Supplementary Material

Diet Can Impact Microbiota Composition in Children With Autism Spectrum Disorder

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1 Supplementary Figures and Tables

1.1 Supplementary Tables

Supplemental Table 1. Primer for Real-time quantitative analysis of microbial populations.

Target Group	Primer	Sequence (5' – 3')	Annealing Temperature (°C)	Reference
Total Bacteria	Uni331F	TCCTACGGGAGGCAGCAGT	60	Nadkarni et al, 2002
	Uin797R	GGACTACCAGGGTATCTATCCTGTT		
Lactobacillus spp.	LacF	AGCAGTAGGGAATCTTCCA	58	Kok et al, 1996
	LacR	CACCGCTACACATGGAG		
Bifidobacterium spp.	Bif164F	GGGTGGTAATGCCGGATG	60	Kok et al, 1996
	Bif662R	CCACCGTTACACCGGGAA		
Prevotella spp.	g_prevF	GGTTCTGAGAGGAAGGTCCCC	55	Rinttila et al., 2004
	g_prevR	TCCTGCACGCTACTTGGCTG		
Clostridium perfringens	s-Clper-F	GGGGGTTTCAACACCTCC	60	Matsuda et al., 2009
	ClPER-R	GCAAGGGATGTCAAGTGT		
Clostridium difficile	Cdiff_F	TTGAGCGATTTACTTCGGTAAAGA	58	Rinttila T, 2004
	Cdiff_R	CCATCCTGTACTGGCTCACCT		
mmdA	mmdAF	AATGACTCGGGIGGIGCIMGNATHCARGA	56	Reichardt et al, 2014
	mmdAR	GATTGTTACYTTIGGIACNGTNGCYTC		
BCoAT	BCoATscrF	GCIGAICATTTCACITGGAAYWSITGGCAYATG	53	Louis and Flint 2007
	BCoATscrR	CCTGCCTTTGCAATRTCIACRAANGC		

Supplemental Table 2.

(A) Relative abundances of bacterial taxa detected in feces of children in the ASD and CONT groups.

Phyla	**ASD (n=7)**	**CONT (n=32)**
Euryarchaeota	0.51 ± 2.03	0.11 ± 0.63
Actinobacteria	5.98 ± 5.96	3.92 ± 5.85
Bacteroidetes	39.88 ± 20.75†	49.5 ± 19.02
Cyanobacteria	0.003 ± 0.007	0.01 ± 0.02
Firmicutes	50.53 ± 18.16*	40.42 ± 14.47
Fusobacteria	0.0002 ± 0.0008	0.0003 ± 0.001
Proteobacteria	0.85 ± 1.03	0.99 ± 1.23
Tenericutes	0.0002 ± 0.0004	0.0002 ± 0.0003
Verrucomicrobia	2.26 ± 4.18	5.39 ± 10.3

Order	**ASD (n=26)**	**CONT (n=32)**
Bacteroidales	0.02 ± 0.06	0.00 ± 0.00
Streptophyta	0.00 ± 0.01†	0.01 ± 0.02
Clostridiales	3.98 ± 2.73†	2.47 ± 1.3
RF32	0.02 ± 0.05	0.01 ± 0.05
RF39	0.00 ± 0.00	0.00 ± 0.00

Family	**ASD (n=26)**	**CONT (n=32)**
Coriobacteriaceae	0.29 ± 0.54*	0.05 ± 0.1
RF16	0.01 ± 0.02	0.00 ± 0.01
Rikenellaceae	2.95 ± 4.67*	3.83 ± 3.27
S247	0.00 ± 0.00	0.03 ± 0.12
Barnesiellaceae	0.88 ± 1.62	1.57 ± 3.58
Christensenellaceae	0.13 ± 0.3	0.19 ± 0.93
Clostridiaceae	1.65 ± 2.96†	0.32 ± 0.36
EtOH8	0.01 ± 0.03	0.00 ± 0.01
Lachnospiraceae	8.39 ± 4.62	6.71 ± 3.48
Peptostreptococcaceae	0.12 ± 0.21*	0.03 ± 0.05
Mogibacteriaceae	0.03 ± 0.06	0.03 ± 0.04
Erysipelotrichaceae	0.86 ± 1.11	0.68 ± 0.89
Enterobacteriaceae	0.26 ± 0.49	0.10 ± 0.25

Genera	**ASD (n=26)**	**CONT (n=32)**
Archea – Euryarchaeota		
Methanobrevibacter	0.51 ± 2.03	0.12 ± 0.63
Actinobacteria		
Bifidobacterium	3.33 ± 3.89	3.20 ± 4.61
Adlercreutzia	0.16 ± 0.24	0.14 ± 0.27
Collinsella	1.93 ± 3.6	0.39 ± 1.6
Eggerthella	0.15 ± 0.27	0.08 ± 0.13
Slackia	0.13 ± 0.39	0.06 ± 0.31

Bacteroidetes		
Bacteroides	30.50 ± 19.44[b]	38.14 ± 19.23
Parabacteroides	2.05 ± 2.85	2.09 ± 1.86
Prevotella	3.16 ± 8.81	2.98 ± 7.97
Alistipes	0.05 ± 0.16	0.02 ± 0.04
Butyricimonas	0.01 ± 0.04*	0.03 ± 0.11
Odoribacter	0.11 ± 0.2	0.29 ± 0.52
Paraprevotella	0.03 ± 0.11	0.15 ± 0.58
Prevotella	0.11 ± 0.4	0.01 ± 0.05
Firmicutes		
Staphylococcus	0.02 ± 0.06	0.01 ± 0.02
Enterococcus	0.16 ± 0.52	0.00 ± 0.02
Lactobacillus	0.09 ± 0.27	0.00 ± 0.00
Leuconostoc	0.03 ± 0.15	0.00 ± 0.00
Lactococcus	0.09 ± 0.26	0.01 ± 0.00
Streptococcus	0.51 ± 0.87	0.29 ± 0.43
Turicibacter	0.34 ± 0.98	0.14 ± 0.46
02d06	0.01 ± 0.01	0.00 ± 0.00
Clostridaceae_Clostridium	0.86 ± 1.87*	0.20 ± 0.41
SMB53	2.64 ± 3.59†	1.24 ± 1.59
Sarcina	0.05 ± 0.13	0.00 ± 0.00
Eubacterium	0.01 ± 0.03	0.00 ± 0.01
Anaerostipes	0.10 ± 0.16	0.12 ± 0.19
Blautia	1.84 ± 1.41*	1.18 ± 0.93
Butyrivibrio	0.01 ± 0.02*	0.05 ± 0.05
Coprococcus	3.88 ± 2.32	2.97 ± 1.9
Dorea	1.13 ± 0.98	0.84 ± 0.57
Lachnospira	0.47 ± 0.9	0.49 ± 0.57
Roseburia	1.02 ± 1.94*	0.43 ± 0.55
Peptococcus	0.06 ± 0.22	0.00 ± 0.01
Anaerotruncus	0.02 ± 0.04	0.01 ± 0.01
Peptostreptococcaceae_Clostridium	0.007 ± 0.03	0.002 ± 0.003
Faecalibacterium	8.02 ± 7.03*	11.13 ± 6.46
Oscillospira	0.49 ± 0.34	0.47 ± 0.26
Ruminococcus	2.83 ± 2.9	2.55 ± 2.97
Acidaminococcus	0.02 ± 0.07	0.00 ± 0.02
Dialister	0.61 ± 1.04*	1.23 ± 1.57
Megamonas	0.57 ± 2.87	0.15 ± 0.59
Megasphaera	0.18 ± 0.92	0.00 ± 0.00
Phascolarctobacterium	0.31 ± 0.92	0.34 ± 0.94
Succiniclasticum	0.00 ± 0.00	0.04 ± 0.24
Veillonella	0.10 ± 0.32	0.04 ± 0.07
Catenibacterium	0.04 ± 0.14	0.00 ± 0.00
Coprobacillus	0.01 ± 0.03	0.01 ± 0.02
Holdemania	0.01 ± 0.01	0.01 ± 0.01
Fusobacteria		
Fusobacterium	0.00 ± 0.00	0.00 ± 0.00

Proteobacteria		
Sutterella	0.28 ± 0.37	0.61 ± 0.76
Bilophila	0.03 ± 0.06*	0.04 ± 0.06
Campylobacter	0.05 ± 0.23	0.00 ± 0.00
Haemophilus	0.22 ± 0.68	0.22 ± 0.87
Verrucomicrobia		
Akkermansia	2.26 ± 4.18	5.39 ± 10.3

(B) Bacterial Densities in feces of children in the CONT and ASD groups.

	ASD	**CONT**
Log_{10} gene copy numbers/g		
Total Bacteria	12.34 ± 0.83	12.71 ± 0.67
Lactobacillus	7.39 ± 1.14	7.59 ± 0.93
Bifidobacterium	9.15 ± 1.04*	9.82 ± 0.56
Prevotella	10.45 ± 0.94	11.03 ± 0.78
Clostridium perfringens	6.62 ± 0.58*	7.38 ± 1.14

Data expressed as mean ± SD; data was analyzed using proc glimmix (non-normal data); within same segment and row, different from CONT at *p≤0.05 and †≤0.1; age, gender, height, weight, BMI and season were included as covariates; ASD=Autism Spectrum Disorder group; CONT=unaffected control group

Supplemental Table 3. Comparison of Nutrient Intake and Food Groups Consumed by Children in the ASD and CONT groups.

(A) Nutrient intake

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Variable	ASD (n=26)	CONT (n=32)
Macronutrients		
Energy	1371 ± 311	1489 ± 306
Total Fat	51 ± 16	56 ± 17
Omega-6 Fatty Acids	11 ± 4.4	11 ± 4.7
Omega-3 Fatty Acids	1.1 ± 0.5	1.2 ± 0.6
SFA	18 ± 7	14 ± 4
MUFA	18 ± 5	20 ± 7
PUFA	12 ± 5	9 ± 2
Total Carbohydrate	184 ± 44	147 ± 67
Total Sugars	79 ± 32	88 ± 28
Added Sugars	44 ± 24	46 ± 24
Total Grains	5.6 ± 2.1	6 ± 1.9
Whole Grains	1.2 ± 1.6	1.3 ± 1.1
Refined Grains	4.4 ± 2	4.7 ± 2
Total Protein	49 ± 16	55 ± 18
Dietary Fiber		
Total Dietary Fiber	12.3 ± 4.9	14.6 ± 5.6
Soluble Dietary Fiber	4 ± 1.6	3.1 ± 1.6
Insoluble Dietary Fiber	8.2 ± 3.6†	10.3 ± 4.2
Pectin	1.5 ± 0.8†	0.6 ± 0.6
Vitamins		
Vitamin A	523 ± 400	405 ± 177
Vitamin D	4.0 ± 3.1	4.8 ± 2.1
Vitamin E	6.4 ± 2.5	5.9 ± 1.8
Vitamin K	43.7 ± 32	58.8 ± 53.1
Vitamin C	52.4 ± 43.6†	80.5 ± 51.1
Thiamin	1.2 ± 0.4	1.3 ± 0.4
Riboflavin	1.5 ± 0.5	1.6 ± 0.4
Niacin	15.3 ± 5.1	15.9 ± 6.1
Pantothenic Acid	3.3 ± 1.2	3.6 ± 1.1
Vitamin B_6	1.3 ± 0.5	1.3 ± 0.6
Folate	304 ± 231	344 ± 195
Vitamin B_{12}	3.5 ± 2.7	3.5 ± 1.4
Minerals		
Calcium	736 ± 318	798 ± 276
Phosphorus	875 ± 310	956 ± 235
Magnesium	178.6 ± 65	202.9 ± 61.4
Iron	12.3 ± 7.8	11.9 ± 6.5
Zinc	6.8 ± 3.4	8 ± 3.1
Copper	0.8 ± 0.6	0.9 ± 0.3
Selenium	67.5 ± 21.1	78.7 ± 25.9

Sodium	2029 ± 669	2092 ± 642
Potassium	1628 ± 628	1821.8 ± 585
Manganese	2.2 ± 0.9	2.7 ± 1.3
Choline	186 ± 71	216 ± 90

(B) Food Groups

Food Group	**ASD (n=26)**	**CONT (n=32)**
Fruit	2.27 ± 1.31	2.4 ± 1.04
Vegetables	1.16 ± 0.82	1.7 ± 0.85
Legumes	0.38 ± 0.49	0.40 ± 0.31
Starchy Foods	0.29 ± 0.33	0.35 ± 0.39
Starchy Vegetables	0.73 ± 0.64	0.53 ± 0.35
Juice	0.66 ± 0.61	0.39 ± 0.35
Sweetened Beverages	0.27 ± 0.31	0.26 ± 0.29
Grains	0.86 ± 0.94	0.8 ± 0.70
Refined Carbohydrates	1.13 ± 0.72	0.99 ± 0.41
Fried Foods	0.33 ± 0.31	0.25 ± 0.19
Protein	1.39 ± 0.89	1.2 ± 0.63
Dairy	3.26 ± 1.68*	4.5 ± 1.94
Snack	1.17 ± 0.71†	0.8 ± 0.51
Sweets	2.17 ± 0.57†	1.7 ± 0.8
Kid's Meal	0.87 ± 0.57	1 ± 0.52
Fish	0.12 ± 0.12	0.16 ± 0.14
Condiments	0.40 ± 0.39	0.38 ± 0.29

Food groups consumed were derived from YAQ; nutrient intake derived from three day food record; Differences between groups examined with proc glimmix (non-normal data) and proc mixed (normal data) with age, gender, height, weight, BMI and season were included as co-variates; within same segment and row, different from CONT at *$p \leq 0.05$ and †≤ 0.1; ASD, Autism Spectrum Disorder group; CONT, unaffected control group

Supplemental Table 4. Correlations between dietary intake and food groups with bacterial phyla/genera in ASD (a) and CONT (b).