Supplemental Materials and Methods

Study Diets

Study diets were composed of 0.8 g/kg high biologic value protein (current Dietary Reference Intake [DRI]), 30% of energy from fat, and the remaining energy from carbohydrate. Total food intake was adjusted to be isocaloric and to provide adequate intakes of macro- and micronutrients (supplemental table 2). All diets met or exceeded the Estimated Average Requirement for methionine plus cysteine and the DRI for vitamins B-12, B-6, and folic acid. A multivitamin supplement (Kirkland Brand Multi-vitamin Multi-mineral) provided vitamins A, D, B-12, and C and thiamine, niacin, and riboflavin at or above the DRI. Subjects were also given a calcium and magnesium supplement. Subjects' weights remained relatively stable, as caloric intake was adjusted for each subject over the course of the study to maintain subject weight (supplemental table 9). In addition to changes in choline levels, all depletion diets included a soy shake containing Benefiber and, thereby, differed from the study diets at other time points. Description of and micronutrient levels of all diets are extensively detailed in Busby et al.[1]

ARISA Analysis Methods

Using extracted DNA, the intergenic region between the 16S rRNA and the 23S rRNA genes was PCR amplified for each of two technical replicates using universal bacterial primers 1406F-FAM (FAM+TGY ACA CAC CGC CCG T) and 125R (GGG TTB CCC CAT TCR G). Reactions were set up using 50ng of template DNA according to a NanoDrop ND-1000 spectrophotometer. Thermalcycling conditions were as follows: an initial denaturation step at 94^{o}C for 2 minutes, followed by 35 cycles of 94^{o}C for 25 seconds; 56.5C for 30 seconds; 72^{o}C for 60 seconds. An extension was carried out at 72^{o}C for 5 minutes. Samples were loaded on an Applied Biosystems 3130 genetic analyzer. Applied Biosystems GeneScan™ 1200 LIZ® size standard was used to determine sizing up to 1200 nucleotides. The labeled fragments were separated by size on an Applied Biosystems 3130 sequencer, resulting in an electropherogram where each signal peak represented a different bacterial signature (supplemental figure 1). The electropherogram from each replicate was analyzed using custom JAVA software (unpublished) that establishes a base-pair scale, calls the data peaks from the spectra, assigns base pair sizes to each

peak and provides summaries of the peak call assignments. Peak calls were validated using Applied Biosystems 3130 analysis software. Resulting data vectors from all samples were compared using hierarchical clustering with custom JAVA code (available upon request).

Sequencing Analysis Methods

The V1-V2 variable regions of the 16S rRNA gene were targeted using the 454 Life Sciences primer B with a "TC" linker and bacterial 27F primer (5'-GCCTTGCCAGCCCGCTCAGTCAGAGTTTGATCCTGGCTCAG-3') and 454 Life Sciences primer A with a "CA" linker, 12 mer barcode and bacterial primer 338R (5'-GCCTCCCTCGCGCCATCAGNNNNNNNNNNNNCATGCTGCCTCCCGTAGGAGT-3') where the N's represent barcodes used to identify each sample.[2] PCRs were set up with Platinum Taq DNA polymerase (Invitrogen) according to the included protocol with 100ng of bacterial genomic DNA as a template. Each reaction was quantitated by PicoGreen on a NanoDrop ND-3300 fluorospectrometer. Samples were pooled in equimolar amounts and concentrated in a vacuum centrifuge before being submitted to the Environmental Genomics Core Facility at the University of South Carolina for 454-FLX sequencing.

Over 200,000 sequences were obtained from the 454-FLX process and were end-trimmed based on the Lucy algorithm at a cut-off of 0.002 corresponding to a quality score of 27.[3] Sequences had to meet the following criteria[4] to be included in the final dataset: (1) no Ns in the trimmed sequence, (2) an exact match to the 5' primer, (3) Lucy's identified region of poor quality at the 0.002 threshold did not extend beyond the 5' primer. The 5' primer (including the barcode) was trimmed from the sequences before analysis. Any sequences that did not meet a length requirement from 180 to 280 bases after trimming were discarded. The 194,781 trimmed, quality-controlled sequences (supplemental table 4) were evaluated for human contamination by using BLASTn[5] searches against the E. coli (J01695) 16S rRNA gene. All but 60 of our sequences matched the E. coli 16S rRNA gene with an e-score threshold of 0.04. For the 60 sequences that did not meet this threshold, a BLASTn search was performed against the entire bacterial 16S rRNA Ribosomal Database Project (RDP)[6] database. Every one of these sequenced matched this database with an e-value of 2.00E-90 or smaller. Taken together, these results

indicate that our quality-controlled, trimmed sequences had little to no human contamination. Rarefaction curves illustrate that the resulting sequences provided sufficient sequence depth in all subjects and in 74 of the 79 samples originally collected (supplemental figures 2-3).

Statistical Methods

The standardized logged sequence abundance was used in statistical analysis of all sequence counts, using the following calculation:

LOG10 ((Frequency/ # sequences in sample)*Average # of sequences per sample +1)

As an example, consider a dataset in which there was an average of 2,500 sequences per sample assigned to phylum at a 50% confidence level. For one sample within this dataset, 1,000 sequences were assigned to phylum and, of those, 300 were assigned to the taxon Firmicutes. The transformation would be:

Log10 ((300 / 1000) * 2500 + 1) = 2.8756

Using this measure, the logged sequence proportion corrects for different samples having different total numbers of sequences.

For hierarchical clustering of OTUs, we estimated the probability of obtaining the perfect clustering of samples by subject that we observed by sequencing by calculating how many different ways there are to achieve perfect clustering and dividing this value by the total number of ways the samples could be clustered. For the purposes of this calculation, we consider the hierarchical cluster as arranging the samples in an unweighted tree and the results of the cluster as an ordered set of our 74 samples represented by the 74 most derived nodes of the tree. In a tree with perfect clustering by subject, there are $x_i!$ possible arrangements of samples for each subject, where x_i is the number of samples for subject i (supplemental table 4). Since there are 15 ways of arranging our subjects while maintaining perfect clusters, the total number of unweighted trees that we could have observed that would have contained perfect clustering by patient is given by:

$$15! \prod_{i=1}^{i=15} X_i \quad (1)$$

Since there are 74 samples, there are 74! possible arrangements of all of our samples. Dividing equation (1) by 74! yields a value of $6.78e^{-65}$. We conclude that we can reject a null hypothesis that we could have observed perfect clustering of our patients by chance with a p-value of essentially zero. For all taxonomic statistical testing, we report results at the most inclusive taxonomic level for which effects were identified. From a practical perspective, we were able to determine that statistical testing at fine levels of detail was unlikely to be productive. In many cases, a particular family or genus would only be present in a small subset of our subjects, sometimes only in one subject, which reduced sample size and, therefore, the power of any statistical analysis, to the point where achieving significance was improbable. We were also aware of the problem created by multiple comparisons and wanted to approach our dataset with that issue in mind. To manage power and to provide some control for multiple comparisons, we chose to conduct statistical testing at the highest taxonomic level present in at least half of our subjects. While we recognized that the range of bacterial types within a group as large as class would likely also represent a range of functional types, our observed correlations between physiological changes and bacterial abundance suggested that that function in the bacterial group was consistent enough to support the host physiology link.

For paired t-tests comparing time points, samples missing one or more time points (subjects 29, 04) were excluded from the analysis. To manage the number of comparisons for which we had to correct, we removed from statistical analysis any taxon which did not have a presence in at least 50% of our subjects (9 categories for the taxonomic class level), a criterion which reduced the possibility of spurious observations that might result from low sample sizes.

To correct for multiple comparisons in statistical testing, we used the Benjamini and Hochberg algorithm[7] to adjust each p-value. The adjusted p-values for a statistical test run over a set of taxa (ordered in ascending rank by p-value) is given by $N * p(k)/k$ where N is the number of taxa for which a null hypothesis is evaluated by the test statistic, p(k) is the p-value produced by the test statistic and k is the rank of the taxa within the p-value ranked list. (Note that, for the top hit with the smallest p-value, the adjusted p-value is identical to Bonferroni correction). The adjusted p-value can then be evaluated against a threshold false discovery rate. For example, an adjusted p-value of 0.05 indicates that a result could be considered significant if a commonly-selected 5% FDR threshold was chosen. We note that

our adjusted p-values can be greater than 1 and hence cannot be strictly considered as classical p-values.

In our PCA models of the relationships between bacterial abundance, host genotype and liver fat changes, we recognized the potential problem of model over-fitting. Because we selected the two taxa having the highest R^2s of the nine regressions we performed (one for each class found in at least 8 of our patients), we would expect that the first component of the PCA could be well matched to the LF:SF ratio % change. We recognize that the reported p-value from the linear regression in Figure 5C is, therefore, likely to be anticonservative. To correct for model over-fitting, we developed a permutation procedure which randomly reassigned the taxa associated with each subject, then performed regressions between the permuted taxa and the LF:SF % change. (For example, in a permutation the taxa that were associated with subject 04, could be assigned to patient 28; for 15 subjects, the number of possible permutations for this procedure is 15! or 1.31E+12.) We then selected the two taxa that had regressions with the highest R^2 values for each permutation and used those taxa in a PCA. We then computed the R^2 of the first component of that PCA against the LF:SF ratio % change. We ran the permutation procedure one million times. The "permuted p-value" reported from this process is the fraction of times that we observed an R^2 with a value greater than or equal to the R^2 we observed in the unpermuted data. To correct for over-fitting in our model in Figure 5D, we performed a similar set of one million permutations to those described for Figure 5C. We maintained the correct SNP genotype assignment for each subject while reassigning taxa to different subjects, as before. The reported permutation based p-value, therefore, tests the null hypothesis that, given the established relationship between the *PEMT* SNP and fatty liver, inclusion of microbiome composition adds no power to the model. All permutations were conducted using custom JAVA software (source code available on request).

Supplemental References

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Supplemental Tables

Supplemental Table 1

Status Post/ Pre	Study #	Age	BMI	Race Ethnicity	PEMT	Depletion Status		Start Date (Day 1)	End Date (Final Study)	Total Days in Study
						Depleted Y/N	LF:SF % Change			
Post	28	73	22.2	White	HET	Y	54.4	1/5/08	3/6/08	62
Post	29	61	27.8	White	WT	N	-14.6	1/7/08	3/8/08	62
Post	30	67	30.5	Black	HET	Y	33.1	3/9/08	5/9/08	62
Post	31	58.0	25.3	White	HET	N	3.1	3/12/08	5/12/08	62
Post	32	56	28.3	White	HO	Y	48.5	3/12/08	5/27/08	77
Post	33	52	28.3	Black	WT	N	-17.6	3/26/08	5/26/08	62
Post	34	61	26.5	White	HET	Y	56.3	5/14/08	7/7/08	55
Post	36	50	29.5	Hispanic	HET	N	-8.8	5/28/08	7/27/08	61
Post	37	78	22.9	Hispanic	HET	Y	38.5	7/16/08	9/29/08	76
Post	38	59	23.3	White	HET	Y	44.2	7/16/08	8/25/08	41
Post	39	61	21.4	White	HET	Y	30.3	8/6/08	10/20/08	76
Pre	03	20	25.4	White	HET	N	23.6	5/4/08	7/4/08	62
Post	41	69	26.6	White	WT	N	18.8	9/3/08	11/3/08	62
Post	42	71	26.9	White	WT	N	2	9/20/08	11/20/08	62
Pre	04	49	30.5	Black	WT	N	-5.2	5/28/08	7/28/08	62

Table 1. Descriptive information by Subject. For the *PEMT* SNP (promoter region: 12325817), WT = Wild Type, HET = Heterozygous, HO = Homozygous.

Supplemental Table 2: Nutrient Content in Sample Baseline, Depletion and Repletion Diets

	Breakfast			Lunch		
Nutrients*	Baseline	Depletion	Repletion	Baseline	Depletion	Repletion
Choline (mg)	129.10	13.14	243.69	150.45	10.54	152.60
Energy (kcal)	484.29	474.29	377.72	700.47	657.64	640.33
Pro (g)	7.12	13.79	9.70	16.45	15.16	18.66
Fat (g)	18.64	15.65	11.14	24.05	21.70	27.17
Carb (g)	72.65	72.82	59.35	103.58	104.63	78.70
Folate (mcg)	72.24	20.01	100.30	157.28	4.80	158.08
Vitamin B-12 (mcg)	0.00	0.00	0.64	0.17	0.17	0.24
Met (g)	0.06	0.00	0.06	0.32	0.13	0.37
Cys (g)	0.07	0.00	0.06	0.20	0.03	0.22
Na (mg)	546.49	266.38	530.36	2560.95	191.20	2709.85
Water (g)	29.78	244.72	308.24	215.45	116.45	219.36
Fiber - total dietary (g)	3.25	2.38	1.18	1.62	1.44	1.62
Ca (mg)	29.83	12.61	43.15	187.40	147.95	242.60
P (mg)	293.67	117.47	263.99	389.20	111.20	440.50
K (mg)	283.45	743.51	274.11	275.14	652.16	291.54
Fe (mg)	2.79	1.24	2.54	5.71	0.28	5.72
Zn (mg)	1.05	0.85	0.85	1.81	0.66	2.09
Mg (mg)	80.79	74.90	21.86	35.49	9.20	38.19
Cu (mg)	0.14	0.12	0.12	0.37	0.04	0.37
Mn (mg)	1.30	1.61	0.78	0.57	0.09	0.57
Se (mcg)	18.69	8.68	25.81	36.22	3.23	37.66
Vit C (mg)	0.02	0.02	0.27	0.02	25.44	0.02
Thiamin (mg)	0.37	0.14	0.27	0.49	0.02	0.50
Riboflavin (mg)	0.24	0.07	0.45	0.56	0.11	0.59
Niacin (mg)	2.64	0.21	1.92	5.42	0.24	5.43
Pantothenic acid (mg)	0.36	0.20	0.99	0.83	0.20	0.88
Vit B-6 (mg)	0.05	0.03	0.10	0.06	0.05	0.07
Vit A (IU) (IU)	764.76	382.20	436.15	2237.49	617.10	2333.59
Vit E (mg_ATE)	2.43	1.11	0.76	1.32	1.11	1.32
Vit D (IU)	0.00	0.00	17.27	0.00	2.40	0.00
Cholesterol (mg)	0.00	0.00	211.50	46.16	21.00	55.56
Vitamin K (mcg)	12.32	6.51	3.26	8.39	6.11	8.66
Protein (%)	5.85	11.32	10.30	9.45	8.99	11.78
Fat (%)	34.45	28.90	26.64	31.07	28.96	38.57
Carbohydrate (%)	59.70	59.77	63.06	59.48	62.06	49.65
Alcohol (%)	0.00	0.00	0.00	0.00	0.00	0.00

*Nutrient values do not include supplements provided to subjects (supplemental methods, study diets)

Supplemental Table 2 (cont.)

Nutrients*	4PM Snack			Dinner		
	Baseline	Depletion	Repletion	Baseline	Depletion	Repletion
Choline (mg)	95.19	2.09	189.67	206.39	19.85	227.68
Energy (kcal)	207.93	220.54	316.32	599.83	521.09	581.57
Pro (g)	1.18	0.16	2.25	24.48	20.11	17.86
Fat (g)	5.00	5.72	10.00	11.02	7.66	11.12
Carb (g)	39.55	43.40	53.61	100.91	92.66	103.17
Folate (mcg)	0.00	0.00	0.00	257.01	89.19	223.70
Vitamin B-12 (mcg)	0.00	0.00	0.00	0.22	0.10	0.14
Met (g)	0.00	0.00	0.00	0.60	0.27	0.40
Cys (g)	0.00	0.00	0.00	0.30	0.15	0.21
Na (mg)	9.48	9.48	9.48	708.16	358.23	556.15
Water (g)	211.17	211.17	211.17	386.85	163.20	361.06
Fiber - total dietary (g)	0.00	0.00	0.00	6.76	0.69	6.33
Ca (mg)	7.11	7.11	7.11	86.30	22.12	76.91
P (mg)	30.81	30.81	30.81	414.80	115.87	287.79
K (mg)	61.11	6.76	119.85	735.64	646.43	684.49
Fe (mg)	0.05	0.05	0.05	4.49	2.19	3.44
Zn (mg)	0.02	0.02	0.02	2.21	1.12	1.74
Mg (mg)	2.37	2.37	2.37	63.35	25.07	54.16
Cu (mg)	0.01	0.01	0.01	0.26	0.12	0.22
Mn (mg)	0.04	0.04	0.04	1.02	0.73	0.92
Se (mcg)	0.24	0.24	0.24	37.51	19.60	26.21
Vit C (mg)	0.00	0.00	0.00	103.35	0.03	103.35
Thiamin (mg)	0.00	0.00	0.00	0.51	0.26	0.39
Riboflavin (mg)	0.00	0.00	0.00	0.44	0.06	0.33
Niacin (mg)	0.00	0.00	0.00	8.10	3.83	5.67
Pantothenic acid (mg)	0.00	0.00	0.00	1.79	0.73	1.55
Vit B-6 (mg)	0.00	0.00	0.00	0.67	0.25	0.55
Vit A (IU) (IU)	0.00	0.00	0.00	2778.25	1.15	2587.30
Vit E (mg_ATE)	0.00	0.00	0.00	1.33	0.01	0.67
Vit D (IU)	0.00	0.00	0.00	0.00	0.00	0.00
Cholesterol (mg)	0.00	0.00	0.00	41.59	16.69	24.99
Vitamin K (mcg)	0.00	0.00	0.00	175.12	0.16	172.30
Protein (%)	2.27	0.29	2.87	16.30	15.47	12.23
Fat (%)	21.65	22.80	28.72	16.51	13.26	17.13
Carbohydrate (%)	76.08	76.91	68.41	67.19	71.28	70.65
Alcohol (%)	0.00	0.00	0.00	0.00	0.00	0.00

*Nutrient values do not include supplements provided to subjects (supplemental methods, study diets)

Supplemental Table 2 (cont.)

Nutrients*	Bedtime Snack			Total		
	Baseline	Depletion	Repletion	Baseline	Depletion	Repletion
Choline (mg)	3.38	3.38	10.28	584.51	49.00	823.92
Energy (kcal)	230.66	150.08	106.68	2223.17	2023.64	2022.61
Pro (g)	1.96	1.96	2.55	51.19	51.18	51.01
Fat (g)	9.69	9.69	0.98	68.39	60.41	60.42
Carb (g)	35.60	14.81	22.18	352.28	328.33	317.02
Folate (mcg)	12.60	12.60	47.88	499.12	126.60	529.95
Vitamin B-12 (mcg)	0.00	0.00	0.00	0.39	0.27	1.03
Met (g)	0.03	0.03	0.05	1.00	0.43	0.87
Cys (g)	0.02	0.02	0.05	0.60	0.20	0.55
Na (mg)	182.91	173.32	487.20	4008.00	998.62	4293.05
Water (g)	216.75	699.83	700.22	1060.00	1435.38	1800.05
Fiber - total dietary (g)	1.26	1.26	0.90	12.89	5.76	10.02
Ca (mg)	13.83	104.72	108.08	324.46	294.50	477.84
P (mg)	46.20	46.20	31.64	1174.68	421.55	1054.72
K (mg)	359.37	357.00	40.88	1714.71	2405.87	1410.87
Fe (mg)	0.88	0.46	1.21	13.91	4.22	12.96
Zn (mg)	0.42	0.31	0.24	5.51	2.96	4.94
Mg (mg)	21.13	18.76	9.80	203.14	130.30	126.39
Cu (mg)	0.13	0.09	0.07	0.91	0.38	0.81
Mn (mg)	0.15	0.12	0.50	3.09	2.60	2.82
Se (mcg)	2.51	2.27	1.62	95.16	34.01	91.54
Vit C (mg)	8.71	8.71	0.00	112.10	34.20	103.65
Thiamin (mg)	0.05	0.05	0.13	1.42	0.46	1.28
Riboflavin (mg)	0.06	0.06	0.17	1.29	0.29	1.55
Niacin (mg)	1.07	1.07	1.47	17.24	5.35	14.49
Pantothenic acid (mg)	0.11	0.11	0.08	3.09	1.24	3.50
Vit B-6 (mg)	0.18	0.18	0.03	0.96	0.51	0.75
Vit A (IU) (IU)	0.00	0.00	0.00	5780.51	1000.45	5357.05
Vit E (mg_ATE)	1.37	1.37	0.00	6.45	3.60	2.76
Vit D (IU)	0.00	0.00	0.00	0.00	2.40	17.27
Cholesterol (mg)	0.00	0.00	0.00	87.75	37.69	292.05
Vitamin K (mcg)	0.00	0.00	0.25	195.83	12.79	184.47
Protein (%)	3.30	5.08	9.46	9.18	9.93	10.12
Fat (%)	36.72	56.52	8.19	27.61	26.37	26.97
Carbohydrate (%)	59.97	38.40	82.35	63.21	63.70	62.90
Alcohol (%)	0.00	0.00	0.00	0.00	0.00	0.00

*Nutrient values do not include supplements provided to subjects (supplemental methods, study diets)

Supplemental Table 3: MRI Measurement Values for Liver Fat and Spleen Fat

Subject	Study Day	In/out Liver MRI mean	In/out Spleen MRI mean	Ratio L/S	% change B1 to D2
28	Baseline	107.3	55.9	1.92	
	Depletion	164.2	55.4	2.96	54.4
29	Baseline	97.3	52.3	1.86	
	Depletion	84.2	53.0	1.59	-14.6
30	Baseline	93.7	61.4	1.53	
	Depletion	129.8	63.9	2.03	33.1
31	Baseline	105.1	66.1	1.59	
	Depletion	102.1	62.3	1.64	3.1
32	Baseline	106.8	60.1	1.78	
	Depletion	161.0	61.0	2.64	48.5
33	Baseline	111.6	63.3	1.76	
	Depletion	90.1	62.0	1.45	-17.6
34	Baseline	104.1	79.9	1.30	
	Depletion	164.1	80.6	2.04	56.3
36	Baseline	101.2	75.4	1.34	
	Depletion	97.4	79.6	1.22	-8.8
37	Baseline	100.4	71.2	1.41	
	Depletion	142.6	73.0	1.95	38.5
38	Baseline	120.6	73.4	1.64	

	Depletion	171.3	72.3	2.37	44.2
39	Baseline	98.6	77.0	1.28	
	Depletion	119.8	71.8	1.67	30.3
03	Baseline	102.5	68.1	1.51	
	Depletion	136.2	73.2	1.86	23.6
41	Baseline	145.6	56.1	2.60	
	Depletion	179.2	58.1	3.08	18.8
42	Baseline	100.2	75.8	1.32	
	Depletion	102.9	76.3	1.35	2.0
04	Baseline	94.4	57.7	1.64	
	Depletion	100.8	65.0	1.55	-5.2

Table 3. Components of the B1 to D2 Liver Fat to Spleen Fat (LF:SF) ratio percent change. For each subject, MRI measurements of liver fat values, spleen fat values and the ratio of liver fat to spleen fat are provided for Baseline and Depletion time points. The percentage change in the LF:SF ratio from B1 to D2 is also calculated.

Supplemental Table 4

Patient #	Usable Samples	Usable Sequences
03	B1, B2, D1, D2, R	8,819
04	B1, B2, D2	7,578
28	B1, B2, D1, D2, R	18,486
29	B1, B2	10,299
30	B1, B2, D1, D2, R	14,448
31	B1, B2, D1, D2, R	6,571
32	B1, B2, D1, D2, R1, R2	14,838
33	B1, B2, D1, D2, R	8,933
34	B1, B2, D1, D2, R1, R2	9,887
36	B1, B2, D1, D2, R	13,321
37	B1, B2, D1, D2, R1, R2	14,970
38	B1, B2, D1, D2, R	13,763
39	B1, B2, D1, D2, R1, R2	20,698
41	B1, B2, D1, D2, R	24,894
42	B1, B2, D1, D2, R	7,366
Total		194,871
Patients		15
Samples		74
Sequences Generated		213,375
Usable Sequences		194,871
OTUs at 97%		4,857

Table 4. Subject and sample descriptive statistics.

Usable sequences met quality standards: no Ns in sequence, exact match to 5' primer, exact match to barcode

tag, no low quality sequence beyond 5' primer. Sequences that passed quality standards and that had a length 180 nt to 280 nt, after quality trimming, were retained.

Supplemental Table 5

Class	R^2	Direction	p-value	adjusted p-value
Gammaproteobacteria	0.5679	negative	0.00118	0.01062
Erysipelotrichi	0.3822	positive	0.01403	0.06314
Deltaproteobacteria	0.1262	positive	0.19383	0.58149
Bacteroidia	0.0992	negative	0.25290	0.56903
Clostridia	0.0253	positive	0.57110	1.02798
Flavobacteria	0.0246	negative	0.57674	0.86511
Betaproteobacteria	0.0062	negative	0.78000	1.00286
Actinobacteria	0.0040	positive	0.82268	0.92552
Bacilli	0.0022	positive	0.86759	0.86759

Table 5. Results from regressions showing predictive value of B1 bacteria abundance for choline deficiency induced fatty liver development.

Correlations, p-values and adjusted p-values from regressions testing the null hypothesis that the slope of the linear relationship between B1 abundance levels of each class and the percentage change in the liver fat to spleen fat ratio from time point B1 (study initiation) to D2 (maximum subject choline deficiency) is zero. The adjusted p-value (see methods) corrects the p-value for multiple comparisons (n=9) and shows that only Gammaproteobacteria B1 abundance shows a significant linear correlation to liver fat change at a false discovery rate of 0.05.

Supplemental Table 6

Unifrac Environmental Distance P-Values

Patient	B1	B2	D1	D2	R1	R2
28	<=0.01	0.38	1.00	0.99	0.99	***
30	<=0.01	0.99	0.97	.081	1.00	***
31	0.76	1.00	0.96	0.52	<=0.01	***
32	0.60	0.63	0.05	0.59	0.89	0.41
33	<=0.01	0.99	0.74	0.75	0.97	***
34	<=0.01	0.96	1.00	0.81	0.83	0.17
36	0.89	0.05	0.79	0.74	0.97	***
37	0.12	0.93	0.97	0.29	0.09	1.00
38	0.70	0.89	0.58	0.58	0.64	***
39	0.01	0.91	0.98	0.88	0.91	0.17
3	0.06	0.49	0.39	0.57	0.96	***
41	0.02	0.70	0.86	0.71	0.98	***
42	0.27	0.35	0.58	0.39	0.98	***
4	0.83	0.90	***	0.87	***	***

Table 6. P-values based on Unifrac analysis of samples within subject. The p-value represents the probability that a sample has more unique phylogenetic branch lengths that would be expected by chance. Low values indicate that the sample is different from the other patient samples. Asterisks designate missing samples. Noteworthy are the B1 samples from patients 28, 30, 33 and 34, where baseline samples are distinct.

Supplemental Table 7

Classes	B1 to B2		D2 to R		B1 to D2		B1 to R	
	p-value	adjusted p-value	p-value	adjusted p-value	p-value	adjusted p-value	p-value	adjusted p-value
Gammaproteobacteria	0.727	0.793	0.006	0.216	0.523	0.856	0.041	0.492
Betaproteobacteria	0.074	0.533	0.008	0.144	0.221	0.530	0.175	0.525
Bacilli	0.059	0.531	0.627	0.806	0.171	0.560	0.079	0.406
Clostridia	0.078	0.468	0.841	0.841	0.205	0.527	0.124	0.496
Deltaproteobacteria	0.528	0.836	0.665	0.798	0.373	0.707	0.086	0.387
Bacteroidia	0.781	0.803	0.503	0.862	0.151	0.544	0.185	0.512
Erysipelotrichi	0.659	0.818	0.610	0.845	0.239	0.538	0.307	0.650
Actinobacteria	0.326	0.652	0.699	0.812	0.595	0.857	0.440	0.792
Flavobacteria	0.725	0.816	0.551	0.827	0.615	0.820	0.755	0.799

Table 7. Significance of changes in bacterial frequencies from one time point to another. P-values and adjusted p-value (see methods) from paired t-tests of the null hypothesis that there was no change between time points in logged standardized sequence abundance for each bacterial class. P-value indicates the probability that the difference could be as significant by chance. Adjusted p-value corrects the p-value for multiple comparisons (n=36) and shows that no difference is significant at a false discovery rate of 0.05.

Supplemental Table 8: Multivariate Regression Effect Testing

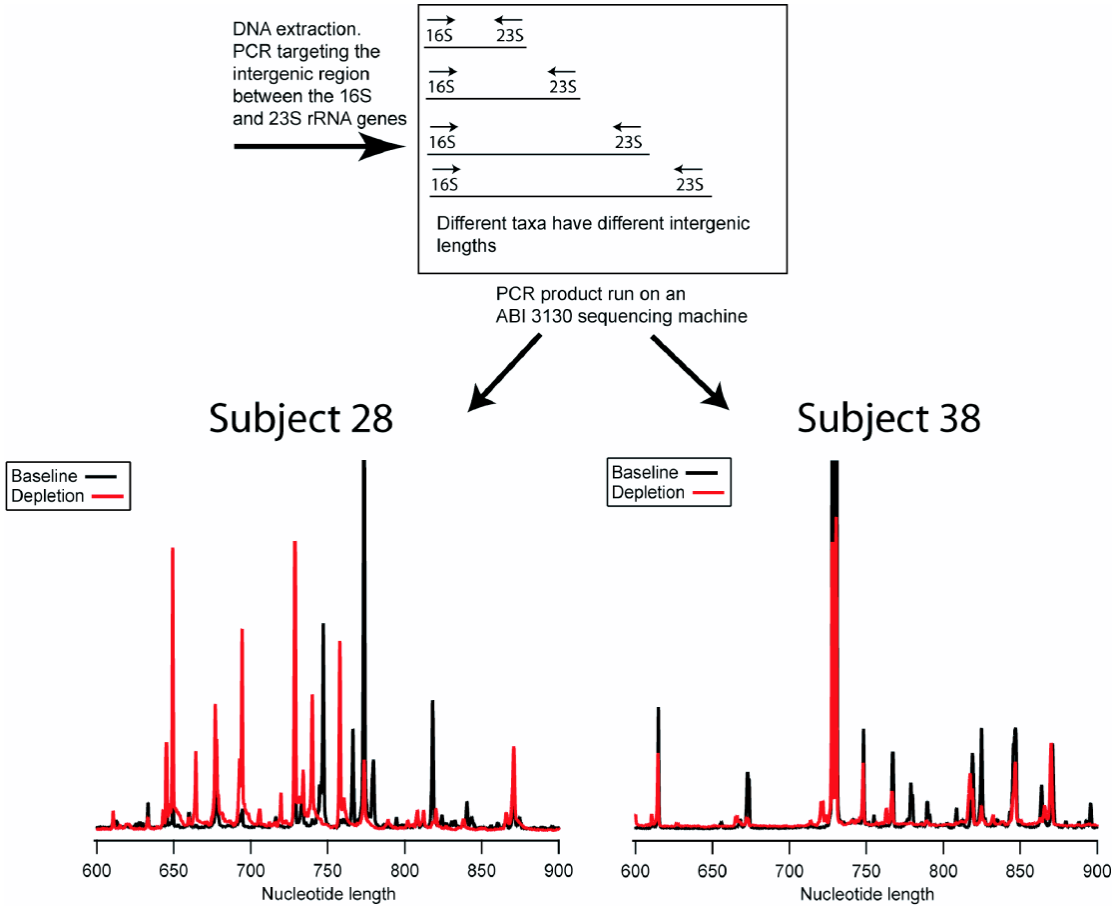
	Sum of Squares	F Ratio	Probability > F
Model 1: R^2=.6545 *P*=.0017			
% change LF:SF ratio against:			
Gammaproteobacteria	2509.47	9.46	0.0096
Erysipelotrichi	798.67	3.01	0.1083
Model 2: R^2=.8716 *P*=3.3E-5			
% change LF:SF ratio against:			
Gammaproteobacteria	1402.09	13.04	0.0041
Erysipelotrichi	711.16	6.61	0.0260
PEMT	2000.6	18.6	0.0012

Table 8. Multivariate regression effect testing shows contribution of each factor to model. Probability > F is the probability that if the null hypothesis is true, a larger F-statistic would occur due to random error – the probability that the actual effect is zero. Model 1 considers only the taxa Gammaproteobacteria and Erysipelotrichi. Model 2 considers both taxa as well as host genotype for PEMT

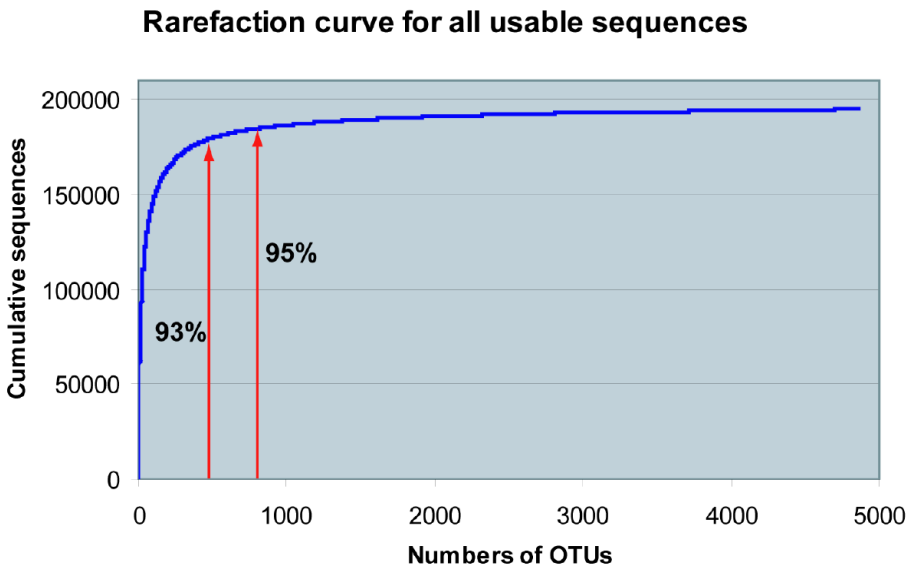
Supplemental Table 9: Weight of Each Subject at Baseline (B1), Depletion (D2) and Repletion (R) Time Points

Subject	Baseline (B1) Weight (kg)	Depletion (D2) Weight (kg)	Repletion (R1) Weight (kg)
28	70.6	71.7	72.5
29	77.6	80.6	81.0
30	75.5	76.0	75.8
32	73.3	72.9	71.3
33	72.8	73.8	73.4
34	64.6	65.6	64.6
36	80.9	82.1	83.4
37	53.5	54.4	54.6
38	63.7	62.3	62.5
39	51.0	51.8	52.3
03	71.2	71.5	72.3
41	63.7	64.4	64.8
42	70.9	72.8	71.7
04	84.0	80.8	81.9

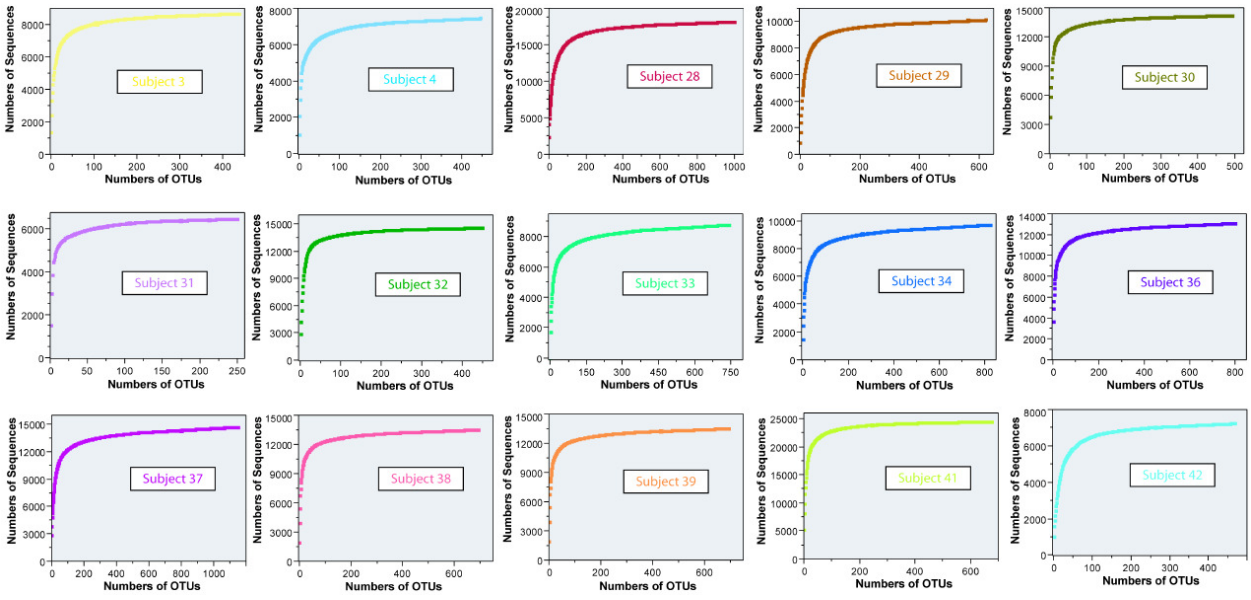
Supplemental Figures



Supplemental Figure 1. Example of Automated Ribosomal Intergenic Spacer Analysis. An ARISA experiment showing changes in the microbial community during the initial baseline stage, when subjects have entered the hospital are placed on a controlled diet and a choline depletion stage when subjects are placed on a low-choline diet.



Supplemental Figure 2. Rarefaction curve for all samples. Rarefaction curve shows that sampling saturates the sequence space. The red arrows indicate the numbers of OTUs at which 93% (566 OTUs) and 95% (843 OTUs) of total sequences are under the curve.



Supplemental Figure 3. Rarefaction curves by patient across samples. Curves illustrate the differing levels of saturation.