



Supplementary Materials: Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome

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Table S1. Differences in richness metrics, Chao1, Shannon and Simpson, between tertiles of baseline body mass index.

Differences in Chao1		
	Tertile 1	Tertile 2
Tertile 2	0.95 #	-
Tertile 3	0.95	0.95
Differences in Shannon		
	Tertile 1	Tertile 2
Tertile 2	0.82	-
Tertile 3	0.82	0.82
Differences in Simpson		
	Tertile 1	Tertile 2
Tertile 2	0.92	-
Tertile 3	0.92	0.92

False discovery rate *P*-value tested by Wilcoxon rank sum test.

Table S2. Differences in beta diversity distances, Bray-Curtis, Jaccard, Weighted Unifrac, Unweighted Unifrac, between tertiles of baseline body mass index.

Differences in Bray-Curtis distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.762	0.381	1.062	0.006	0.318
Residuals	361	129.461	0.359		0.994	
Total	363	130.223			1.000	
Differences in Jaccard distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.863	0.431	1.033	0.006	0.323
Residuals	361	150.728	0.417		0.994	
Total	363	152.591			1.000	
Differences in Weighted Unifrac distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.004	0.002	0.633	0.003	0.535
Residuals	361	1.267	0.003		0.996	
Total	363	1.271			1.000	
Differences in Unweighted Unifrac distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.218	0.109	0.904	0.005	0.716
Residuals	361	43.552	0.121		0.995	
Total	363	43.770			1.000	

Df, degrees of freedom; Sqs, squares; Pr(>F), significance on F-test. Differences in beta diversity distances were tested using PERMANOVA test. Permutations: free; number of permutations 999; terms added sequentially (first to last).

Table S3. Variability in beta diversity distances among tertiles of baseline body mass index.

Bray-Curtis distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0002	0.0001	0.050	999	0.955
Residuals	361	0.911	0.002			
Jaccard distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.00008	0.00004	0.044	999	0.953
Residuals	361	0.316	0.0009			
Weighted Unifrac distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0009	0.0004	0.439	999	0.644
Residuals	361	0.357	0.001			
Unweighted Unifrac distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0005	0.0002	0.066	999	0.939
Residuals	361	1.323	0.004			

Df, degrees of freedom; Sq, square; N.Perm, number of permutations; Pr(>F), significance on F-test. Permutation test for homogeneity of multivariate dispersions performed to statistically test variability among groups. Permutation: free; number of permutations: 999; response: distances.

Table S4. Pairwise comparisons of baseline body mass index tertiles mean dispersion.

Bray-Curtis distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.885 [#]	0.871
Tertile 2	0.879	-	0.743
Tertile 3	0.871	0.747	-
Jaccard distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.823	0.929
Tertile 2	0.838	-	0.769
Tertile 3	0.931	0.768	-
Weighted Unifrac distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.374	0.910
Tertile 2	0.392	-	0.435
Tertile 3	0.927	0.438	-
Unweighted Unifrac distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.762	0.738
Tertile 2	0.779	-	0.977
Tertile 3	0.735	0.972	-

[#] Observed P-value below diagonal. permuted P-value above diagonal, calculated using t-test performed on the pairwise group dispersions.

Table S5. Results of log normalized *Firmicutes*-to-*Bacteroidetes* ratio and *Prevotella*-to-*Bacteroides* ratio between tertiles of baseline body mass index.

	N	Mean	Std dev	Min	Max
<u>Log2_FB_ratio</u>					
Tertile 1	121	0.861	1.388	−5.500	3.536
Tertile 2	122	0.864	1.298	−4.917	2.964
Tertile 3	121	0.915	1.310	−3.917	2.839
<u>Log2_PB_ratio</u>					
Tertile 1	121	−0.224	2.786	−11.459	10.071
Tertile 2	122	−0.146	2.543	−9.515	6.104
Tertile 3	121	−0.758	3.154	−12.788	5.687

Log2_FB_ratio one-way ANOVA (F (2, 361) = 0.064, *P* = 0.938).
 Log2_PB_ratio one-way ANOVA (F (2, 361) = 1.388, *P* = 0.332).

Table S6. Results of log normalized *Firmicutes*-to-*Bacteroidetes* ratio and *Prevotella*-to-*Bacteroides* ratio between tertiles of changes in body weight after 12-month intervention.

	N	Mean	Std dev	Min	Max
<u>Log2_FB_ratio</u>					
Tertile 1	115	0.783	1.367	−2.96	5.22
Tertile 2	115	1.046	1.223	−2.74	4.21
Tertile 3	115	0.759	1.358	−3.54	4.11
<u>Log2_PB_ratio</u>					
Tertile 1	115	−0.289	2.756	−12.47	6.00
Tertile 2	115	−0.270	2.576	−12.70	6.06
Tertile 3	115	−0.295	2.778	−11.46	5.69
<u>Delta_FB_ratio</u>					
Tertile 1	115	0.041	1.404	−4.05	4.59
Tertile 2	115	−0.229	1.325	−7.16	2.44
Tertile 3	115	0.116	1.478	−6.86	3.83
<u>Delta_PB_ratio</u>					
Tertile 1	115	−0.164	3.297	−12.62	12.47
Tertile 2	115	0.034	2.838	−13.26	9.72
Tertile 3	115	0.293	3.399	−11.79	11.46

Log2_FB_ratio one-way ANOVA (F (2, 342) = 1.680, *P* = 0.188)
 Log2_PB_ratio one-way ANOVA (F (2, 342) = 0.003, *P* = 0.997).
 Delta_FB_ratio one-way ANOVA (F (2, 342) = 1.931, *P* = 0.147).
 Delta_PB_ratio one-way ANOVA (F (2, 342) = 0.598, *P* = 0.550).

Table S7. Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of baseline body mass index.

ASV ID	baseMean	log₂FoldChange	lfcSE	stat	pvalue	padj	Genus
T1 vs T2							
5b1b613f2b 8a8224ffb8 f4df91585f 76	10.099	29.200	3.568	8.185	2.7×10^{-16}	1.2×10^{-12}	Prevotella 2
T1 vs T3							
6c03f9ce7c 4e3251103 817d98a64 4563	0.442	21.623	3.585	6.032	1.6×10^{-9}	6.9×10^{-6}	Bacteroides
T2 vs T3							
6c03f9ce7c 4e3251103 817d98a64 4563	0.442	29.927	3.540	8.453	2.8×10^{-17}	1.2×10^{-13}	Bacteroides
5b1b613f2b 8a8224ffb8 f4df91585f 76	10.099	-18.876	3.541	-5.33	9.8×10^{-18}	2.1×10^{-14}	Prevotella 2

Table 8. Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of changes in body weight after 12-month intervention.

ASV ID	baseMean	log2FoldChange	lfcSE	stat	p-value	padj	Genus
T1 vs T2							
de204b44fc231cd402652f4d87380471	0.234	23.705	3.671	6.457	1.1×10^{-10}	7.3×10^{-7}	Prevotella 9
d7cc2fea278ab0156b4e6e72dcdbe327	0.103	22.482	3.671	6.123	9.2×10^{-10}	2.0×10^{-6}	uncultured
c2a9c6e2349c0166d4d1f321354baae3	0.095	22.355	3.671	6.088	1.1×10^{-9}	2.0×10^{-6}	Lachnospiraceae UCG-001
733ac0a6c00a457793ea9c9d1a428aa9	0.102	22.323	3.671	6.080	1.2×10^{-9}	2.0×10^{-6}	NA
cc5d6923fc7f1124160533a11bcfbb76	0.078	22.072	3.671	6.011	1.8×10^{-9}	2.5×10^{-6}	Bacteroides
5b1b613f2b8a8224ffb8f4df91585f76	8.046	-18.829	3.670	-5.13	2.9×10^{-7}	3.3×10^{-4}	Prevotella 2
T1 vs T3							
de204b44fc231cd402652f4d87380471	0.234	24.512	4.303	5.697	1.2×10^{-8}	8.3×10^{-5}	Prevotella 9
d7cc2fea278ab0156b4e6e72dcdbe327	0.103	23.473	4.303	5.454	4.9×10^{-8}	1.2×10^{-4}	uncultured
c2a9c6e2349c0166d4d1f321354baae3	0.095	23.330	4.303	5.421	5.9×10^{-8}	1.2×10^{-4}	Lachnospiraceae UCG-001
cc5d6923fc7f1124160533a11bcfbb76	0.078	23.103	4.303	5.368	7.9×10^{-8}	1.2×10^{-4}	Bacteroides
733ac0a6c00a457793ea9c9d1a428aa9	0.102	23.018	4.303	5.349	8.8×10^{-8}	1.2×10^{-4}	NA
d00c1161004d40e7b94bbbda74a867cf	0.355	22.966	4.303	5.105	3.3×10^{-7}	3.7×10^{-4}	Bacteroides
T2 vs T3							

d00c1161004d40e7b94b bbda74a867cf	0.356	28.676	3.830	7.488	7.0×10^{-14}	1.2×10^{-10}	Bacteroides
5b1b613f2b8a8224ffb8f 4df91585f76	8.046	26.343	3.831	6.876	6.1×10^{-12}	4.7×10^{-9}	Prevotella 2
c24bf620a7e511901684 4066114196fc	0.070	−23.898	3.832	−6.237	4.5×10^{-10}	1.7×10^{-7}	Sutterella
68ec1aefa0103ae766daf c814b38894b	0.142	−24.646	3.832	−6.432	1.3×10^{-10}	5.0×10^{-8}	Bacteroides
26cb357d46b7cb7c7fc6 0dec29530cba	0.077	−24.767	3.832	−6.464	1.0×10^{-10}	4.4×10^{-8}	Prevotella 2
89595c082e6cf4a47edb b41a7d13bd3a	0.095	−24.929	3.832	−6.506	7.7×10^{-11}	3.5×10^{-8}	Dialister
a10c31f88f53ba0b7319 de39d21e0ee6	0.099	−24.931	3.832	−6.506	7.7×10^{-11}	3.5×10^{-8}	Prevotella 9
21ab7f3a71ced7896203 e4bfeca1289f	0.113	−24.988	3.832	−6.521	6.7×10^{-11}	3.5×10^{-8}	Prevotella 9
579a75cbf324508b5a74 d6533dd9169c	0.181	−25.015	3.832	−6.528	6.6×10^{-11}	3.5×10^{-8}	Bacteroides
fb5d414fd6bd7d6dc753 f0b889a3c473	0.167	−26.198	3.832	−6.837	8.1×10^{-12}	5.0×10^{-9}	Bacteroides
88bff18bedc7d38cd6ff3 b46201c90d0	0.167	−26.198	3.832	−6.837	8.1×10^{-12}	5.0×10^{-9}	Bacteroides
a188c60c7eab567a12ae 4318c1a54694	0.107	−26.339	3.832	−6.874	6.2×10^{-12}	4.7×10^{-9}	Bacteroides
9e7912a029eca5c8e694 a06acaa15ef3	0.111	−28.364	3.832	−7.4	1.3×10^{-13}	1.3×10^{-10}	Bacteroides
a58697475d24d4d265ea a922b346d6f5	0.088	−28.490	3.832	−7.435	1.0×10^{-13}	1.2×10^{-10}	Prevotella 2
4c6f7e347c7157738bafc b4ac98c385f	0.103	−28.547	3.832	−7.450	9.3×10^{-14}	1.2×10^{-10}	NA
eb1e06232c80ba2efd64 dfa172203d5c	0.171	−28.813	3.832	−7.520	5.5×10^{-14}	1.2×10^{-10}	Prevotella 9
d49ea76b83aacea8576b fc30b28f0caa	0.165	−29.180	3.832	−7.615	2.6×10^{-14}	9.0×10^{-11}	Bacteroides
02500c8e7dff746267b9 b6542b9f5140	0.225	−33.036	3.832	−8.622	6.6×10^{-18}	4.5×10^{-14}	Bacteroides

lfcSE, log fold change Standard Error; stat, Wald test statistic; padj, Benjamini-Hochberg adjusted *P*-value.

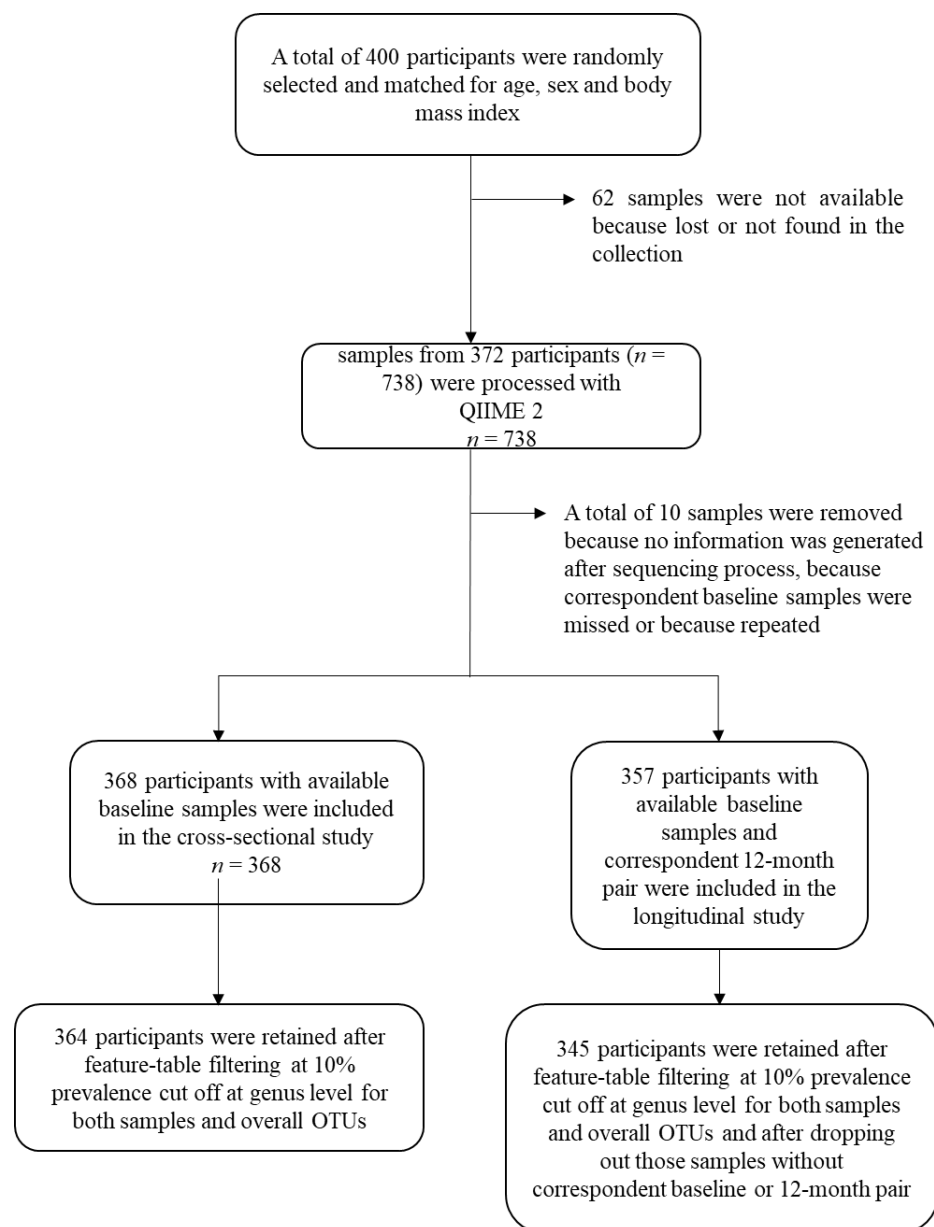


Figure S1. Flowchart of study participants.