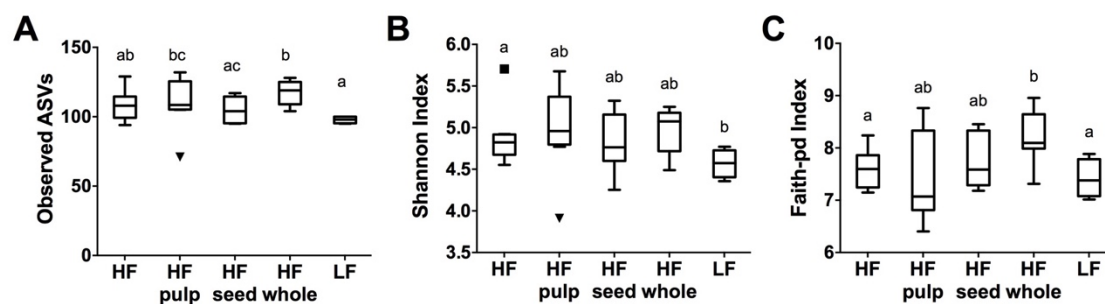


**Supplementary Material for**

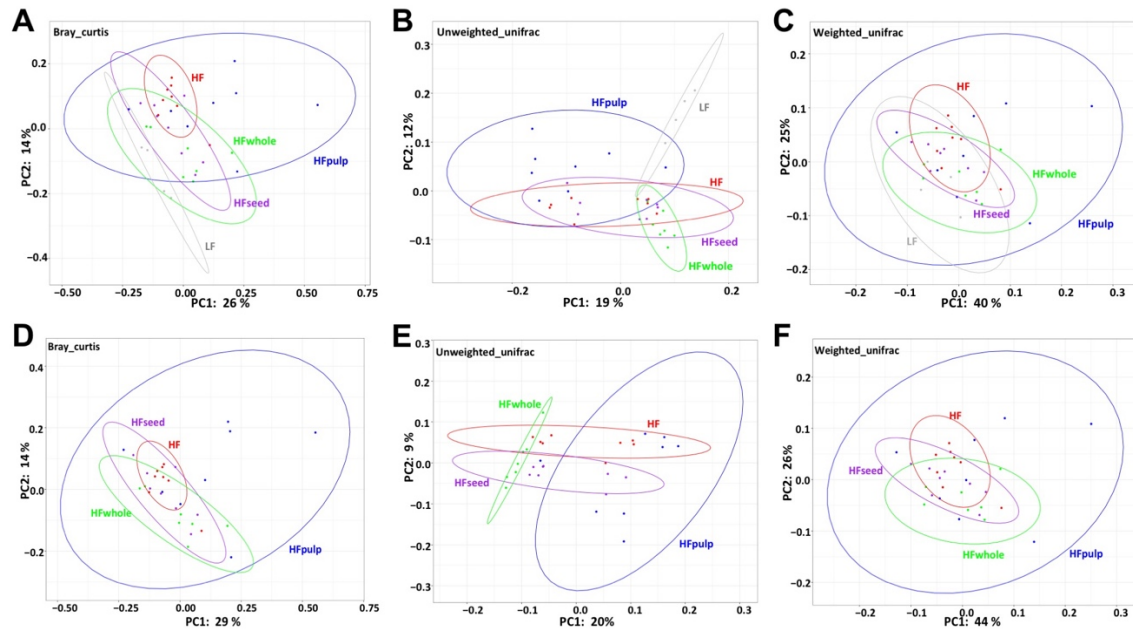
**Polyphenolic fractions isolated from red raspberry whole fruit, pulp, and seed differentially  
alter the gut microbiota of mice with diet-induced obesity**

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Amanda E. Ramer-Tait<sup>1,2,#</sup>

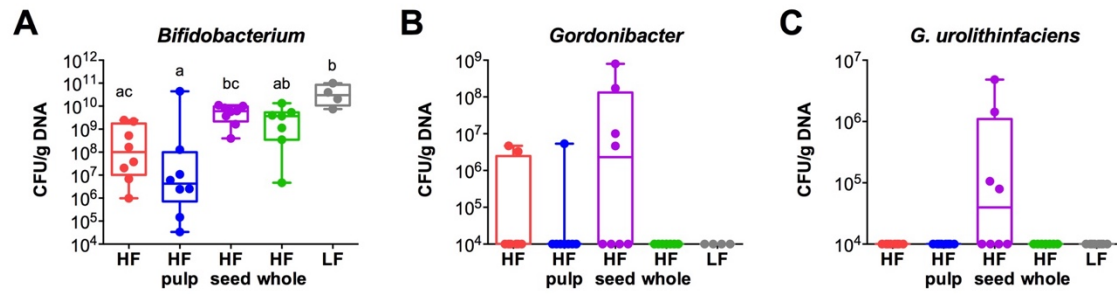
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**Supplementary Figure 1. Alpha diversity plots by dietary treatment.** All data were analyzed using a non-parametric Kruskal-Wallis one-way ANOVA test followed by Dunn's post-hoc test. Outliers were plotted as individual dots. Treatments with different letters are significantly different from one another at  $p < 0.05$ . LF = low-fat diet; HF = high-fat diet; HFwhole = whole raspberry polyphenols; HFseed, raspberry seed polyphenols; HFpulp, raspberry pulp polyphenols.



**Supplementary Figure 2. Beta diversity analyses.** Principal Coordinate Analyses (PCoA) plot of  $\beta$ -diversity based on Bray-Curtis (A, D), unweighted Unifrac (B, E) and weighted Unifrac (C, F) for all dietary treatments (A-C) or high-fat diets supplemented with or without raspberry polyphenols (D-F). Ellipses represent the 95% confidence interval for each dietary treatment. LF = low-fat diet; HF = high-fat diet; HFwhole = whole raspberry polyphenols; HFseed, raspberry seed polyphenols; HFpulp, raspberry pulp polyphenols.



**Supplementary Figure 3. Quantification via qPCR of select bacterial taxa.** Absolute abundance (CFU/gDNA) of (A) *Bifidobacterium* species, (B) *Gordonibacter* species, and (C) *Gordonibacter urolithinfaciens* in mouse cecal contents. Treatments with different letters are significantly different from one another at  $p < 0.05$ . LF = low-fat diet; HF = high-fat diet; HFwhole = whole raspberry polyphenols; HFseed, raspberry seed polyphenols; HFpulp, raspberry pulp polyphenols.

**Supplementary Table 1. Composition of experimental diets.**

	<b>HF</b>	<b>HFpulp</b>	<b>HFseed</b>	<b>HFwhole</b>	<b>LF</b>
	<b>g (%)</b>	<b>g (%)</b>	<b>g (%)</b>	<b>g (%)</b>	<b>g (%)</b>
Protein	19.8	19.8	19.8	19.8	19.8
Carbohydrate	45.0	45.0	45.0	45.0	65.0
Fat	23.7	23.7	23.7	23.7	4.6
	<b>kcal (%)</b>	<b>kcal (%)</b>	<b>kcal (%)</b>	<b>kcal (%)</b>	<b>kcal (%)</b>
Protein	16.76	16.76	16.76	16.76	20.81
Carbohydrate	38.10	38.10	38.10	38.10	68.31
Fat	45.49	45.49	45.49	45.49	9.90
<b>Ingredient quantity</b>	<b>g</b>	<b>g</b>	<b>g</b>	<b>g</b>	<b>g</b>
Casein	195	195	195	195	195
L-Cystine	3	3	3	3	3
Corn Starch	300	300	300	300	600
Maltodextrin	50	50	50	50	50
sucrose	100	100	100	100	0
Palm Oil Refined	187	187	187	187	21
Soybean oil	50	50	50	50	25
Cellulose	64	61	63	60	55
Mineral Mix	35	35	35	35	35
Calcium Carbonate	4	4	4	4	4
Vitamin Mix	10	10	10	10	10
Choline bitartrate	2	2	2	2	2
Raspberry extracts	0	3	1	4	0
Total	1000	1000	1000	1000	1000
Energy density (Kcal/g)	4.7	4.7	4.7	4.7	3.8

**Supplementary Table 2. Phenolic composition of red raspberry dry extracts and supplemented diets.**

	RR dry extracts <sup>#</sup>			RR supplemented diets <sup>*</sup>					
	Pulp	Seed	Whole	HFpulp		HFseed		HFwhole	
	mg/g dry extract			mg/1000g diet	% in total GAE	mg/1000g diet	% in total GAE	mg/1000g diet	% in total GAE
Phenol									
Quercetin	1.21	3.13	2.73	3.63	0.36	3.13	0.95	10.92	0.98
Myricetin	27.7	14	19.1	83.1	8.18	14	4.23	76.4	6.84
Ellagic acid	49.7	134.8	66.1	149.1	14.67	134.8	40.74	264.4	23.69
(+)-Catechin	20.4	20.1	11.4	61.2	6.02	20.1	6.08	45.6	4.09
(-)-Epicatechin	67.4	11.7	75.4	202.2	19.89	11.7	3.54	301.6	27.02
Anthocyanin									
Cyanidin-3-o-b-D-glucoside	13.6	10.3	8.73	40.8	4.01	10.3	3.11	34.92	3.13
Cyanidin-3-o-b-D-glucoside equivalent	112	31.3	39.6	336	33.05	31.3	9.46	158.4	14.19
Total phenolic content (GAE)	338.83	330.85	279.04	1016.49	100	330.85	100	1116.16	100

<sup>#</sup> Data for the dry extracts are expressed as means ± standard deviations of triplicate tests.

<sup>\*</sup> Data for the supplemented diets are calculated based on the amount of dry extracts added into the diets.

GAE = gallic acid equivalent

**Supplementary Table 3. Pairwise Adonis test results for beta diversity metrics.**

Treatment 1	Treatment 2	Sample size	Permutations	p-values			
				Jaccard	Bray Curtis	Weighted Unifrac	Unweighted Unifrac
HF	HFpulp	16	999	0.001	0.003	0.276	0.01
HF	HFseed	16	999	0.002	0.001	0.085	0.005
HF	HFwhole	15	999	0.002	0.001	0.018	0.001
HF	LF	12	999	0.005	0.002	0.002	0.002
HFpulp	HFseed	16	999	0.001	0.018	0.137	0.006
HFpulp	HFwhole	15	999	0.002	0.016	0.246	0.001
HFpulp	LF	12	999	0.002	0.009	0.072	0.004
HFseed	HFwhole	15	999	0.002	0.024	0.176	0.001
HFseed	LF	12	999	0.002	0.003	0.021	0.003
HFwhole	LF	11	999	0.003	0.002	0.037	0.002

**Supplementary Table 4. Relative abundance of bacterial taxa.**

Taxa	p value	Mean					SEM				
		HF	HFpulp	HFseed	HFwhole	LF	HF	HFpulp	HFseed	HFwhole	LF
<i>Bifidobacterium</i>	0.0014	0.002598	0.009682	0.01929*	0.01312	0.07426**	0.00134	0.009411	0.004211	0.004347	0.02205
<i>uncl. Coriobacteriaceae</i>	0.0002	0.003377	0.007065	0.01923	0	0.01332	0.00142	0.007065	0.004281	0	0.003688
<i>Adlercreutzia</i>	0.0474	0.000552	0.001358	0.0007049	0.000995	0.000381	0.000142	0.000227	0.000131	0.000375	0.000261
<i>Bacteroides</i>	0.1042	0.01015	0.0152	0.01165	0.01157	0.02382*	0.001903	0.00586	0.001511	0.002152	0.002824
<i>uncl. Rikenellaceae</i>	0.2642	0.01035	0.02582	0.01081	0.01089	0.01265	0.001749	0.006998	0.001132	0.002913	0.002935
<i>uncl. S24-7</i>	0.0796	0.1257	0.1296	0.1539	0.1776	0.1987	0.01234	0.02888	0.009108	0.01149	0.02649
<i>uncl. Bacillaceae</i>	0.0297	0	0.0158	0	0	0	0	0.01007	0	0	0
<i>Staphylococcus</i>	0.0132	0	0.0006788	0.0001621	0	0.002051	0	6.79E-05	0.000135	0	0.00177
<i>Enterococcus</i>	0.5729	0.000401	0.0003678	0.0002253	0.000138	0.0002013	0.000183	0.000158	8.64E-05	0.000138	0.000201
<i>Lactobacillus</i>	0.1309	0.07151	0.03346	0.07609	0.08215	0.07019	0.0106	0.01773	0.01505	0.01294	0.007861
<i>Lactococcus</i>	0.0113	0.001122	0.00007888**	0.0007465	0.000996	0*	0.000305	7.89E-05	0.000255	0.000317	0
<i>Streptococcus</i>	0.2801	0.000864	0.0003168	0.0001999	0.00068	0	0.000342	0.000195	0.00011	0.000273	0
<i>Turicibacter</i>	0.6112	0.01077	0.01696	0.01969	0.01914	0.02611	0.003853	0.007035	0.003623	0.004751	0.01423
<i>uncl. Clostridiales</i>	0.0463	0.08001	0.05298*	0.05511*	0.05164*	0.03621**	0.008964	0.01259	0.004265	0.007978	0.003656
<i>uncl. Clostridiales_1</i>	0.0613	0.1624	0.1987	0.1433	0.1682	0.09322	0.01365	0.0322	0.01434	0.0232	0.005643
<i>uncl. Christensenellaceae</i>	0.1997	0.001681	0.001635	0.001271	0.001968	0.0009115	0.000233	0.000295	0.000144	0.000412	0.000229
<i>uncl. Clostridiaceae</i>	0.0663	0.0143	0.003158	0.01398	0.01467	0.02324	0.003911	0.001622	0.004436	0.003408	0.009816
<i>Dehalobacterium</i>	<0.0001	0.003175	0.00192**	0.003133	0.002254*	0.004495**	0.000187	0.000268	0.000333	0.000305	0.000361
<i>uncl. Lachnospiraceae</i>	0.0018	0.01556	0.02161	0.02841	0.01239	0.04938**	0.003852	0.00399	0.003179	0.003101	0.00773
<i>uncl. Lachnospiraceae_1</i>	0.1739	0.01947	0.03269	0.02778	0.04157*	0.02446	0.0026	0.009216	0.005492	0.008767	0.004399
<i>Coprococcus</i>	0.0201	0.01083	0.01449	0.0104	0.008026	0.008565	0.001421	0.001353	0.000862	0.00089	0.001661
<i>Dorea</i>	0.0069	0.009239	0.007857	0.005744	0.006092	0.000841*	0.002166	0.00061	0.000756	0.000521	0.00022
<i>Roseburia</i>	0.0003	0.006483	0.01512***	0.01275**	0.008738	0.002457	0.001223	0.001481	0.00255	0.001027	0.000762
<i>Ruminococcus_1</i>	<0.0001	0.05304	0.03739***	0.04156*	0.03252***	0.02702***	0.003381	0.003265	0.001568	0.002681	0.006236
<i>uncl. Peptostreptococcaceae</i>	0.2727	0.0179	0.01799	0.01587	0.02067	0.0001463	0.005633	0.007802	0.003467	0.004592	0.000146
<i>uncl. Ruminococcaceae</i>	0.1584	0.006058	0.006956	0.004978	0.004373	0.002001	0.000638	0.001723	0.00128	0.000816	0.001069
<i>Anaerotruncus</i>	0.3071	0.003586	0.005398	0.002406	0.005517	0.001885	0.000726	0.001815	0.000519	0.00212	0.000432
<i>Oscillospira</i>	0.0254	0.06827	0.1062	0.05989	0.07026	0.06337	0.005893	0.01554	0.005256	0.002818	0.003438
<i>Ruminococcus</i>	0.0018	0.01918	0.01467	0.01358*	0.01947	0.008382***	0.001755	0.001707	0.000876	0.002152	0.001866
<i>uncl. Mogibacteriaceae</i>	<0.0001	0.001901	0.0006839***	0.001165**	0.00155	0.0001695***	0.000193	0.000153	0.00018	0.00017	0.000131
<i>uncl. Erysipelotrichaceae</i>	0.047	0.007703	0.007054	0.003539	0.002802*	0.003221	0.001799	0.001982	0.000746	0.000502	0.000926
<i>Coprobacillus</i>	0.1609	0.000395	0.0002174	0.00005825	0.000115	0	0.000128	0.000148	4.33E-05	5.5E-05	0
<i>Sutterella</i>	0.0045	0.000398	0.000019	0.002851	0.003135	0.001203	0.000198	0.000019	0.001489	0.001071	0.000318
<i>uncl. RF39</i>	0.0014	0.000261	0.002159	0.00002063	0.000748	0	0.000141	0.002159	2.06E-05	0.000212	0
<i>Akkermansia</i>	0.6232	0.2607	0.1953	0.2395	0.2061	0.2137	0.02296	0.04425	0.02819	0.02755	0.02435

\* p<0.05, \*\* p<0.01 and \*\*\* p<0.001 vs HF