

Supplementary Information Files

Gut microbiota and metabolic health among overweight and obese individuals

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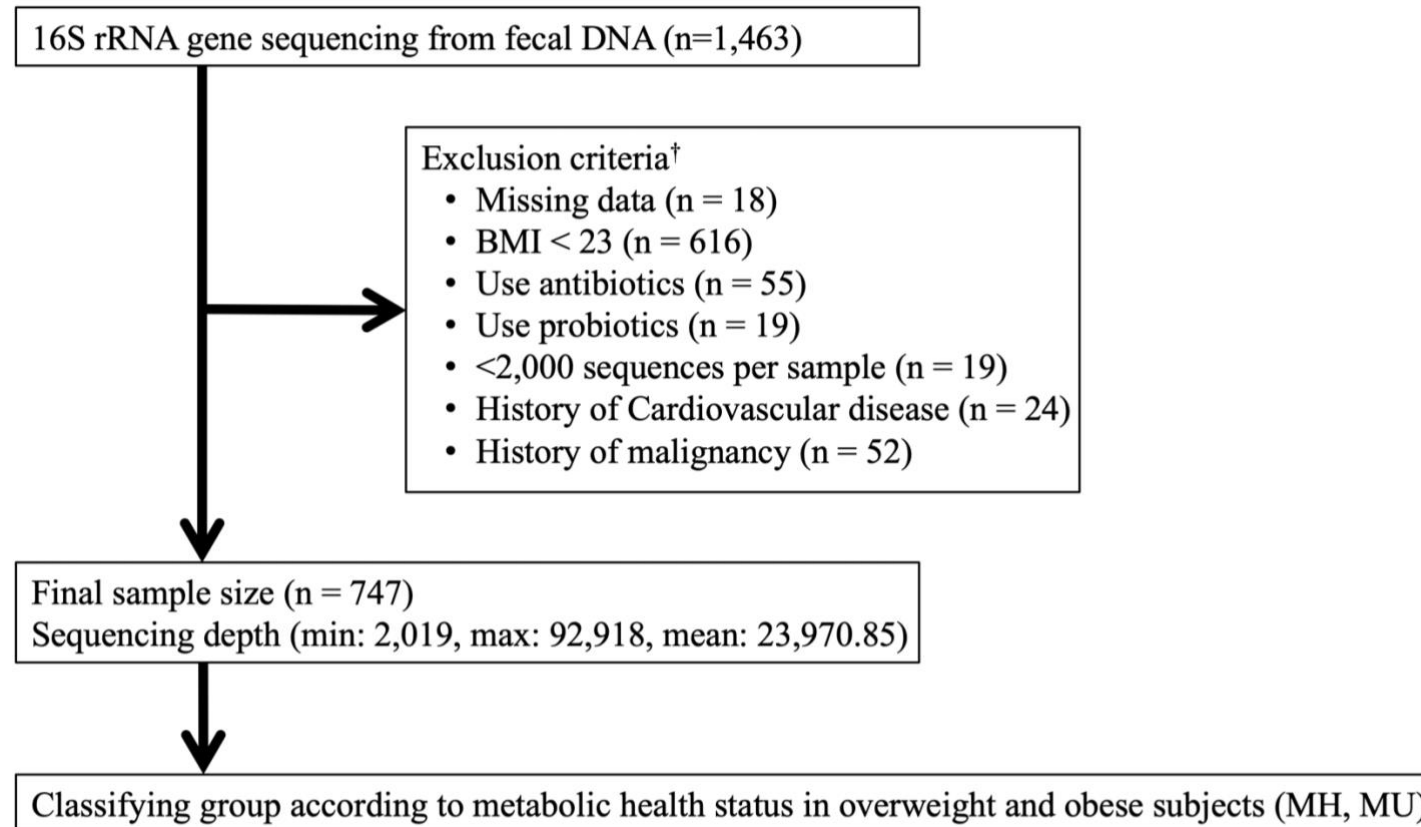
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Supplementary Figure S1. Enrollment of subjects. †Some subjects met several exclusion criteria. MH, metabolically healthy; MU, metabolically unhealthy.

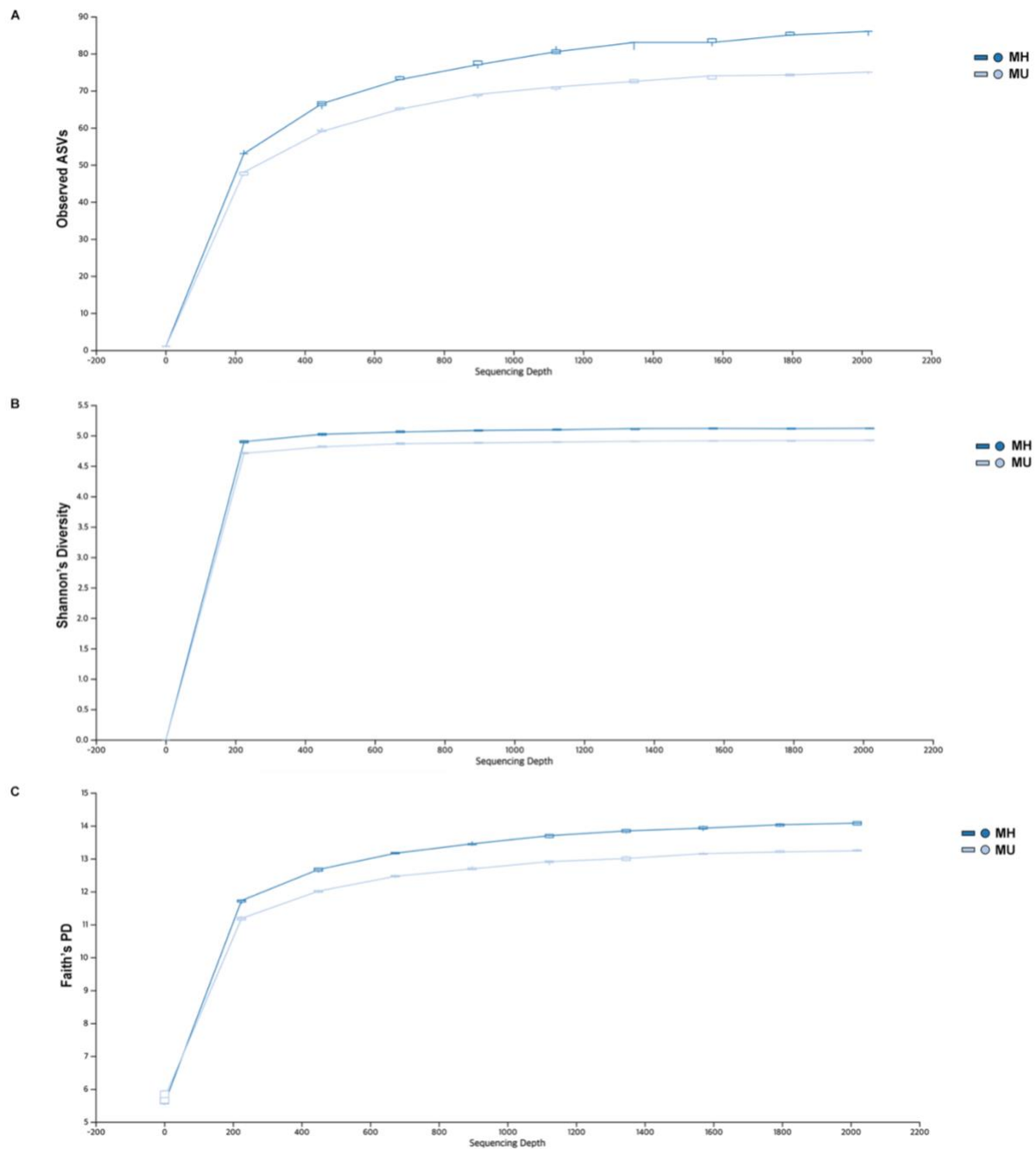


Supplementary Table S1. Nutrient characteristics of study participants according to metabolic health in obesity and obese individuals.

	Total (N = 561)	Metabolically healthy (N = 236)	Metabolically unhealthy (N = 325)	<i>p</i> -value
Total energy, kcal/day	1472.8 ± 652.7	1522.1 ± 608.7	1436.9 ± 681.6	0.13
Total protein, g/day	50.8 ± 25.5	51.6 ± 23.9	50.1 ± 26.6	0.50
Total fat, g/day	29.2 ± 18.8	30.9 ± 19.0	28.0 ± 18.7	0.08
Total carbohydrate, g/day	247.7 ± 113.4	255.6 ± 105.7	241.9 ± 118.5	0.16
Total calcium, mg/day	314.2 ± 195.4	314.3 ± 175.3	314.2 ± 209.0	1.00
Total phosphorus, mg/day	738.2 ± 344.0	745.1 ± 315.4	733.1 ± 363.8	0.68
Total Vitamin A, ug/day	341.5 ± 248.2	338.4 ± 223.9	343.8 ± 264.9	0.80
Total sodium, mg/day	1698.4 ± 1034.4	1715.7 ± 948.3	1685.8 ± 1093.9	0.74
Vitamin B1, mg/day	0.9 ± 0.4	0.9 ± 0.4	0.8 ± 0.5	0.26
Vitamin C, mg/day	71.1 ± 58.2	73.2 ± 59.7	69.5 ± 57.1	0.45
Folate, mg/day	155.0 ± 101.9	158.2 ± 99.2	152.7 ± 103.9	0.53
Retinol, ug/day	71.9 ± 58.3	73.5 ± 53.8	70.8 ± 61.5	0.58
Fiber, g/day	3.8 ± 2.3	3.8 ± 2.1	3.8 ± 2.4	1.00
Cholesterol, mg/day	175.0 ± 138.0	180.1 ± 137.9	171.3 ± 138.2	0.46

Mean (standard deviation) and *p*-value from t-test are shown

Supplementary Fig. S2. Rarefaction curves based on alpha diversity metrics. a) The number of observed ASVs, b) Shannon's diversity, and c) Faith's PD, indicated that 2019 sequences per sample are sufficient for capturing the alpha diversity of microbial communities in both MH and MU groups. The x-axis shows the number of sequences per sample. The rarefaction curves construction (10 replicates/depth) were performed using the "diversity alpha-rarefaction" plugin QIIME2.



Supplementary Table S2. Statistical significance among metabolically healthy non-obese (MHN), metabolically healthy obese (MH), and metabolically unhealthy obese (MU) groups using distance matrices for alpha-diversity.

	MHN vs. MH	MHN vs. MU	MH vs. MU
Diversity index	<i>p</i> -value ^a	<i>p</i> -value ^a	<i>p</i> -value ^a
Observed ASVs	0.700	0.036*	0.016*
Faith's PD	0.683	0.003**	0.001*
Shannon index	0.580	0.011*	0.004*
Pielou's evenness	0.886	0.495	0.442

^a Statistics were calculated using the Kruskal-Wallis Test. * $p < 0.05$, ** $p < 0.01$

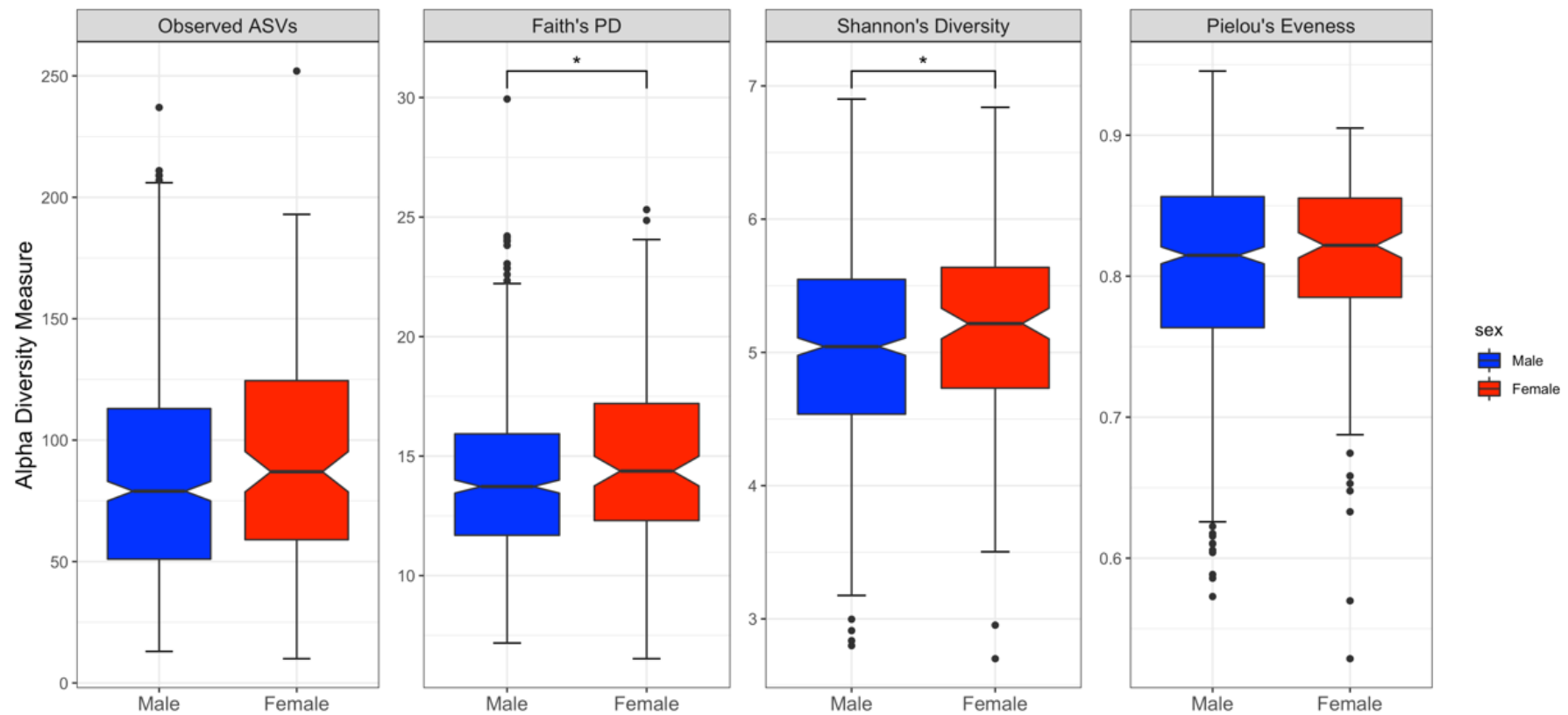
Supplementary Table S3. Statistical significance among metabolically healthy non-obese (MHN), metabolically healthy (MH), and metabolically unhealthy (MU) groups using distance matrices for beta-diversity.

Diversity index	MHN vs MH		MHN vs MU		MH vs MU	
	<i>Pseudo-F</i> ^a	<i>p-value</i> ^a	<i>Pseudo-F</i> ^a	<i>p-value</i> ^a	<i>Pseudo-F</i> ^a	<i>p-value</i> ^a
Bray-Curtis dissimilarity	2.137	0.001*	3.834	0.001*	1.565	0.007*
Jaccard distance	1.499	0.001*	2.777	0.001*	1.509	0.001*
Weighted UniFrac distance	4.825	0.001*	10.382	0.001*	2.159	0.085
Unweighted UniFrac distance	2.677	0.003*	7.250	0.001*	3.294	0.001*

^a Statistics were calculated using pairwise PERMANOVA with 999 permutations.

* $p < 0.05$, ** $p < 0.01$

Supplementary Fig. S3. Alpha diversity between males and females. Diversity was significant for observed features (ASVs) ($p = 0.080$, Mann-Whitney U-test), phylogenetic diversity ($p = 0.013$, Mann-Whitney U-test), Shannon index ($p = 0.035$, Mann-Whitney U-test), and Pielou's evenness ($p = 0.486$, Mann-Whitney U-test). * $p < 0.05$. Notched boxes indicate interquartile range (IQR) for 25th to 75th percentiles. The median value is shown as a line within the box, and the notch indicates the 95% confidence interval of the median. Whiskers extend to the most extreme value within $1.5 \times \text{IQR}$. Possible outliers are shown as dots.



Supplementary Table S4. Summary of results for the sex-separated analysis: Alpha diversity

Diversity index	Male (mean rank)			Female (mean rank)		
	MH	MU	<i>p</i> -value ^a	MH	MU	<i>p</i> -value ^a
Observed ASVs	315.33	282.83	0.022*	86.00	71.75	0.050*
Faith's PD	319.56	279.76	0.005**	88.72	69.62	0.009**
Shannon index	314.66	283.32	0.028*	90.07	68.56	0.003**
Pielou's evenness	296.24	296.69	0.975	84.18	73.17	0.130

N = male, 592; female, 155

MH, metabolically healthy group; MU, metabolically unhealthy group

^a Statistics were calculated using Kruskal-Wallis Test. * $p < 0.05$, ** $p < 0.01$

Supplementary Table S5. Summary of results for the sex-separated analysis: Beta diversity

Diversity index	Male (MH vs. MU)		Female (MH vs. MU)	
	<i>Pseudo-F</i> ^a	<i>p</i> -value ^a	<i>Pseudo-F</i> ^a	<i>p</i> -value ^a
Bray-Curtis dissimilarity	1.363	0.034*	0.967	0.543
Jaccard distance	1.558	0.001*	1.089	0.157
Weighted UniFrac distance	1.017	0.345	2.251	0.072
Unweighted UniFrac distance	2.978	0.002*	1.856	0.029*

MH, metabolically healthy group; MU, metabolically unhealthy group

^a Statistics were calculated using pairwise PERMANOVA with 999 permutations. * $p < 0.05$, ** $p < 0.01$

Supplementary Table S6. Association between covariates and the phylum Actinobacteria and their sub-taxa.

Taxa	age			sex			BMI		
	Coef. ^a	<i>p</i> -value	<i>q</i> -value	Coef. ^a	<i>p</i> -value	<i>q</i> -value	Coef. ^a	<i>p</i> -value	<i>q</i> -value
p__Actinobacteria	-0.001	1.86.E-06	2.60.E-05	-0.036	7.53.E-09	2.11.E-07	-0.002	9.41.E-02	2.03.E-01
p__Actinobacteria;c__Actinobacteria	-0.001	8.37.E-07	4.02.E-05	-0.027	2.66.E-06	6.38.E-05	-0.002	5.87.E-02	1.76.E-01
p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales	-0.001	7.37.E-07	4.13.E-05	-0.027	2.50.E-06	6.99.E-05	-0.002	6.23.E-02	1.94.E-01
p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales	0.000	2.30.E-01	4.29.E-01	-0.001	3.78.E-01	5.35.E-01	0.000	7.30.E-01	8.02.E-01
p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__ <i>Bifidobacteriaceae</i>	-0.001	7.37.E-07	9.72.E-05	-0.027	2.50.E-06	1.65.E-04	-0.002	6.23.E-02	1.61.E-01
p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__ <i>Coriobacteriaceae</i>	0.000	2.30.E-01	3.84.E-01	-0.001	3.78.E-01	5.25.E-01	0.000	7.30.E-01	8.03.E-01

^a The coefficients from the generalized linear model using MaAsLin.

Supplementary Fig. S4. Firmicutes/Bacteroidetes ratios in the metabolically healthy (MH) and metabolically unhealthy (MU) groups.

The p -value was calculated using t-test.

