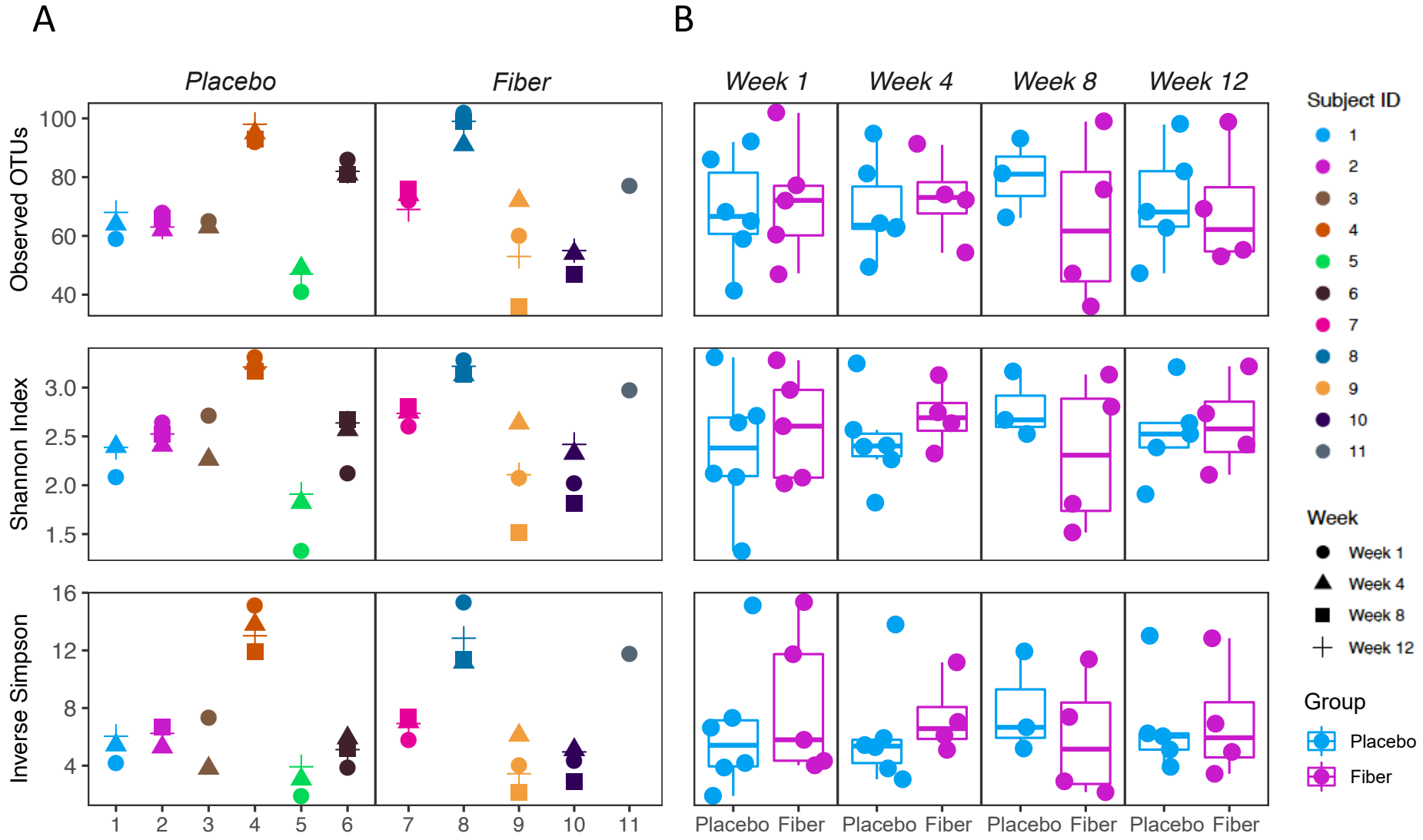
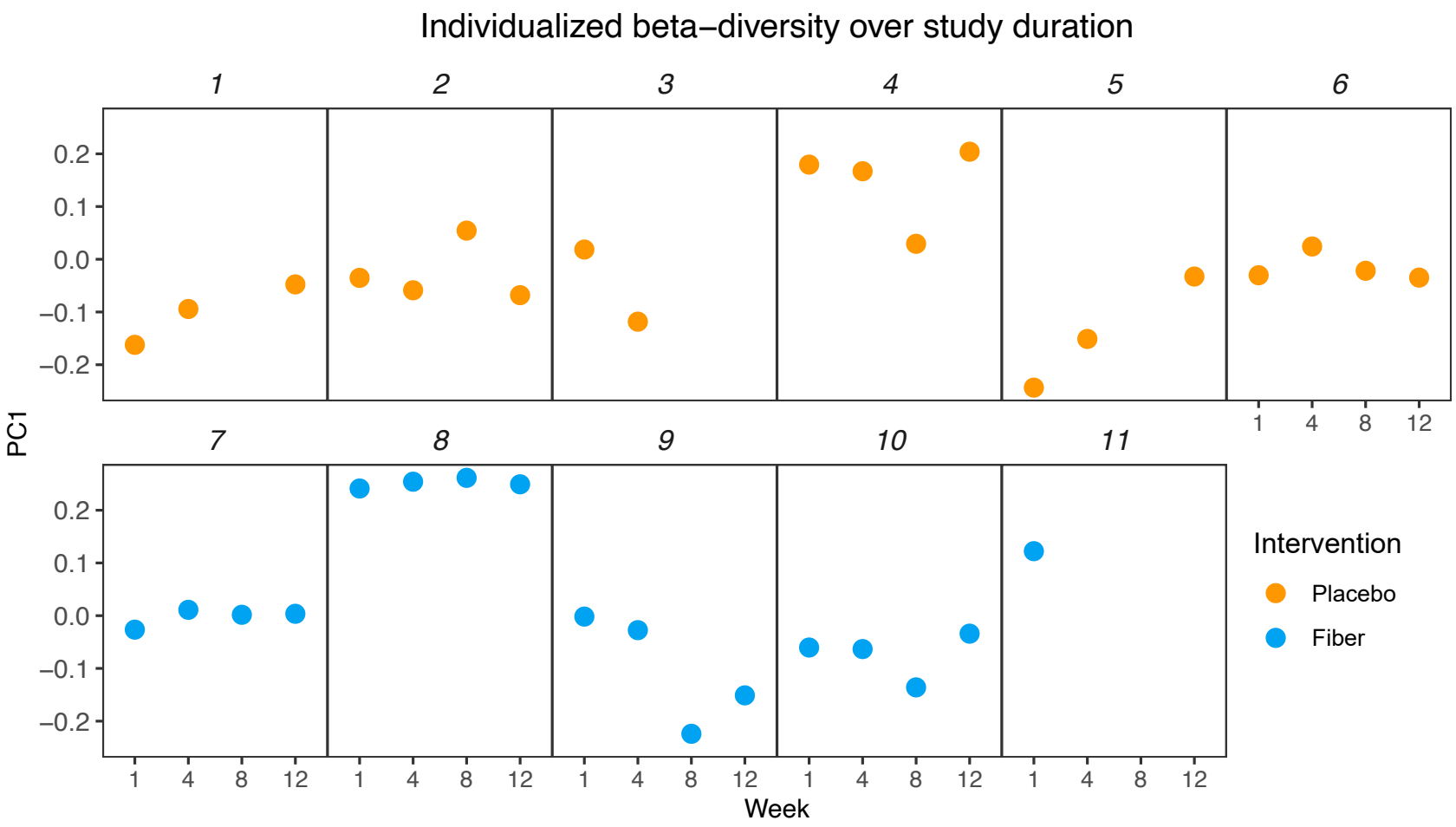
Figures

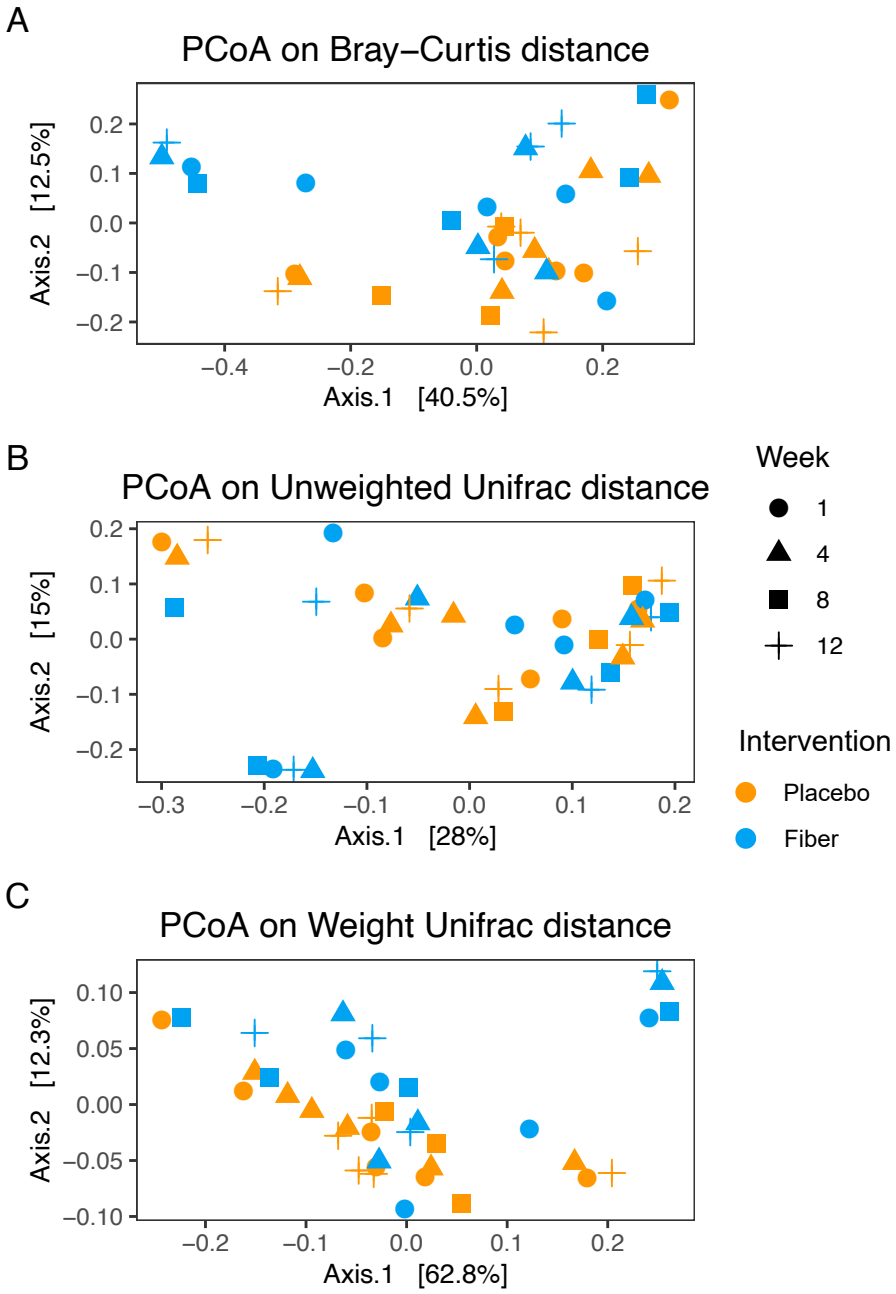
Fiber-Microbiome Study

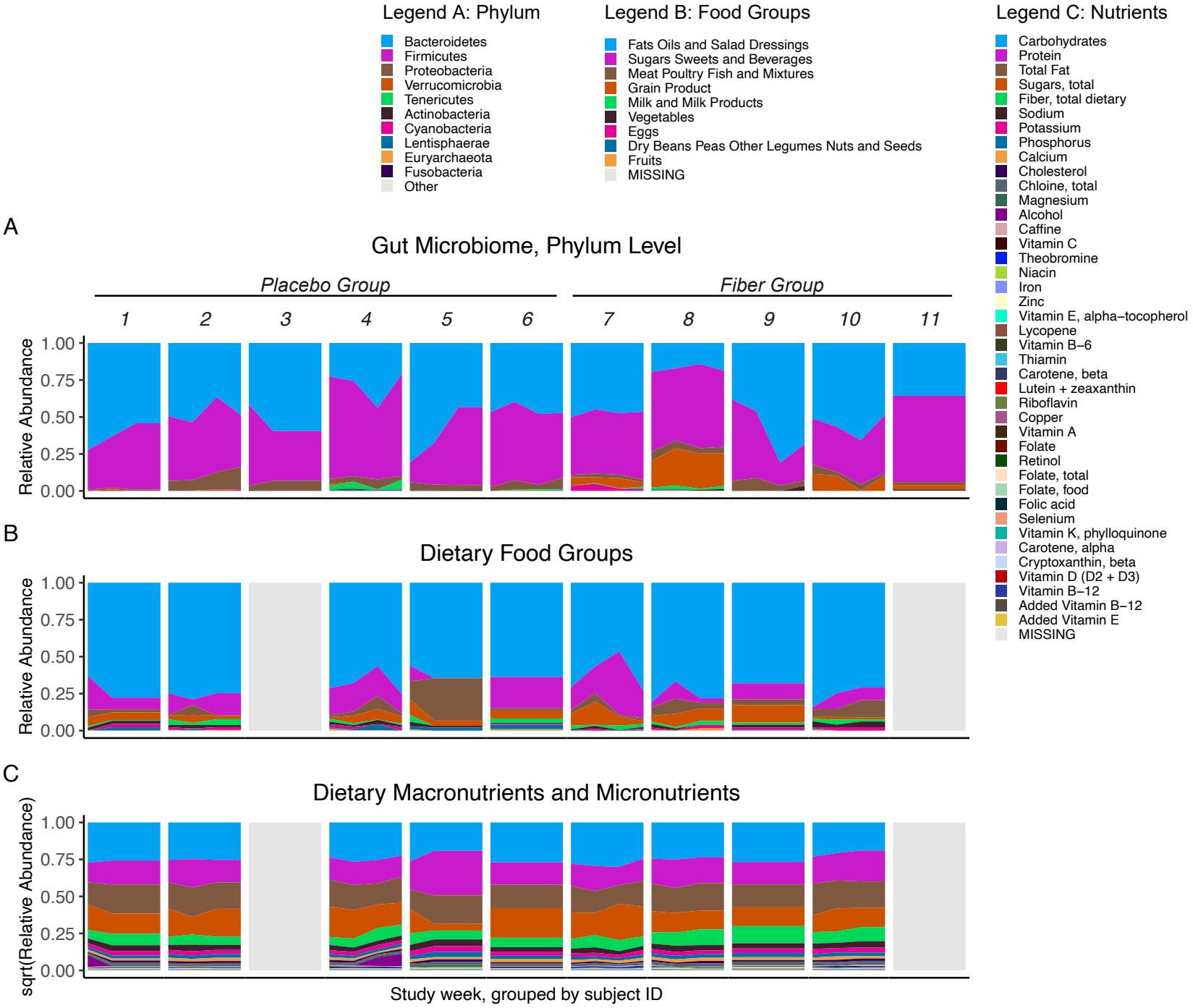
Questions to be addressed

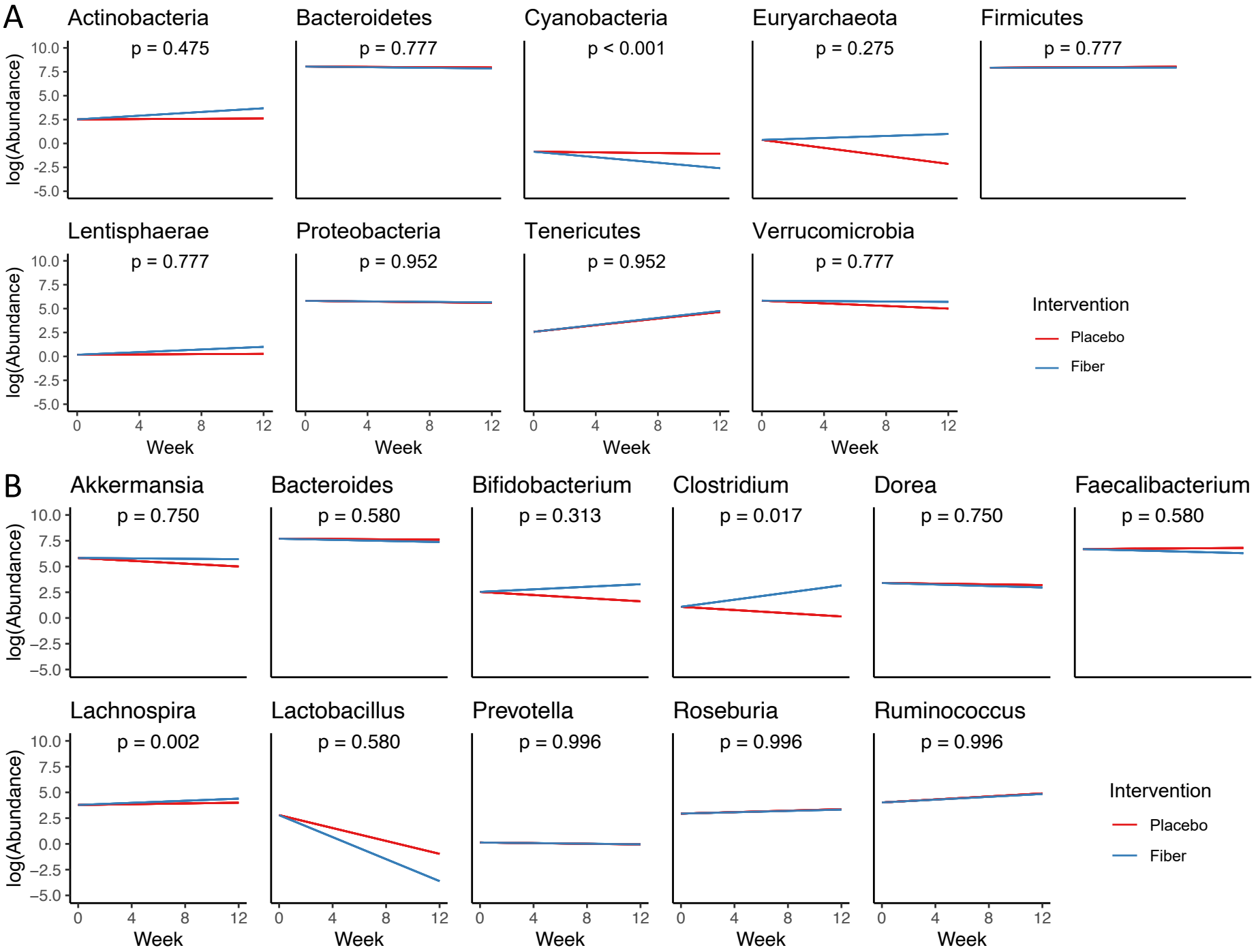
- What is the effect of Prebiotin vs placebo on the gut microbiome (controlling for, age, ethnicity)
  - Stability over time - pre vs post intervention (Johnson paper – Fig. 6E)
  - Diversity over time - pre vs post intervention
    - Alpha – **FIG 1**
    - Beta – **FIG 2/3**
    - Phylogenetic - **FIG 4**
- What is the effect of Prebiotin vs placebo on anthropometrics (controlling for diet, age, ethnicity)- Did the intervention mitigate excess weight gain? NO EFFECTS --> **Table 3**
  - BMI
  - Lean mass
  - Fat mass
  - Weight, overall
  - Weight change
- What is the effect of Prebiotin vs placebo on measures of stress
  - What is the correlation between the microbiome and stress? **Table 3**
- What is the effect of Prebiotin vs placebo on clinical variables (blood measures - lipids, PYY) **Table 3**


A
B
Placebo
Fiber
Week 1
Week 4
Week 8
Week 12
Observed OTUs
Shannon Index
Inverse Simpson
100
80
60
40
3.0
2.5
2.0
1.5
16
12
8
4
1
2
3
4
5
6
7
8
9
10
11
Placebo
Fiber
Subject ID
Week
Week 1
Week 4
Week 8
Week 12
Group
Placebo
Fiber

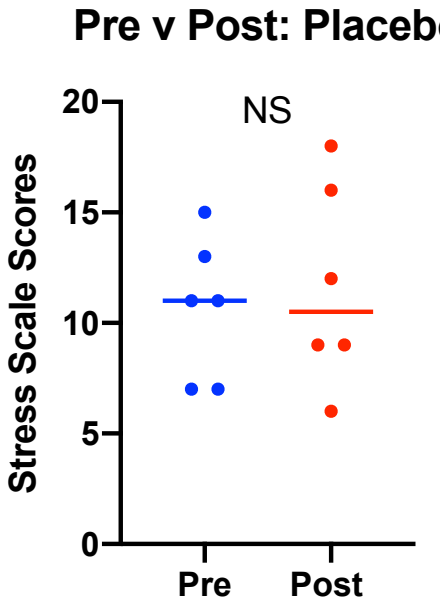

Individualized beta−diversity over study duration
1
2
3
4
5
6
7
8
9
10
11
PC1
0.2
0.1
0.0
−0.1
−0.2
1
4
8
12
Week
Intervention
Placebo
Fiber

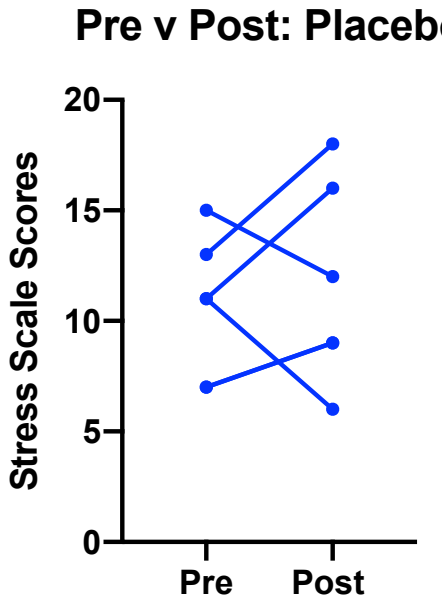

A
PCoA on Bray–Curtis distance
Axis.2 [12.5%]
0.2
0.1
0.0
−0.1
−0.2
−0.4
−0.2
0.0
0.2
Axis.1 [40.5%]
B
PCoA on Unweighted Unifrac distance
Axis.2 [15%]
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.2
−0.1
0.0
0.1
0.2
Axis.1 [28%]
Week
1
4
8
12
Intervention
Placebo
Fiber
C
PCoA on Weight Unifrac distance
Axis.2 [12.3%]
0.10
0.05
0.00
−0.05
−0.10
−0.2
−0.1
0.0
0.1
0.2
Axis.1 [62.8%]


Legend A: Phylum
Bacteroidetes
Firmicutes
Proteobacteria
Verrucomicrobia
Tenericutes
Actinobacteria
Cyanobacteria
Lentisphaerae
Euryarchaeota
Fusobacteria
Other
Legend B: Food Groups
Fats Oils and Salad Dressings
Sugars Sweets and Beverages
Meat Poultry Fish and Mixtures
Grain Product
Milk and Milk Products
Vegetables
Eggs
Dry Beans Peas Other Legumes Nuts and Seeds
Fruits
MISSING
Legend C: Nutrients
Carbohydrates
Protein
Total Fat
Sugars, total
Fiber, total dietary
Sodium
Potassium
Phosphorus
Calcium
Cholesterol
Chloine, total
Magnesium
Alcohol
Caffine
Vitamin C
Theobromine
Niacin
Iron
Zinc
Vitamin E, alpha−tocopherol
Lycopene
Vitamin B−6
Thiamin
Carotene, beta
Lutein + zeaxanthin
Riboflavin
Copper
Vitamin A
Folate
Retinol
Folate, total
Folate, food
Folic acid
Selenium
Vitamin K, phylloquinone
Carotene, alpha
Cryptoxanthin, beta
Vitamin D (D2 + D3)
Vitamin B−12
Added Vitamin B−12
Added Vitamin E
MISSING
A
Gut Microbiome, Phylum Level
Placebo Group
Fiber Group
1
2
3
4
5
6
7
8
9
10
11
Relative Abundance
1.00
0.75
0.50
0.25
0.00
B
Dietary Food Groups
Relative Abundance
1.00
0.75
0.50
0.25
0.00
C
Dietary Macronutrients and Micronutrients
sqrt(Relative Abundance)
1.00
0.75
0.50
0.25
0.00
Study week, grouped by subject ID

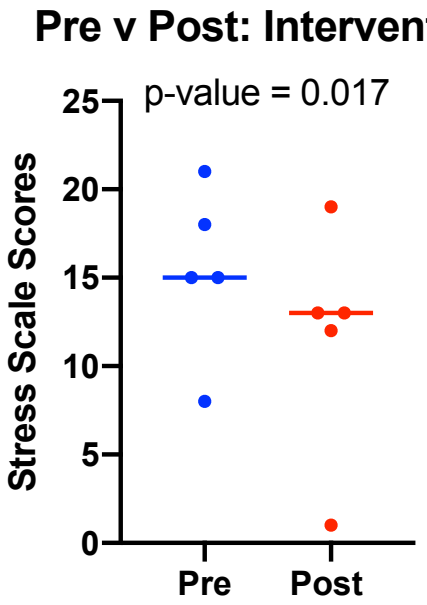

A
Actinobacteria
p = 0.475
Bacteroidetes
p = 0.777
Cyanobacteria
p < 0.001
Euryarchaeota
p = 0.275
Firmicutes
p = 0.777
Lentisphaerae
p = 0.777
Proteobacteria
p = 0.952
Tenericutes
p = 0.952
Verrucomicrobia
p = 0.777
log(Abundance)
10.0
7.5
5.0
2.5
0.0
-2.5
-5.0
0
4
8
12
Week
Intervention
Placebo
Fiber
B
Akkermansia
p = 0.750
Bacteroides
p = 0.580
Bifidobacterium
p = 0.313
Clostridium
p = 0.017
Dorea
p = 0.750
Faecalibacterium
p = 0.580
Lachnospira
p = 0.002
Lactobacillus
p = 0.580
Prevotella
p = 0.996
Roseburia
p = 0.996
Ruminococcus
p = 0.996

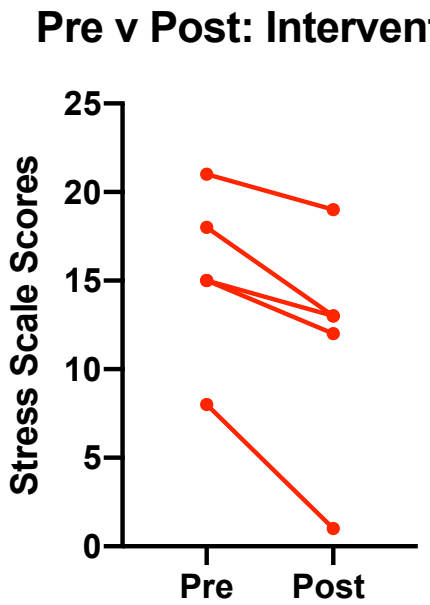
A


Pre v Post: Placebo
NS
Stress Scale Scores
20
15
10
5
0
Pre
Post


Pre v Post: Placebo
Stress Scale Scores
20
15
10
5
0
Pre
Post

B


Pre v Post: Intervention
p-value = 0.017
Stress Scale Scores
25
20
15
10
5
0
Pre
Post


Pre v Post: Intervention
Stress Scale Scores
25
20
15
10
5
0
Pre
Post

Tables

Tables 1 & S1

Table 1. Population Demographics

	Placebo mean (SD)	Intervention mean (SD)	P-value
N	6	5.00	
% Male	33.33	40.00	
Age	27.33(1.5)	28.4(2.6)	0.638
Weight (kg)	68.96(12.19)	73.88(9.23)	0.537
Height (cm)	164.26(10.06)	163.58(6.3)	0.927
BMI	25.45(3.12)	27.51(1.72)	0.247
VFL	8.83(4.3)	10.2(2.94)	0.405
% Body fat	29.75(8.55)	32.34(4.99)	0.464
% Lean fat	26.86(6.9)	27.86(3.99)	0.407
HEI score	89.36(0.44)	89.81(0.36)	0.201

P-values calculted from Student's t-tests

VFL=visceral fat level

Supplemental Table 1

Week	ASA24	FFQ*	Stool Samples	Blood Samples*
Baseline (Pre)	9	9	11	11
4	7	-	10	-
8	4	-	7	-
12 (Post)	1	-	9	11

*FFQ was only given once at baseline

*Blood Samples only collected Pre-Post

Table S2 - Intervention Effect of Change in Alpha Diversity

Table S2: Intervention effect on changes in alpha diversity

Metric	Estimate	Std. Error	df	t value	Pr(>|t|)	p.adjust
Observed	-2.291	2.097	24.504	-1.093	0.285	0.569
Shannon	-0.065	0.072	24.78	-0.905	0.374	0.569
InvSimpson	-0.309	0.382	24.367	-0.807	0.427	0.569

Table S3: Intervention Effect on Change in Clinical Biomarkers

Table S3. Effect of intervention on change in clinical biomarkers

Outcome	Estimate*	Std. Error	t value	Pr(>|t|)	p.adj
Glucose	13.47	7.29	1.85	0.082	0.489
BUN	-3.60	1.83	-1.97	0.065	0.474
Creatinine	-0.07	0.08	-0.91	0.376	0.786
Sodium	-0.70	1.45	-0.48	0.636	0.898
Potassium	0.12	0.34	0.34	0.736	0.903
Chloride	1.47	1.67	0.88	0.393	0.786
Carbon_Dioxid	-1.00	2.02	-0.49	0.628	0.898
Calcium	-0.30	0.23	-1.32	0.206	0.609
Cholesterol	-2.47	30.98	-0.08	0.937	0.957
Triglycerides	38.77	72.27	0.54	0.599	0.898
HDL	2.43	13.11	0.19	0.855	0.929
LDL	-10.13	26.73	-0.38	0.709	0.903
LDL_HDL_Rati	-0.50	0.63	-0.80	0.436	0.804
hsCRP	2.90	2.21	1.32	0.206	0.609
Insulin	-0.27	24.02	-0.01	0.991	0.991
PYY	26.90	160.88	0.17	0.869	0.929

*multivariable model adjusted for age, ethnicity, and stress scores

Table S4

Table S4. Effect of intervention on phylum level relative abundance

Phylum	Estimate	Std. Error	z value	Pr(>|z|)	p.adjust
Actinobacteria	0.356	0.285	1.250	0.211	0.475
Bacteroidetes	-0.032	0.066	-0.486	0.627	0.777
Cyanobacteria	-0.504	0.069	-7.302	< .001	< .001
Euryarchaeota	1.043	0.618	1.687	0.092	0.275
Firmicutes	-0.026	0.047	-0.564	0.573	0.777
Lentisphaerae	0.243	0.509	0.477	0.633	0.777
Proteobacteria	0.018	0.131	0.134	0.893	0.952
Tenericutes	0.035	0.579	0.060	0.952	0.952
Verrucomicrobia	0.236	0.468	0.504	0.614	0.777

Table S5

Table S5. Effect of intervention on changes in relative abundance

Genus	Estimate	Std. Error	z value	Pr(>|z|)	p.adjust
Akkermansia	0.236	0.468	0.504	0.614	0.750
Bacteroides	-0.076	0.085	-0.898	0.369	0.580
Bifidobacterium	0.553	0.368	1.504	0.133	0.313
Clostridium	**1.004**	**0.363**	**2.766**	**0.006**	**0.017**
Dorea	-0.078	0.149	-0.524	0.601	0.750
Faecalibacterium	-0.170	0.183	-0.932	0.351	0.580
Lachnospira	**0.131**	**0.037**	**3.500**	**0.000**	**0.002**
Lactobacillus	-0.883	0.976	-0.906	0.365	0.580
Prevotella	0.003	0.613	0.005	0.996	0.996
Roseburia	-0.011	0.270	-0.043	0.966	0.996
Ruminococcus	-0.018	0.307	-0.059	0.953	0.996

Supplemental Figures

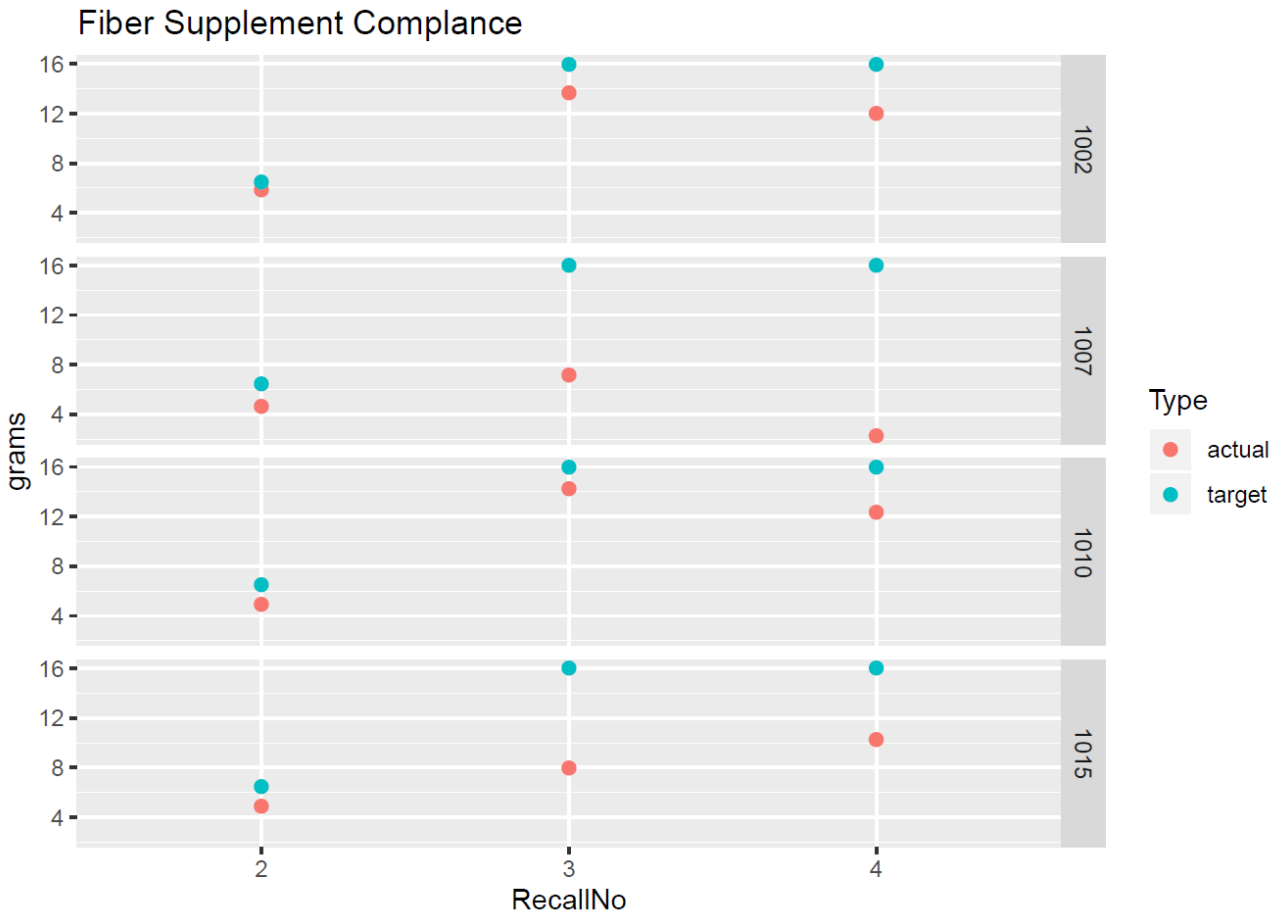


Fig. S1. Fiber Supplement Compliance. *Note: Only participants that received actual supplements are displayed in this chart. Participants receiving placebo were not included.

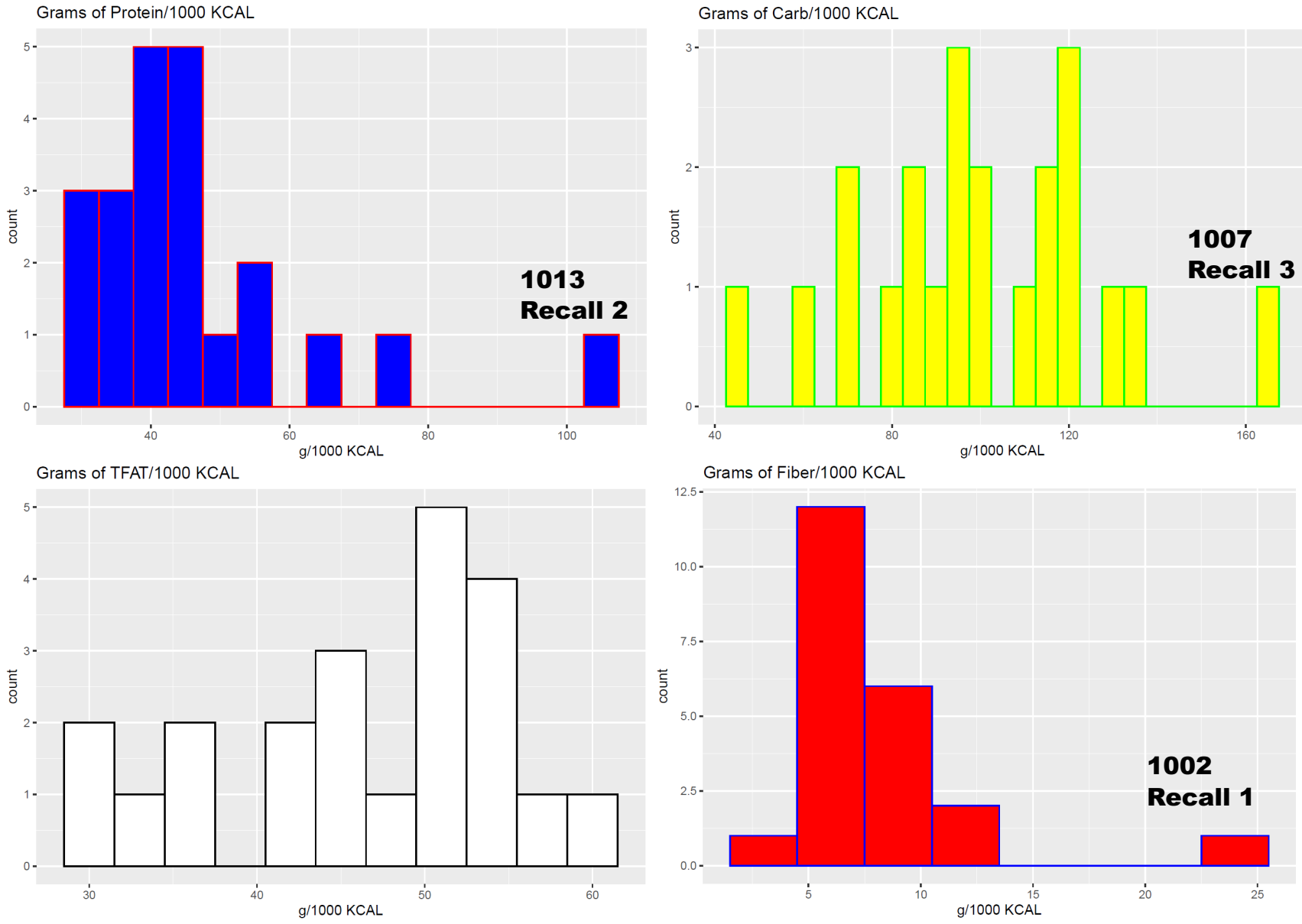


Fig. S2. Assessment of outliers from ASA24 dietary recalls. NHANES interquartile range was used to evaluate outliers along with visual inspection of dietary food reports.

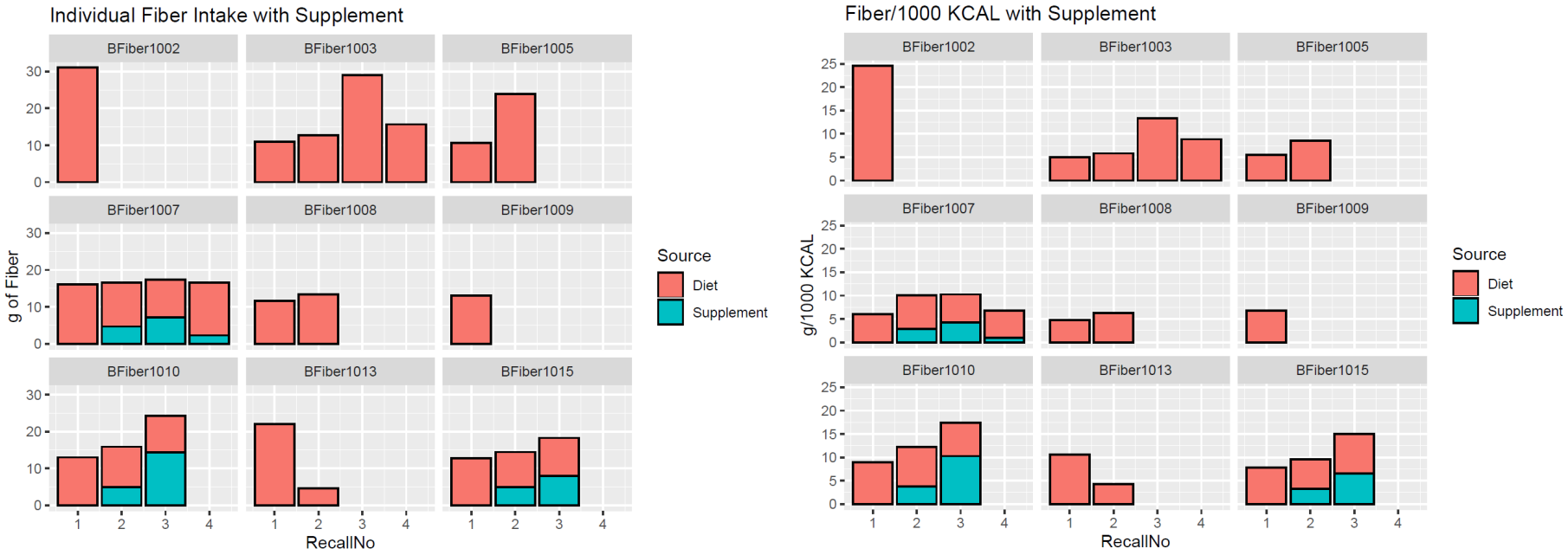


Fig. S3. Individual dietary and fiber supplement intake. Left panel are values by grams of dietary fiber; Right panel is fiber intake per 1000 kcals. *Note: Fiber1002 did not complete ASA24 after Recall 1, thus fiber supplement could not be added. 1007, 1010, and 1015 show supplemental fiber intake. 1003, 1005, 1008, 1009, 1013 received placebo, thus no supplemental fiber was added to their data.

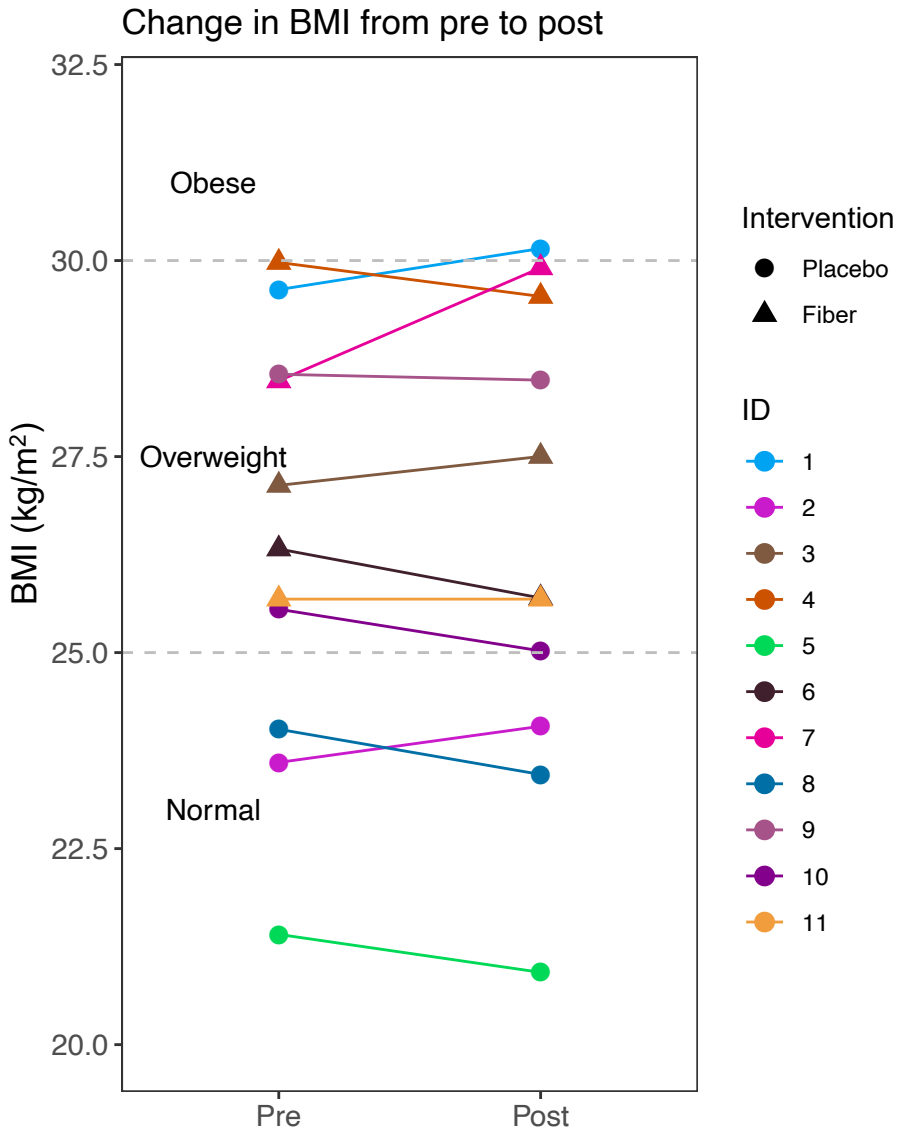
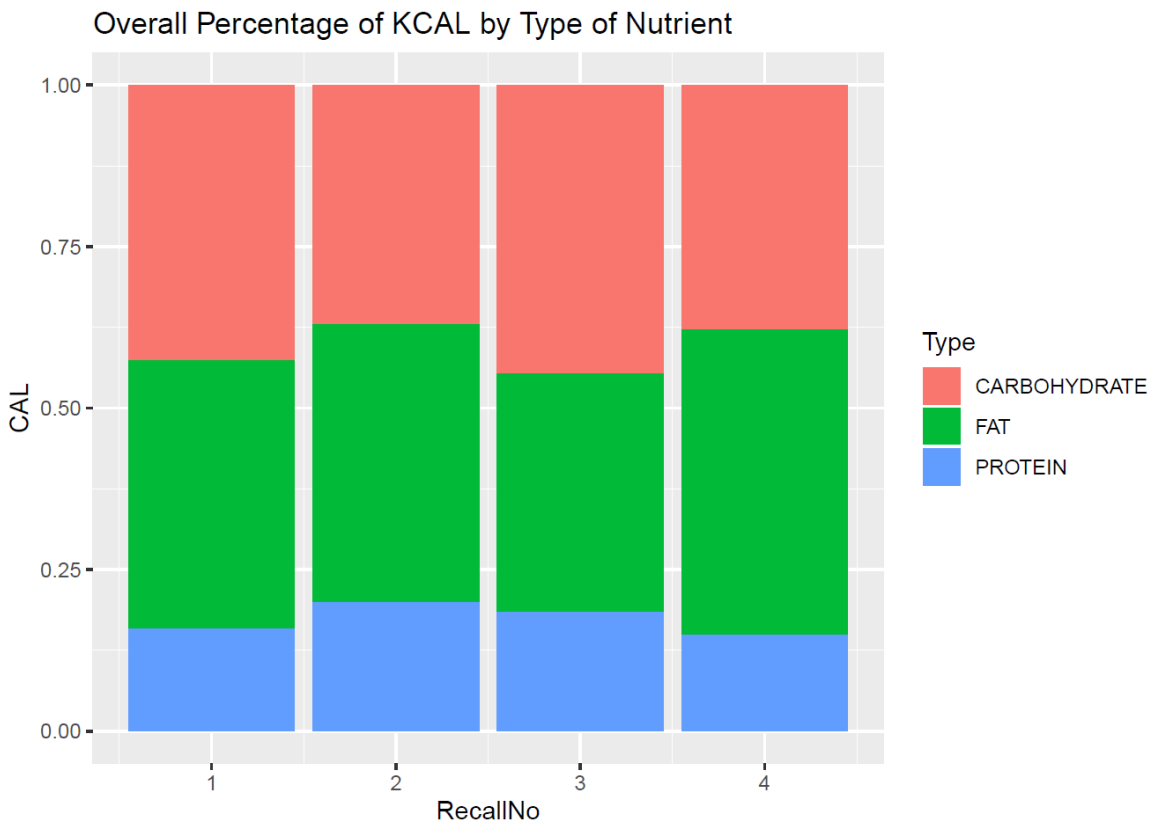


Fig. S4. Individual changes in BMI at baseline at end of the intervention. Red lines are the WHO guidelines for differentiating normal, overweight, and obese BMI.

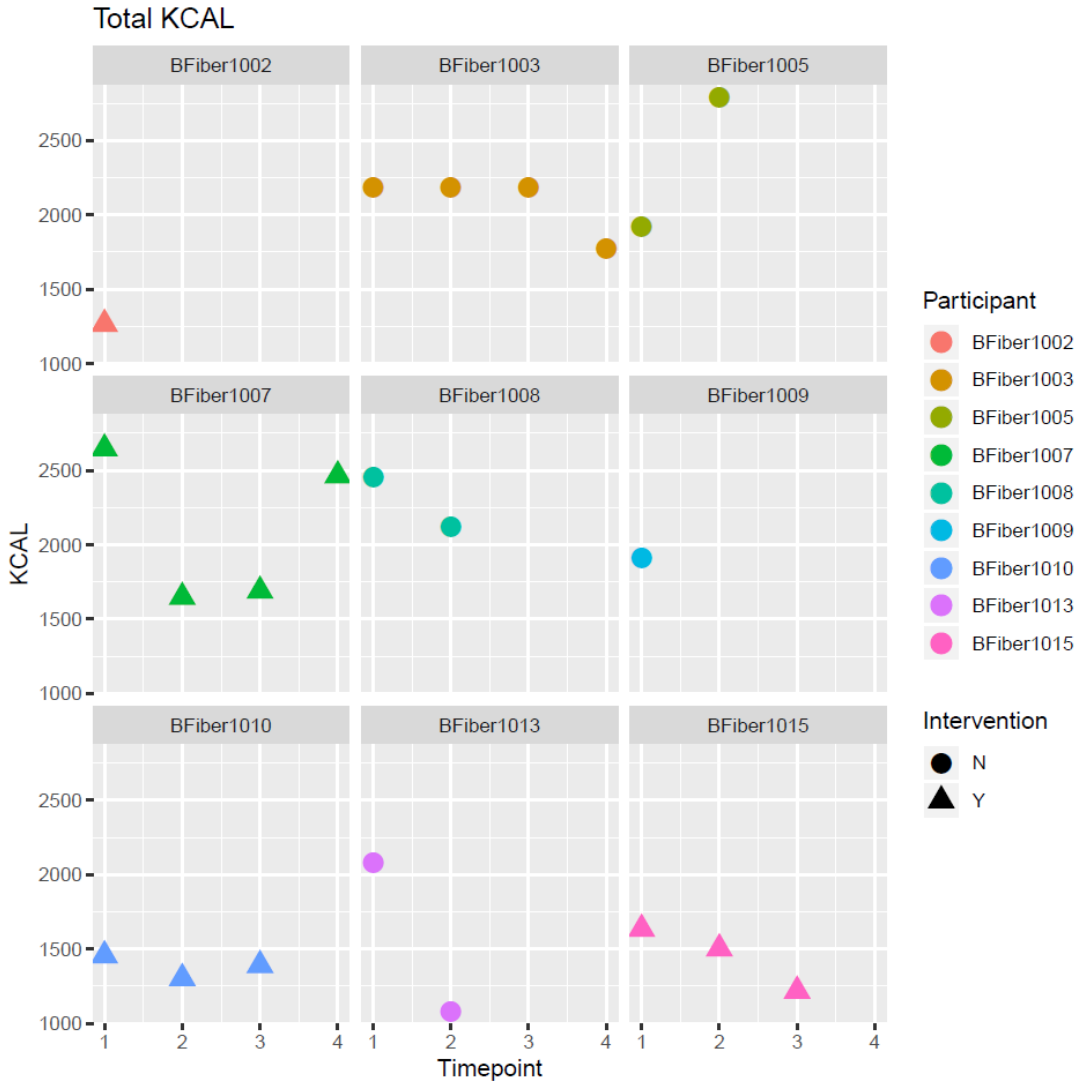
Discussion

- Cookies were not accounted for
  - Some participants logged the cookies. Others did not
  - Those that logged the cookies logged them all differently
  - How would we like to handle this?
- Most participants given supplement were very compliant with the exception of 1007 over the last 4 weeks
- Most of placebo group appeared to be fairly noncompliant during the last 4 weeks with the exception of 1003
- Supplement did appear to make a difference in overall fiber consumption in test participants with the exception of 1007, who was noncompliant during the last 4 weeks, and 1002, who did not complete enough ASA_24 to analyze.

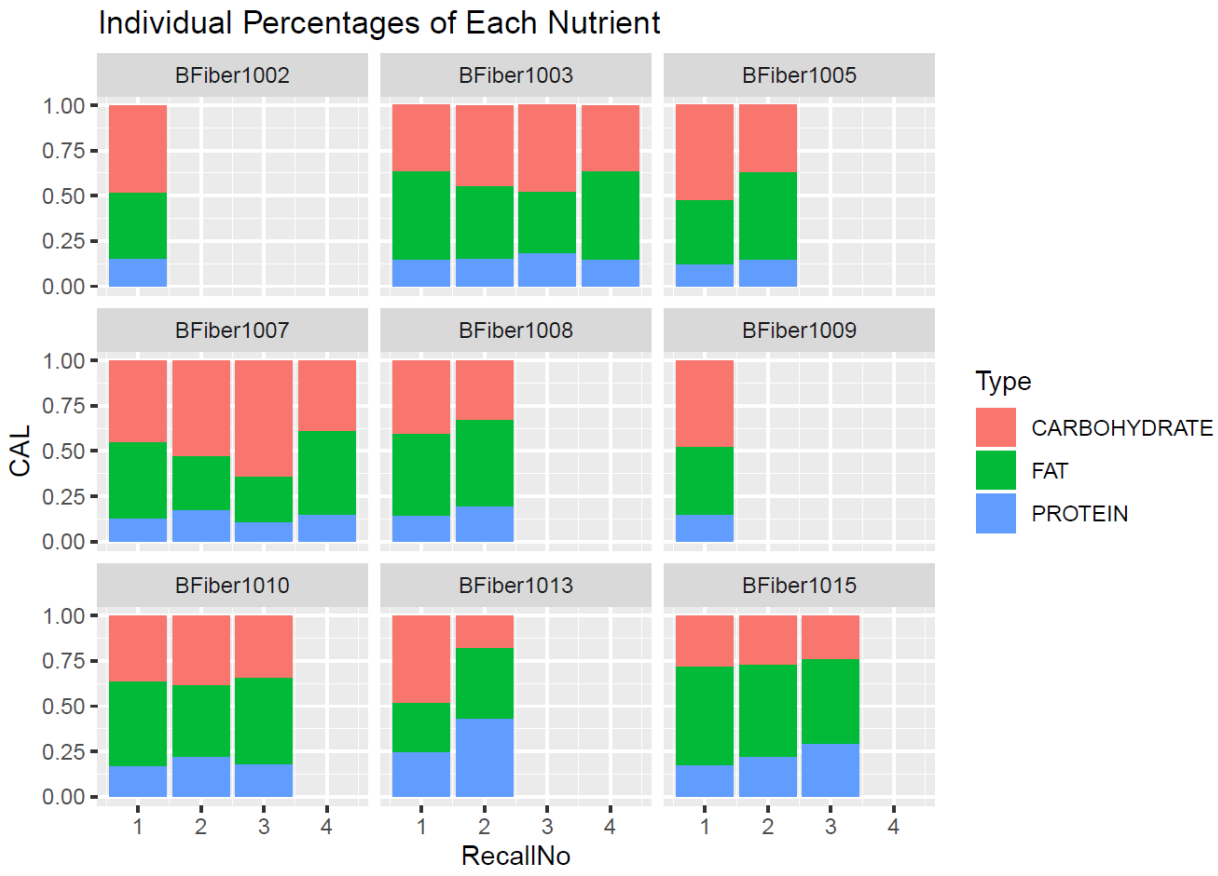
Overall Macronutrient Percentages



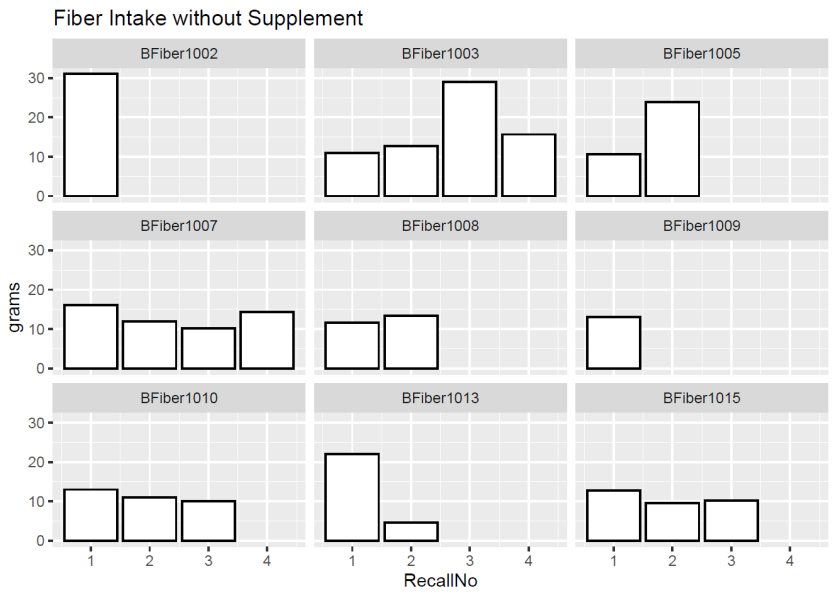
Total KCAL Intake (ASA24)

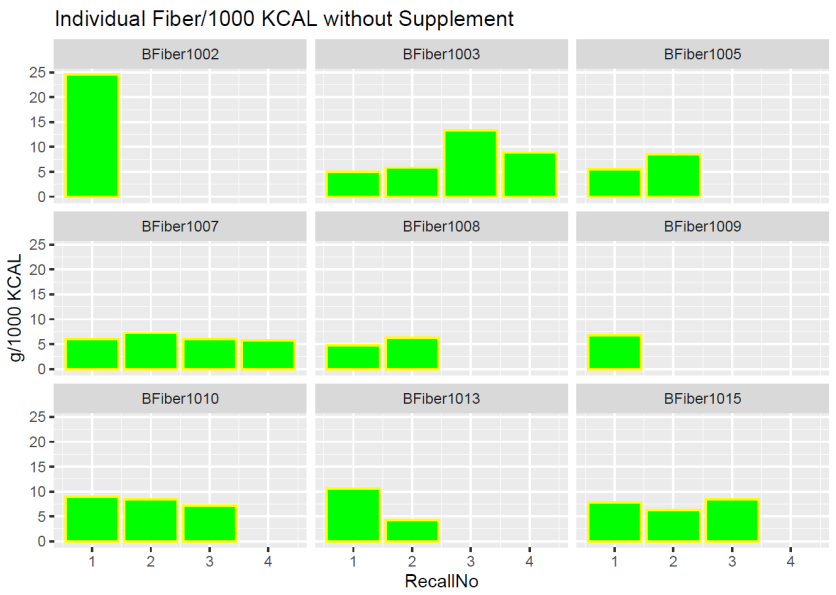


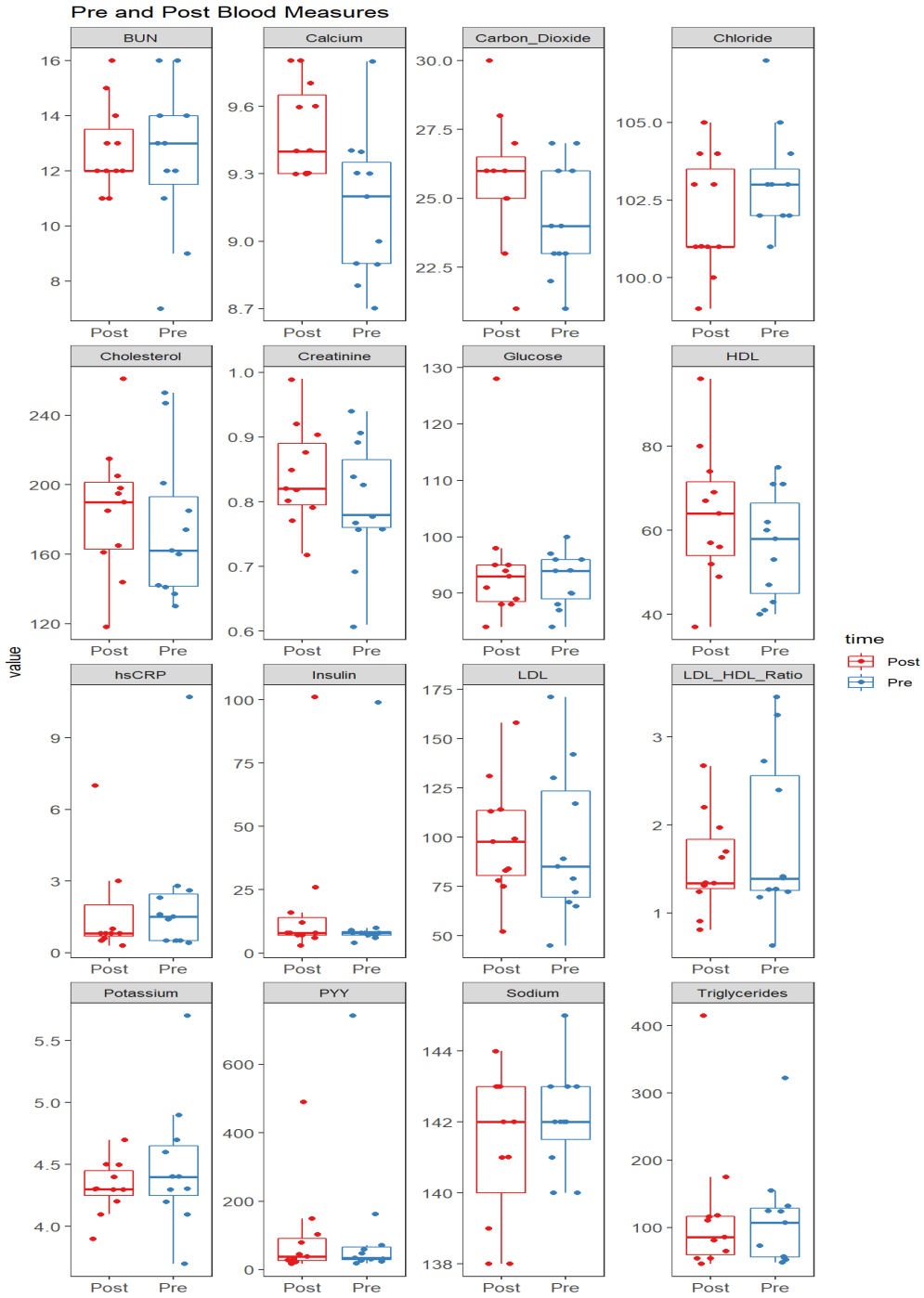
Individual Percentages of Each Nutrient



Individual Fiber Intake Without Supplement






Pre and Post Blood Measures
BUN
Calcium
Carbon_Dioxide
Chloride
Cholesterol
Creatinine
Glucose
HDL
hsCRP
Insulin
LDL
LDL_HDL_Ratio
Potassium
PYY
Sodium
Triglycerides
value
Post
Pre
time
Post
Pre

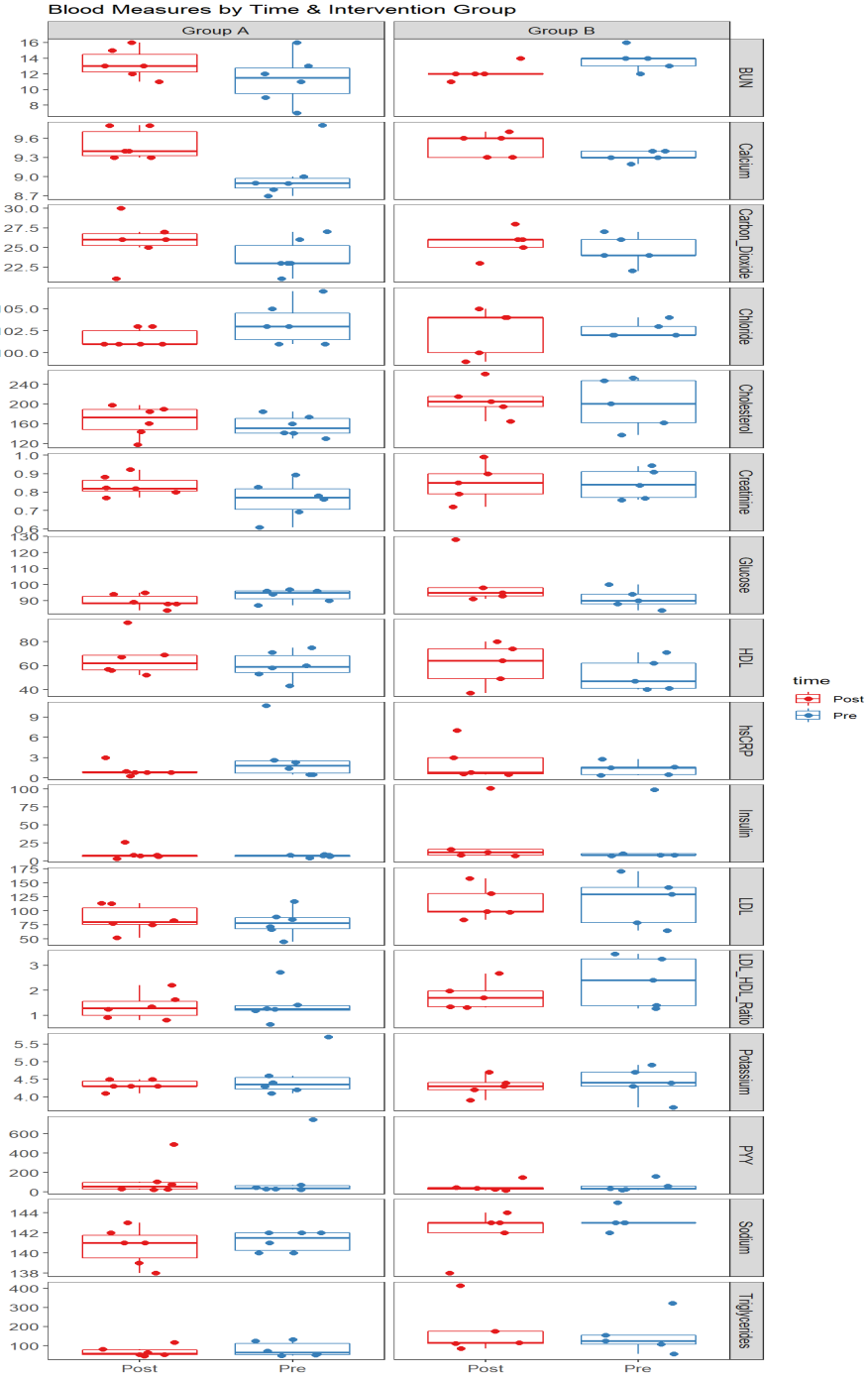

Blood Measures by Time & Intervention Group
Group A
Group B
BUN
Calcium
Carbon_Dioxide
Chloride
Cholesterol
Creatinine
Glucose
HDL
hsCRP
Insulin
LDL
LDL_HDL_Ratio
Potassium
PYY
Sodium
Triglycerides
Post
Pre
time
Post
Pre

Table S3

Supplemental Table 1				
	Baseline		1.5 Year Follow Up	
Variables	Mean	Standard Deviation	Mean	Standard Deviation
Weight	76.92	17.61	3.02	4.99
Waist	34.90	4.00	0.87	2.95
DXA	26.33	8.79	3.35	2.51
Fat weight	44.69	16.62	9.27	6.45
Lean weight	127.52	32.82	-3.50	9.95
Visceral fat	328.24	144.84	54.67	65.35
Total cholesterol	183.04	29.28	8.00	27.95
LDLC	111.21	24.94	7.60	23.51
HDLC	55.88	14.80	-5.82	5.77
Triglycerides	95.92	52.53	17.30	59.64
Glucose	89.92	7.58	9.95	13.74

Preliminary Findings: Findings from an on-going study by co-investigator, Dr. Grandjean, indicate that over a short 18-month period, residents experience an increase in body weight, fat mass, waist circumference and loss of lean mass. Also of concern is that their lipid profiles change unfavorably and glucose levels increase. Please see table 1 for details.