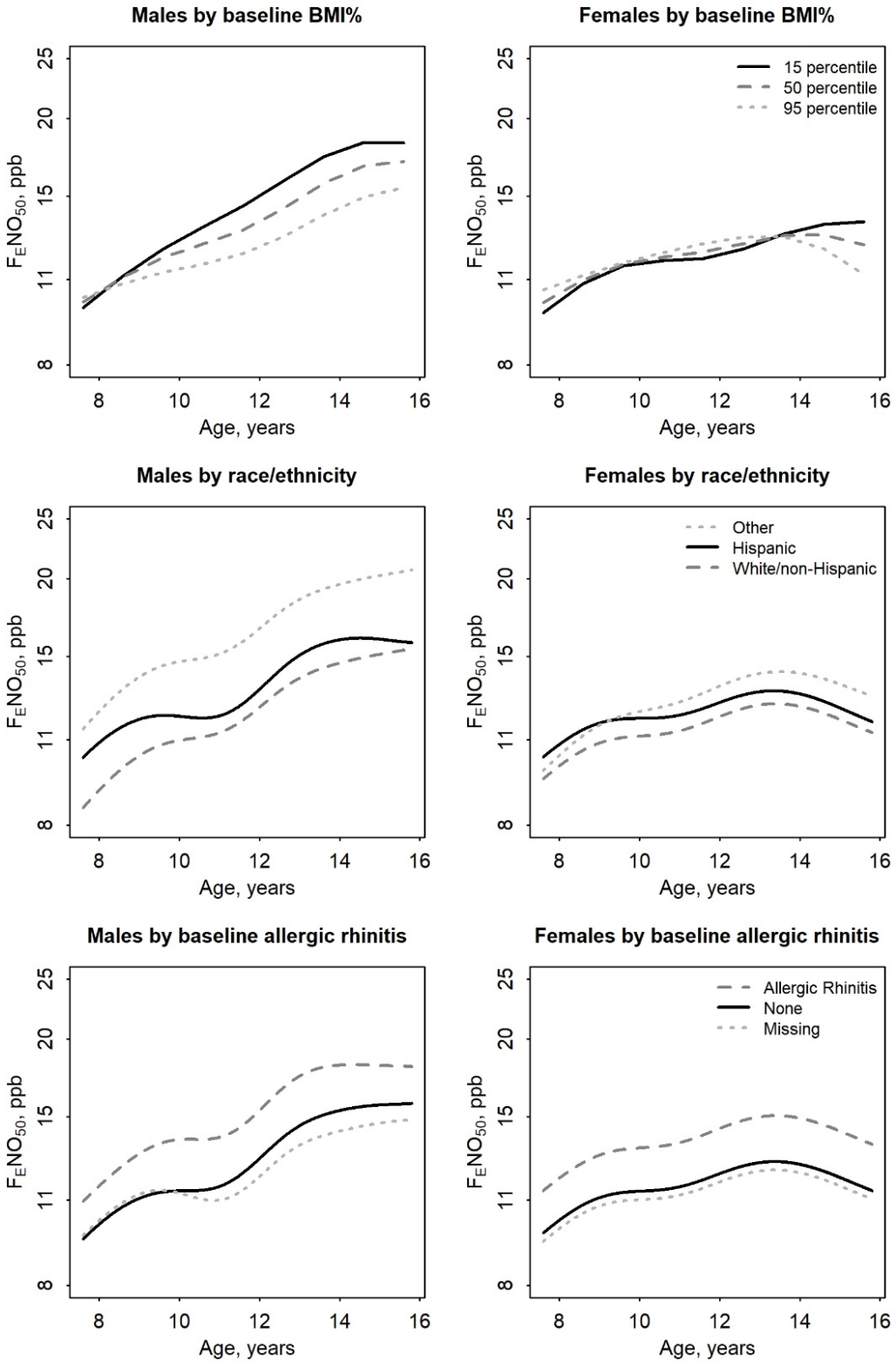

Figure E8. Modeled sex-specific trajectory of F_ENO_{50} as a joint smooth of age and year 3 BMI percentile or as smooths of age that vary by race/ethnicity among participants who never reported wheeze or whistling in the chest through year 10 of the study and without missing year 3 BMI percentile (N=697 males, N=768 females).

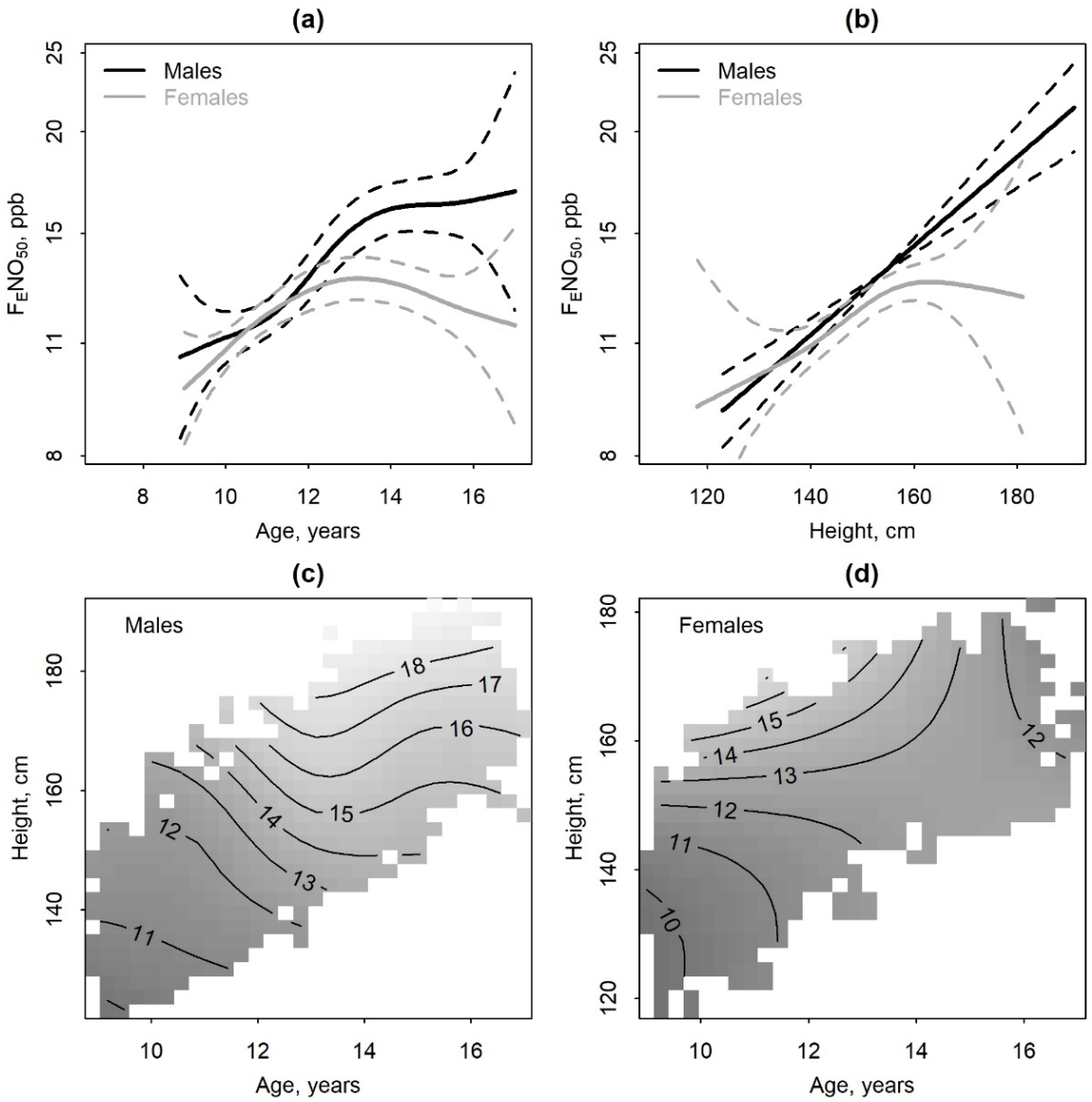

Figure E9. Modeled sex-specific trajectory of F_ENO_{50} as a: (a) smooth function of age only*, (b) a smooth function of height only*, or a joint smooth of age and height for males (c) or females (d) restricting to online F_ENO_{50} measurements (N=740 males, N=818 females).

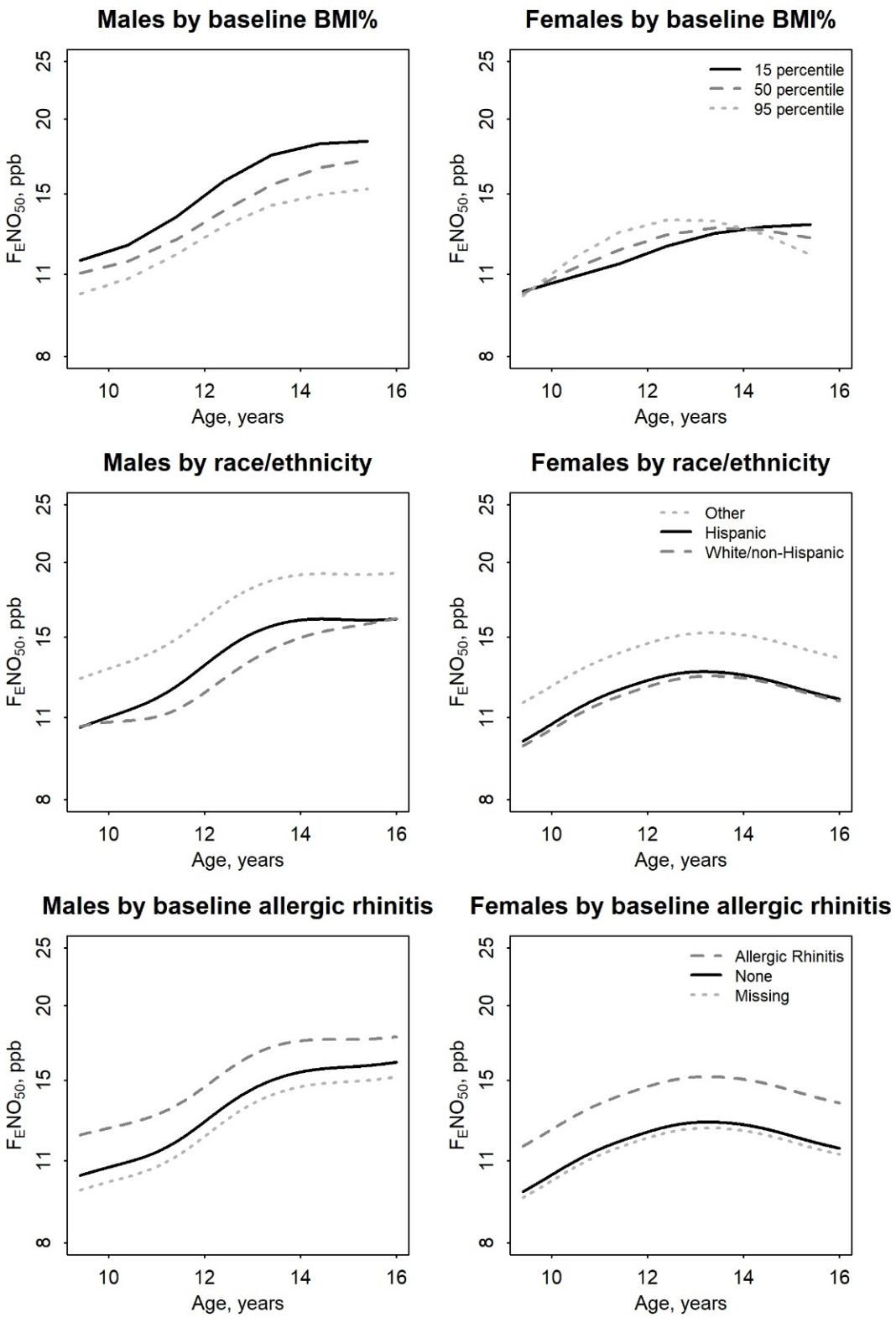

Figure E10. Modeled sex-specific trajectory of F_ENO_{50} as a joint smooth of age and year 3 BMI percentile or as smooths of age that vary by race/ethnicity among participants without missing year 3 BMI percentile and restricting to online F_ENO_{50} measurements (N=700 males, N=788 females).

Supplementary references

1. Gurka MJ. Selecting the best linear mixed model under REML. *The American Statistician* 2006: 60(1): 19-26.
2. Wood SN. Generalized additive models: an introduction with R. CRC press, 2017.