

Supplementary Data File

Greater Microbial Translocation and Vulnerability to Metabolic Disease in Healthy Aged Female Monkeys

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Supplementary Table S1.

Basic demographics of young and old monkeys evaluated in Study 1.

	Young (n=9)	Old (n=10)	p-value
Age (yrs)	10.7 (0.63)	22.1 (0.38)	<0.001
BW (kg)	4.87 (0.41)	4.94 (0.26)	0.45
Waist (cm)	34.3 (2.32)	38.6 (0.83)	0.04
Fasting glucose (mg/dL)*	136 (31)	119 (17)	0.30
Fasting insulin (μ IU/mL)	32.5 (12.6)	16.1 (2.87)	0.09
HOMA score (AU)	8.95 (3.12)	5.14 (1.40)	0.12
Total plasma cholesterol (mg/dL)	162 (11.9)	168 (13.2)	0.36
High density lipoprotein cholesterol (mg/dL)	56.6 (5.73)	57.2 (3.49)	0.46
Plasma triglycerides (mg/dL)	80.6 (6.75)	80.6 (9.44)	0.50

*High blood glucose values are attributed to the terminal nature of the blood collection procedure.

Supplementary Table S2.

The ascending colonic mucosal-associated microbiome was not different between young and old monkeys. This table outlines the lack of significance associated with the first 3 MDS levels at the phylum, class, order, family and genus levels.

		Variance	Age Group	Sequence Depth	Adjusted for Depth Group
Taxonomic					
Level		%	p-value	p-value	p-value
Phylum	MDS1	31.03	0.213	0.803	0.621
Phylum	MDS2	23.00	0.643	0.002	0.883
Phylum	MDS3	13.58	0.235	0.127	0.383
Class	MDS1	29.15	0.314	0.021	0.856
Class	MDS2	18.89	0.676	0.053	0.837
Class	MDS3	11.37	0.563	0.263	0.837
Order	MDS1	29.74	0.474	0.001	0.959
Order	MDS2	16.41	0.330	0.388	0.630
Order	MDS3	11.85	0.657	0.534	0.895
Family	MDS1	25.40	0.338	0.000	0.897
Family	MDS2	15.07	0.614	0.571	0.852
Family	MDS3	11.33	0.328	0.750	0.852
Genus	MDS1	24.88	0.557	0.000	0.980
Genus	MDS2	14.61	0.972	0.149	0.980
Genus	MDS3	11.69	0.502	0.668	0.980

Supplementary Table S3.

The ascending colonic lumen microbiome was not different between young and old monkeys. This table outlines the lack of significance associated with the first 3 MDS levels at the phylum, class, order, family and genus levels.

		Variance	Age Group	Sequence Depth	Adjusted for Depth Group
Taxonomic					
Level		%	p-value	p-value	p-value
Phylum	MDS1	41.32	0.194	0.365	0.500
Phylum	MDS2	23.58	0.590	0.059	0.780
Phylum	MDS3	12.79	0.238	0.784	0.500
Class	MDS1	37.71	0.122	0.374	0.469
Class	MDS2	19.78	0.702	0.054	0.989
Class	MDS3	12.77	0.337	0.251	0.715
Order	MDS1	31.72	0.081	0.261	0.566
Order	MDS2	20.30	0.516	0.217	0.994
Order	MDS3	15.09	0.722	0.024	0.994
Family	MDS1	26.90	0.066	0.562	0.437
Family	MDS2	17.69	0.943	0.021	0.974
Family	MDS3	13.28	0.933	0.149	0.974
Genus	MDS1	30.07	0.162	0.917	0.601
Genus	MDS2	14.63	0.953	0.002	0.719
Genus	MDS3	13.19	0.428	0.902	0.719

Supplementary Table S4.

The fecal microbiome was not different between young and old monkeys. This table outlines the lack of significance associated with the first 3 MDS levels at the phylum, class, order, family and genus levels.

Taxonomic		Sequence		Adjusted for Depth	
Level		Variance	Age Group	Depth	 Group
		%	p-value	p-value	p-value
Phylum	MDS1	57.99	0.146	0.018	0.504
Phylum	MDS2	14.22	0.330	0.297	0.504
Phylum	MDS3	8.29	0.181	0.533	0.504
Class	MDS1	46.12	0.193	0.007	0.557
Class	MDS2	15.58	0.262	0.734	0.557
Class	MDS3	8.48	0.038	0.313	0.557
Order	MDS1	38.52	0.242	0.011	0.682
Order	MDS2	15.95	0.186	0.592	0.682
Order	MDS3	9.31	0.192	0.104	0.682
Family	MDS1	34.78	0.144	0.017	0.493
Family	MDS2	11.75	0.330	0.381	0.493
Family	MDS3	10.21	0.119	0.240	0.493
Genus	MDS1	32.22	0.088	0.019	0.373
Genus	MDS2	11.75	0.905	0.532	0.991
Genus	MDS3	8.88	0.067	0.804	0.260

Supplementary Table S5.**Breakdown of dietary conditions used in Studies 1, 2, and 3.**

Dietary Component	Chow Diet	Western Diet
Total Protein	18.2% of calories	19 % of calories
Total Fat	13.1% of calories	35.5 % of calories
Total Carbohydrates	68.7% of calories	46% of calories
Simple Sugars	5.25% of calories	22% of calories
Cholesterol	0.75 ppm	600ppm
Saturated Fat	0.2% of calories	14% of calories
Sodium	0.72 mg/kCal	0.30 mg/kCal
Crude Fiber	> 6 %	6.1%
Caloric Density	3.43 kcal/g	3.56 kcal/g

Supplementary Table S6.**Basic demographics of young and old monkeys evaluated in Study 2.**

	N	Age	BW	Waist	Glucose	Insulin	TPC	ALT	ALP	LBP-1	sCD14
		years	kg	cm	mg/dL	(μ IU/L)	(mg/dL)	(U/L)	(U/L)	ng/mL	ng/mL
Young	6	8.12 (1.27)	5.2 (0.38)	30.9 (2.02)	78.8 (6.94)	33.7 (15.2)	135 (9.46)	58.2 (0.93)	83.5 (12.4)	2.93 (1.15)	2.13 (0.09)
Old	12	21.7 (0.68)	5.34 (0.34)	35.9 (1.62)	63.1 (6.88)	8.21 (2.76)	158 (10.0)	49.2 (5.83)	92.2 (9.79)	3.97 (0.78)	2.61 (0.13)
p-value		<0.001	0.80	0.04	0.17	0.04	0.08	0.41	0.60	0.53	0.02

Supplementary Table S7.

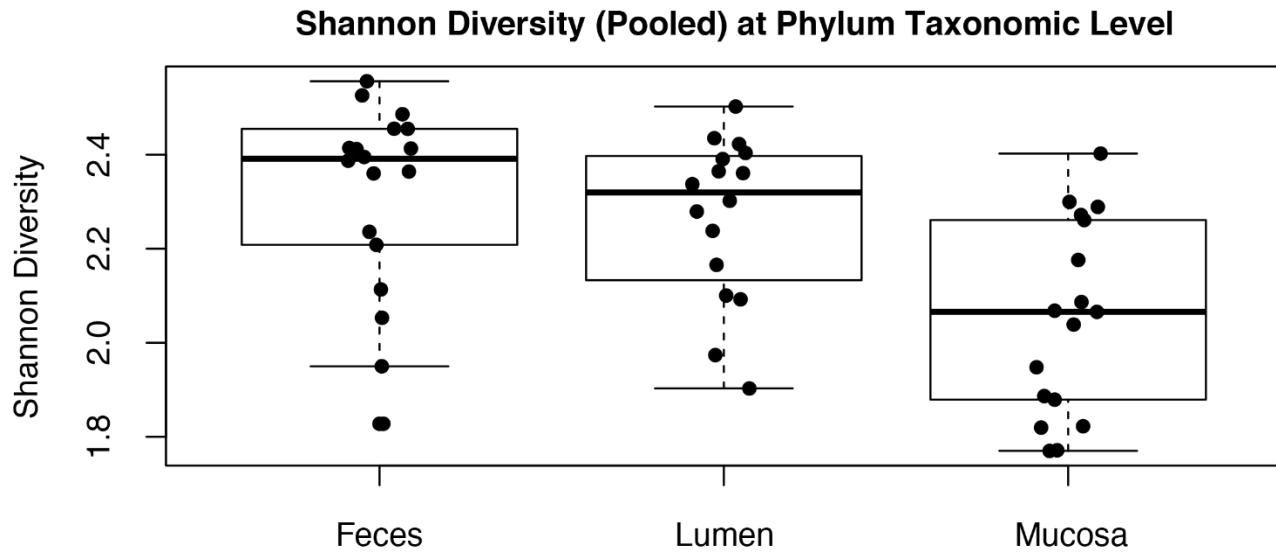
Old monkeys in Study 2 had elevated baseline markers of microbial translocation (lipopolysaccharide binding protein 1 and soluble CD14 [LBP-1, sCD14]) which did not further elevate following 8 weeks consumption of a Western diet.

*p<0.05; #p<0.10 Old vs Young (n=6/group) at baseline. Overall p-value is for ANCOVA.

	LBP-1 (ng/mL)		sCD14 (ng/mL)	
	Baseline	Post-Diet	Baseline	Post-Diet
Young Western Diet	2.93 (1.15)	3.73 (1.49)	2.13 (0.09)	1.81 (0.14)
Old Western Diet	5.15 (1.17) [#]	4.88 (1.03)	2.85 (0.16) [*]	2.46 (0.25)
p-value		0.95		0.25

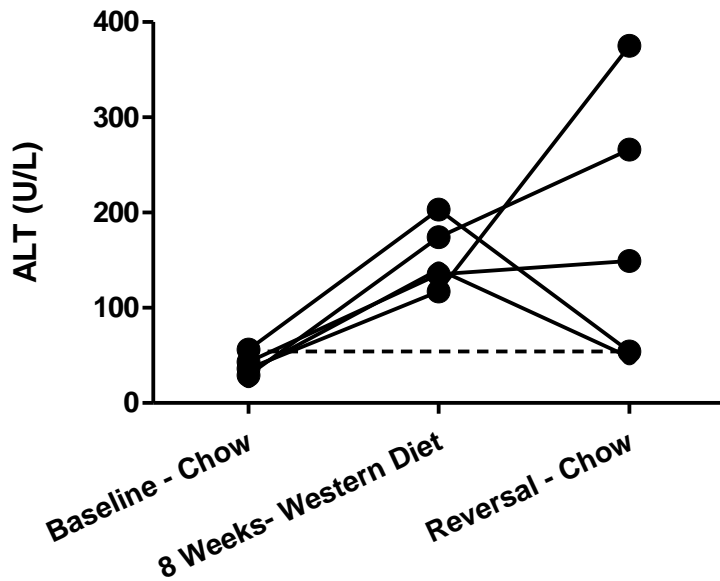
Supplementary Figure S1.

There were significant site differences in the diversity of the microbiome with the ascending colon mucosal microbiome being significantly less diverse than that of feces, or samples from the lumen of the ascending colon ($p=7.35 \times 10^{-6}$).



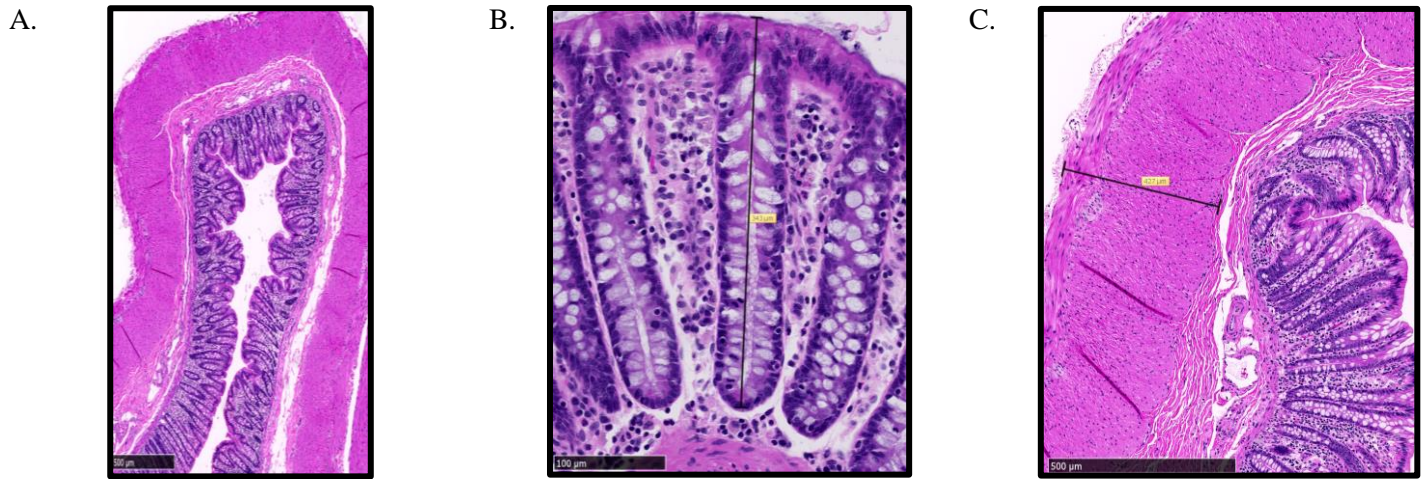
Supplementary Figure S2.

Liver alanine aminotransferase (ALT) levels in monkeys assessed in Study 2 had variable return to baseline values after the Western diet challenge was discontinued for 8 weeks and chow was consumed. Only 40% (2/5) females evaluated returned to baseline and 40% continued to show increasing hepatic damage. The broken line, (----), indicates a median value for age-matched monkeys from the Vervet Research Colony.



Supplementary Figure S3.

Example histology and measurements from Study 1. Overall structure of the colon is shown in panel A. An example of crypt depth and visualization used to manually count goblet cells shown in panel B. Panel C shows measurement of the muscularis layer thickness.



Representative immunostaining for occludin protein abundance is shown in panel D, and claudin-1 in panel E. Co-staining by periodic acid Schiff and Alcian blue for proteoglycan content of mucins is shown in panel F.

