Appendix

Heart Rate Variability Coefficient of Variation During Sleep as a Digital Biomarker That Reflects Behavior and Varies by Age and Sex

Running head: HRV Coefficient of Variation as a Digital Biomarker

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Table S1. Sleep-derived HRV (ms) percentiles by age and biological sex

Percentile (%)	20–29 yrs	30–39 yrs	40–49 yrs	50–59 yrs	60–69 yrs	70–79 yrs
Females						
90	112.7	89.1	71.4	59.7	56.7	67.6
80	93.8	72.9	58.0	49.8	47.2	52.5
70	82.7	63.9	50.8	43.8	41.5	42.4
60	73.5	56.3	45.0	39.9	37.8	38.6
50	65.0	50.1	40.1	36.6	34.7	35.2
40	57.4	45.4	36.9	33.5	31.7	32.5
30	50.1	40.3	33.2	30.3	29.0	29.7
20	43.3	35.6	29.7	27.6	26.3	27.2
10	37.0	30.1	25.4	24.2	22.9	23.2
Males						
90	109.4	90.8	70.5	58.2	61.8	85.1
80	94.3	75.4	57.7	48.3	47.9	60.5
70	84.0	66.9	51.5	42.9	41.0	50.1
60	76.3	59.9	46.5	38.6	37.0	43.5
50	68.9	53.9	41.4	35.1	33.7	38.6
40	61.1	48.9	37.4	32.0	30.9	34.3
30	55.1	43.3	33.8	29.6	28.2	30.4
20	49.0	38.0	30.4	26.4	25.2	27.3
10	40.6	32.1	25.5	22.7	22.2	23.3

HRV was computed as a seven-day rolling coefficient of variation of nocturnal HRV and then averaged per user across 13 weeks. Percentiles (10th-90th) were calculated within each age-sex stratum from these user-level averages using continuous data from 10,561 females and 11,338 males.

Table S2. Sleep-derived HRV-CV (%) percentiles by age and biological sex

Percentile (%)	20-29 yrs	30-39 yrs	40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
Females						
90	25.3	24.0	23.9	23.2	23.8	26.2
80	21.6	20.9	20.3	20.1	20.6	21.9
70	19.4	18.7	18.6	18.3	18.8	19.2
60	17.9	17.3	17.3	17.1	17.4	17.7
50	16.2	15.9	16.1	16.0	16.2	16.4
40	15.0	14.7	14.9	14.9	15.1	15.3
30	13.7	13.4	13.8	13.9	14.2	14.4
20	12.2	12.3	12.4	12.7	13.1	13.0
10	10.4	10.6	11.1	11.4	11.8	11.6
Males						
90	27.5	26.6	27.1	26.6	28.0	29.3
80	23.2	22.8	22.9	23.2	23.7	24.3
70	20.3	20.1	20.5	21.0	21.5	21.8
60	18.0	18.2	18.7	19.1	19.7	20.0
50	16.2	16.6	17.3	17.8	18.2	18.4
40	14.7	15.3	15.9	16.6	17.0	16.8
30	13.2	14.0	14.6	15.5	15.7	15.5
20	11.8	12.5	13.2	14.1	14.3	14.1
10	9.9	10.6	11.6	12.6	12.7	12.4

HRV-CV was computed as a seven-day rolling coefficient of variation of nocturnal HRV and then averaged per user across 13 weeks. Percentiles (10th-90th) were calculated within each age-sex stratum from these user-level averages using continuous data from 10,561 females and 11,338 males.

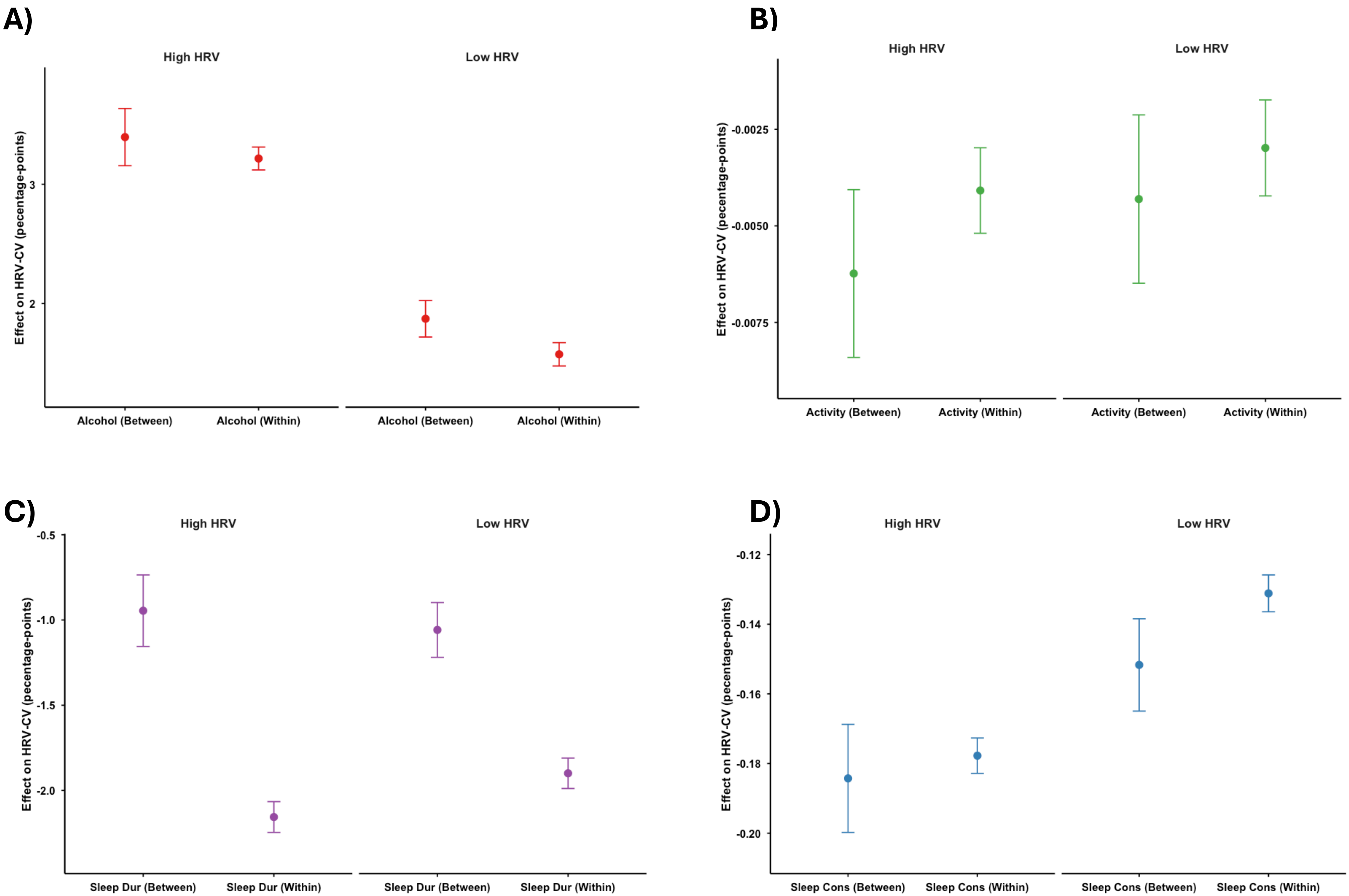


Figure S1. Associations between weekly averages of behavioral predictors and HRV-CV from multi-level linear mixed-effects models stratified by sample-median HRV (i.e., High HRV and Low HRV; 42.9 ms). Point estimates represent covariate-adjusted fixed effects from separate linear mixed-effects models predicting HRV-CV from weekly averages of daily alcohol use (number of drinks; **A**), physical activity (AU; **B**), sleep duration (hours; **C**), and sleep consistency (percentage-points; **D**). Each model controlled for age, sex, strap generation, and BMI, included random intercepts for participant, and decomposed predictors into between- and within-person effects. To avoid conflating nonresponse with no alcohol, alcohol models included two covariates: the weekly alcohol-item completion proportion and a binary indicator for whether any alcohol responses were provided that week. Error bars represent 95% confidence intervals.

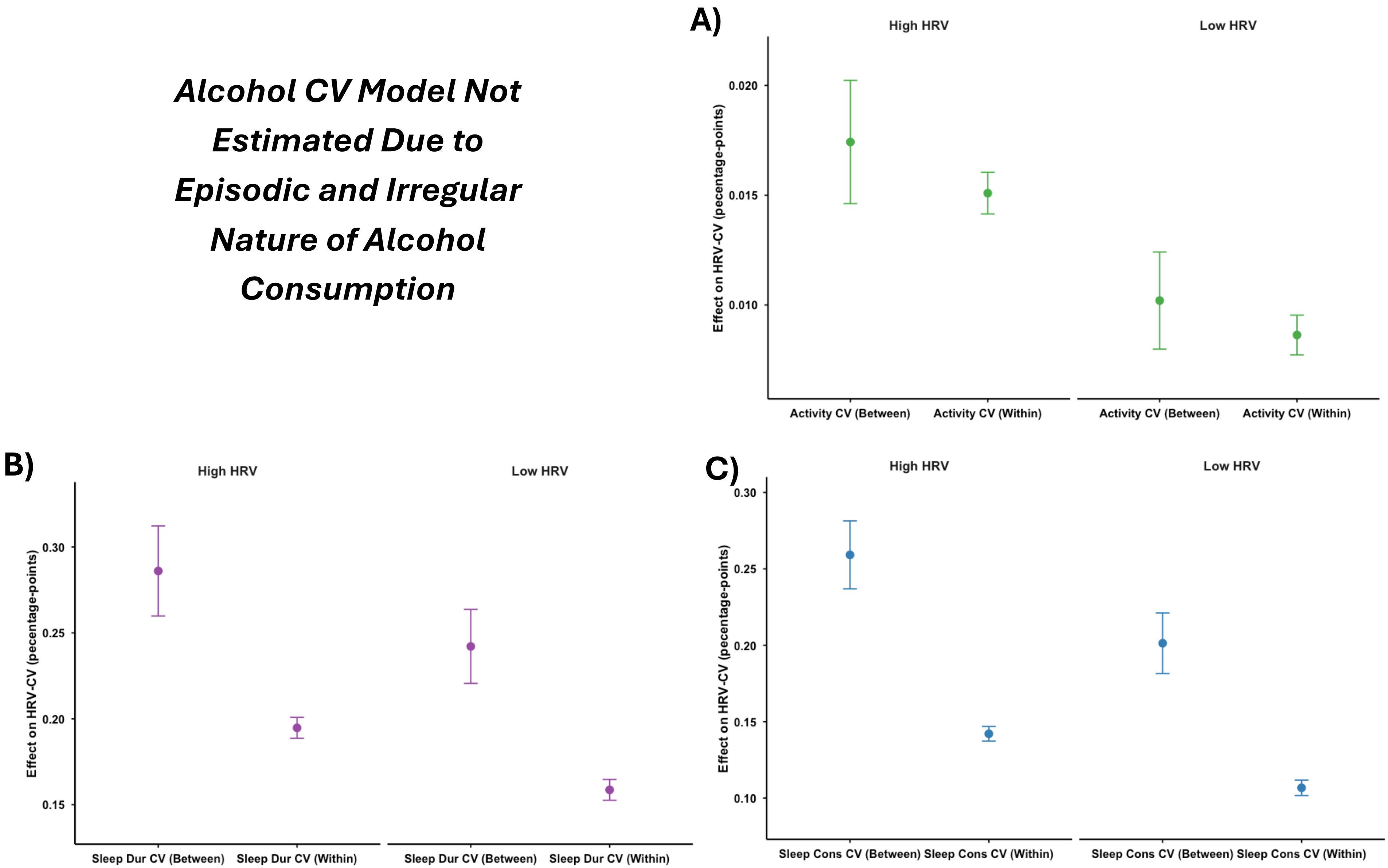


Figure S2. Associations between variability (CV) in behavioral predictors with HRV-CV from multi-level linear mixed-effects models stratified by sample-median HRV (i.e., High HRV and Low HRV; 42.9 ms). Point estimates represent covariate-adjusted fixed effects from separate linear mixed-effects models predicting HRV-CV from the CV of physical activity (**A**), sleep duration (**B**), and sleep consistency (**C**). Each model controlled for age, biological sex, strap generation, and BMI, included random intercepts for participant, and decomposed predictors into between- and within-person effects. To avoid conflating nonresponse with no alcohol, alcohol models included two covariates: the weekly alcohol-item completion proportion and a binary indicator for whether any alcohol responses were provided that week. Error bars show 95% confidence intervals. Variability in alcohol use (CV) was not examined due to the episodic and irregular nature of drinking.

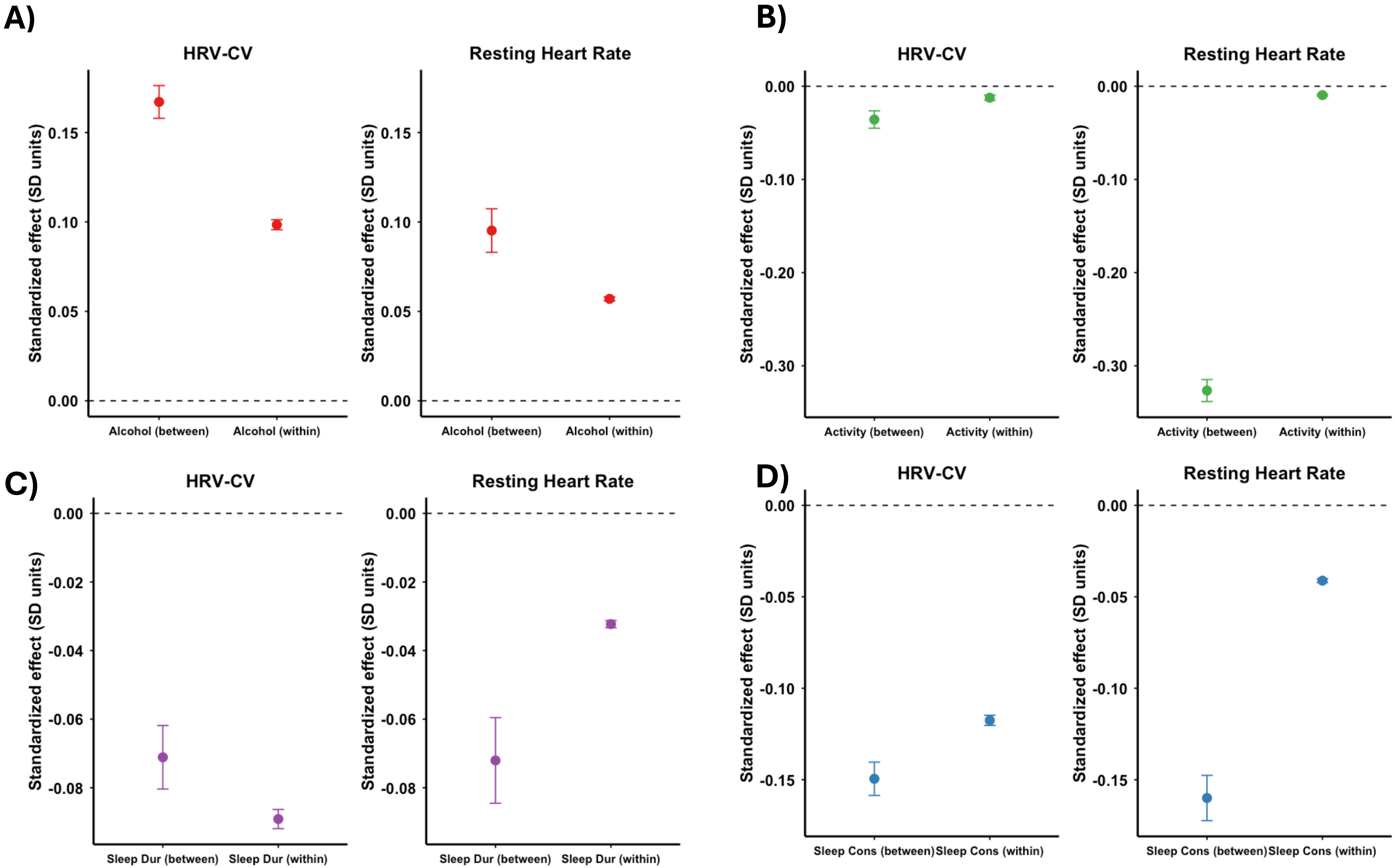
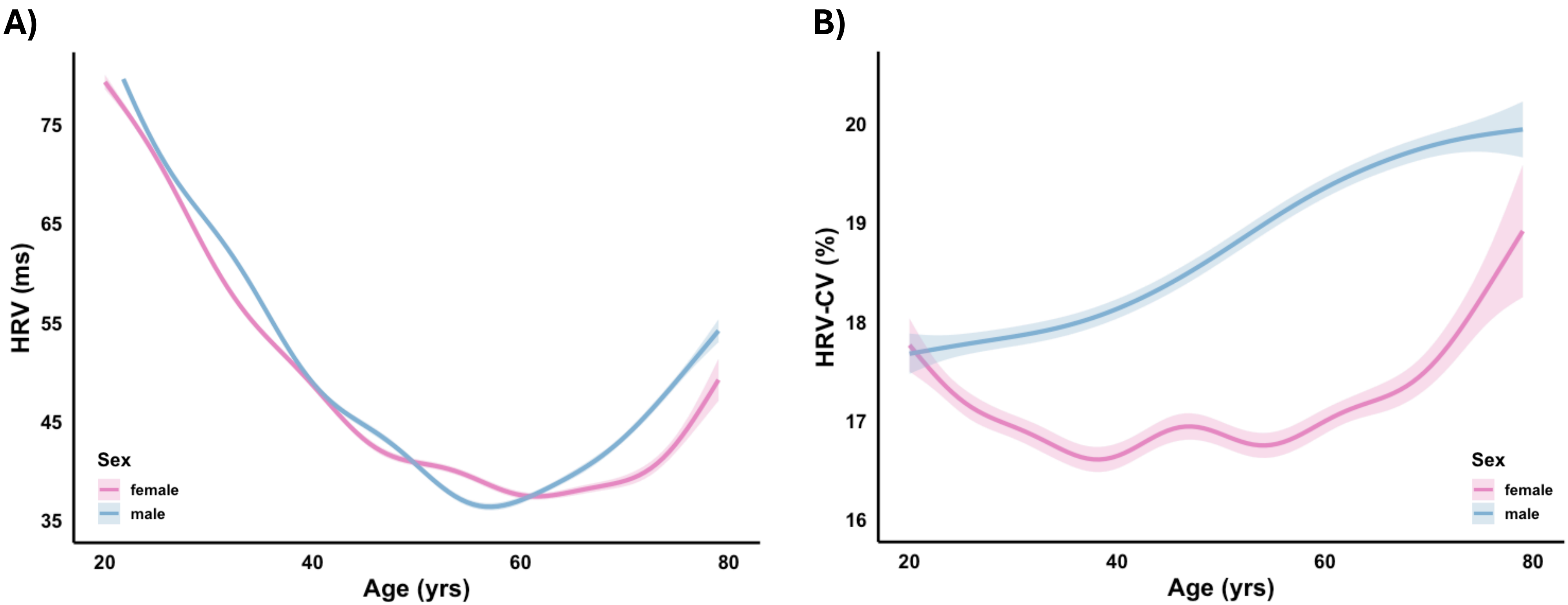


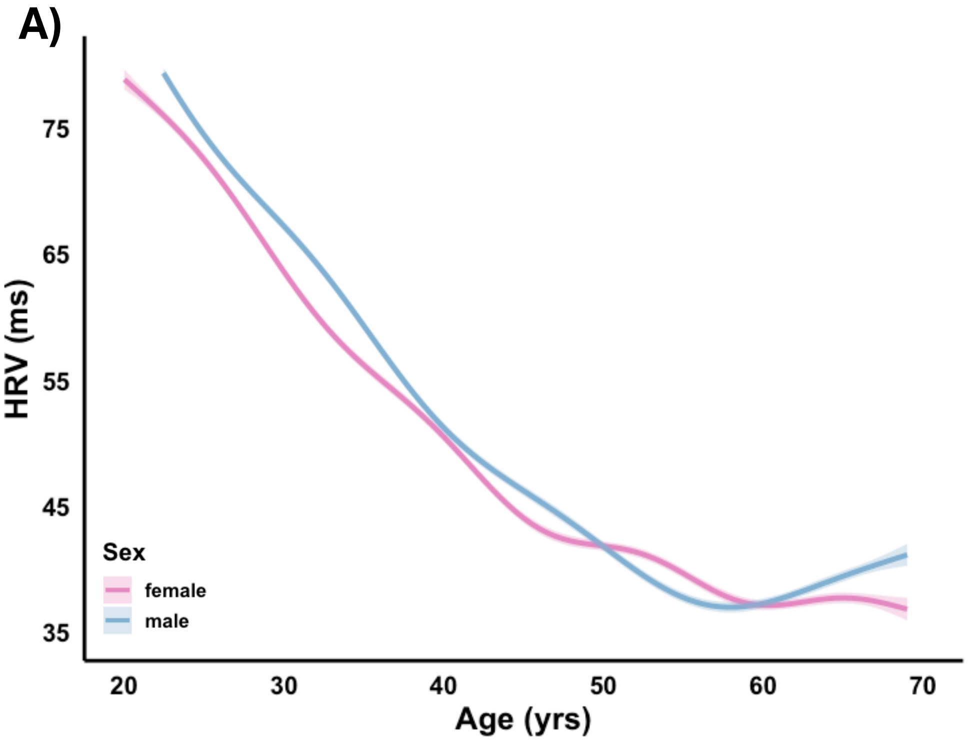
Figure S3. Standardized effects of alcohol (**A**), physical activity (**B**), sleep duration (**C**), and sleep consistency (**D**) on HRV-CV versus RHR, controlling for age, sex, strap generation, and BMI. To avoid conflating nonresponse with no alcohol, alcohol models included two covariates: the weekly alcohol-item completion proportion and a binary indicator for whether any alcohol responses were provided that week. Plots compare standardized coefficients from identical mixed-effects models predicting HRV coefficient of variation (HRV-CV) and mean RHR. Predictors were decomposed into between-person and within-person effects. Coefficients are expressed in SD units of the outcome per 1 SD increase in the predictor. Points show estimates and bars show 95% confidence intervals.



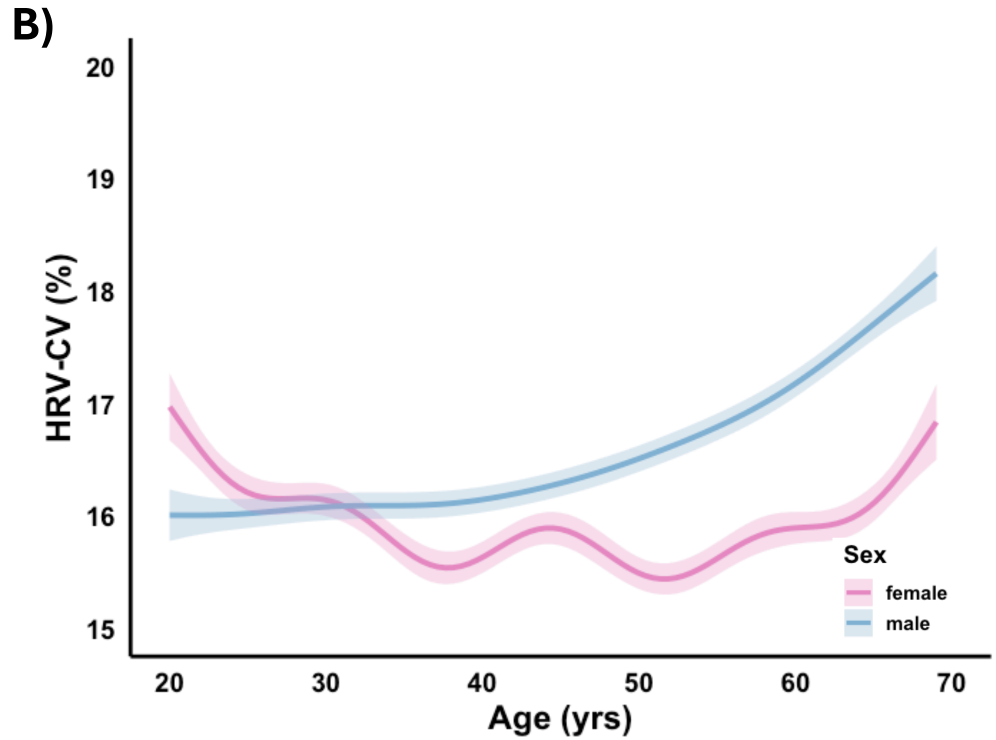
Age (yrs)	Sex Comparison	Female Group	Male Group
20 (a)	<0.001	b,c,d,e,f	b,c,d,e,f
30 (b)	<0.001	a,c,d,e,f	a,c,d,e,f
40 (c)	0.464	a,b,d,e,f	a,b,d,e,f
50 (d)	0.724	a,b,c,e,f	a,b,c,e,f
60 (e)	0.123	a,b,c,d,f	a,b,c,d,f
70 (f)	<0.001	a,b,c,d,e	a,b,c,d,e

Age (yrs)	Sex Comparison	Female Group	Male Group
20 (a)	<0.001	b,c,d,e,f	b,c,d,e,f
30 (b)	<0.001	a,c,d,e,f	a,c,d,e,f
40 (c)	0.856	a,b,d,e,f	a,b,d,e,f
50 (d)	<0.001	a,b,c,e,f	a,b,c,e,f
60 (e)	<0.001	a,b,c,d,f	a,b,c,d,f
70 (f)	<0.001	a,b,c,d,e	a,b,c,d,e

Figure S4. Age-related trends in heart rate variability (HRV; **A**) and HRV coefficient of variation (HRV-CV; **B**), visualized using generalized additive mixed models that included sex both as a main effect (controlling for baseline differences) and an interaction with smooth terms (sex-specific trends), controlling for strap generation. Figures depict unadjusted models and correspond to models adjusted for behaviors (average and CV) and BMI shown in **Figure 5**. Shaded areas represent 95% confidence intervals. Post-hoc pairwise comparisons were conducted using estimated marginal means with multivariate t adjustment for multiple comparisons. Comparisons tested for between-sex differences at selected ages as well as within-sex differences across ages. Tables beneath each panel display p-values for between-sex comparisons and groupings based on within-sex age differences. Letters indicate statistically significant differences (P<0.05) from the corresponding labeled age (e.g., ‘a’ = different from age 20).

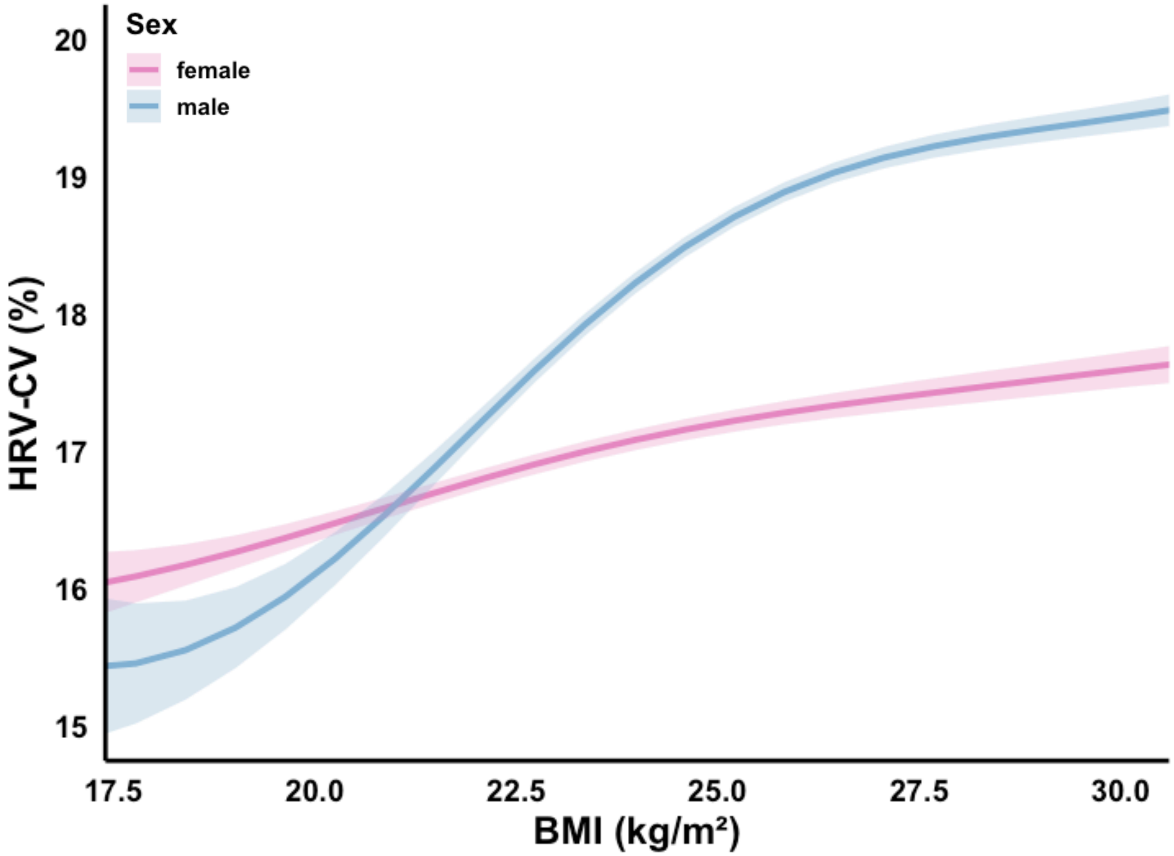


Age (yrs)	Sex Comparison	Female Group	Male Group
20 (a)	<0.001	b,c,d,e	b,c,d,e
30 (b)	<0.001	a,c,d,e	a,c,d,e
40 (c)	0.005	a,b,d,e	a,b,d,e
50 (d)	0.866	a,b,c,e	a,b,c,e
60 (e)	0.480	a,b,c,d	a,b,c,d



Age (yrs)	Sex Comparison	Female Group	Male Group
20 (a)	<0.001	a,b,c,d	d,e
30 (b)	0.628	a,c,d	d,e
40 (c)	<0.001	a,b	d,e
50 (d)	<0.001	a,b,e	a,b,c,e
60 (e)	<0.001	a,d	a,b,c,d

Figure S5. Age-related trends in heart rate variability (HRV; **A**) and HRV coefficient of variation (HRV-CV; **B**), visualized using generalized additive mixed models that included sex as both a main effect (controlling for baseline differences) and an interaction with smooth terms (sex-specific trends). These correspond to the same model specifications as **Figure 5** in the manuscript, with the 70-79 year age group removed. HRV models were adjusted for behavior (average and CV), strap generation, alcohol-item completion proportion and a binary indicator for whether any alcohol responses were provided that week, and BMI, while HRV-CV models were additionally adjusted for HRV. Shaded areas represent 95% confidence intervals. Post-hoc pairwise comparisons were conducted using estimated marginal means with multivariate t adjustment for multiple comparisons. Comparisons tested for between-sex differences at selected ages as well as within-sex differences across ages. Tables beneath each panel display p-values for between-sex comparisons and groupings based on within-sex age differences. Letters indicate statistically significant differences (P<0.05) from the corresponding labeled age (e.g., ‘a’ = different from age 20).



Body Mass Index (kg/m²)	Sex Comparison	Female Group	Male Group
18.4 (a)	0.002	b,c,d	b,c,d
22.0 (b)	<0.001	a,c,d	a,c,d
27.5 (c)	<0.001	a,b,d	a,b,d
30.0 (d)	<0.001	a,b,c	a,b,c

Figure S6. Body mass index (BMI) trajectories of HRV-CV visualized using generalized additive mixed models that included sex both as a main effect (controlling for baseline differences) and an interaction with smooth terms (sex-specific trends), controlling for strap generation. Figures depict unadjusted models and correspond to models adjusted for behaviors (average and CV), age, and HRV shown in **Figure 6**. Shaded areas represent 95% confidence intervals. Post-hoc pairwise comparisons were conducted using estimated marginal means with multivariate t adjustment for multiple comparisons. Comparisons tested for between-sex differences at selected BMI values as well as within-sex differences across BMI values. Tables beneath each panel display p-values for between-sex comparisons and groupings based on within-sex BMI differences. Letters indicate statistically significant differences ($P<0.05$) from the corresponding labeled BMI (e.g., ‘a’ = different from 18.4 kg/m^2).