

Supplementary Information

Impact of Medium- and Long-Chain Triacylglycerols and *sn*-2 Palmitate on Temperament Development in Term Infants: Potential Role of Gut *Bifidobacterium*

Ying-Zhen Qiu^{1#}, Meng-Tao Yang^{1#}, Zhengdong Liu^{2#}, Xiao-Yan Chen¹², Jie-Dong Chen¹, Si-Yu Huang¹, Qiu-Ye Lan¹³, Yan Hu⁴, Xuezheng Zhou², Wei Wei⁵, Xingguo Wang⁵, Xingwang Ye^{2*}, Hui-Lian Zhu^{12*}

¹ Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China.

² Sun Yat-sen University-Mengniu Joint Research Center of Nutrition and Health for Middle-Aged and Elderly, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China.

³ School of Public Health, Guangdong Pharmaceutical University, Guangzhou 510006, China.

⁴ Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangdong Provincial Clinical Research Center for Child Health, Guangzhou 510623, China

⁵ State Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi 214122, China.

Ying-Zhen Qiu, Meng-Tao Yang and Zhengdong Liu contributed equally to this work.

*** Corresponding Author**

Dr. Xingwang Ye

Address: Sun Yat-sen University-Mengniu Joint Research Center of Nutrition and Health for Middle-Aged and Elderly, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China

E-mail: yexingwang@mengniu.cn

Prof. Hui-Lian Zhu

Address: School of Public Health, Sun Yat-sen University, No.74 of Zhongshanerlu Road, Guangzhou, 510080, China

Tel no. +86-20-8733-1811

E-mail: zhuhl@mail.sysu.edu.cn

Table S1. Demographic characteristics of participants

	Novel-F n = 65	Contr-F n = 46	BF n = 66	<i>p</i>
Birth characteristics				
Gestational age, weeks	39.1±1.3	39.4±1.4	40.3±1.4	<0.001
Male infants, n (%)	36 (55.4%)	27 (58.7%)	38 (57.6%)	0.936
Vaginal delivery, n (%)	26 (40.0%)	28 (60.9%)	25 (37.9%)	0.035
Weight, g	3291±390	3294±388	3429±336	0.063
Length, cm	50.2±1.3	50.4±1.2	50.5±1.6	0.419
Baseline				
Z _{w/a}	0.09±1.00	-0.02±0.92	0.18±0.89	0.518
Z _{l/a}	0.21±0.97	0.14±0.85	0.17±0.75	0.919
Z _{w/l}	-0.33±0.85	-0.29±1.25	-0.08±1.00	0.348
Infectious diseases, n (%)	4 (6.2%)	1 (2.2%)	2 (3.0%)	0.557
Endpoint				
Z _{w/a}	0.26±0.77	-0.26±0.76	0.14±0.90	0.004
Z _{l/a}	0.26±0.97	-0.09±0.92	-0.05±1.00	0.095
Z _{w/l}	0.14±0.92	-0.92±0.98	0.37±1.15	0.048
Infectious diseases, n (%)	3 (4.6%)	3 (6.5%)	2 (3.0%)	0.536
Maternal characteristics				
Age, years	28.9±4.9	28.6±4.9	27.6±4.4	0.259
Primipara, n (%)	18 (27.7%)	16 (34.8%)	28 (42.4%)	0.210
Education, completed senior high school, n (%)	19 (29.2%)	26 (56.5%)	33 (50.0%)	0.008
Socio-economic conditions				
Living space per capita (m ²)	30.51±10.72	30.96±9.92	34.78±9.79	0.051
Daily formula intake, mL				
Baseline	676±70	648±80	/	0.058
Endpoint	924±102	897±118	/	0.219

Mean ± SD for continuous variables and *n* (%) for categorical variables. One-way ANOVA or *t* test was used to compare continuous variables, and chi-square test or Fisher's exact tests was used for categorical variables, as appropriate. Abbreviations: Z_{w/a}, weight-for-age z-scores; Z_{l/a}, length-for-age z-scores; Z_{w/l}, weight-for-length z-scores.

Table S2. Relative abundance of gut microbiome at genus level during the intervention (%)

Genus	Time Point	IG	CG	BG	p_{Baseline}	p^1	p^2	p^3
<i>Bifidobacterium</i>	Baseline	56.45±22.03	63.24±23.24	53.64±27.32	0.164	0.011	0.759	0.004
	Endpoint	69.20±16.17	60.18±27.67	68.28±20.67				
<i>Streptococcus</i>	Baseline	14.16±19.39	10.44±19.18	14.98±18.68	0.067	0.183	0.202	0.867
	Endpoint	3.77±5.56	5.88±12.61	9.42±10.53				
<i>Escherichia-Shigella</i>	Baseline	8.31±10.26	6.27±11.58	8.95±14.13	0.249	0.454	0.736	0.282
	Endpoint	7.13±8.98	7.53±15.60	6.98±13.21				
<i>Klebsiella</i>	Baseline	3.59±8.79	2.92±7.69	8.03±20.53	0.214	0.758	0.189	0.125
	Endpoint	1.84±5.40	2.21±8.92	2.35±11.08				
<i>Enterococcus</i>	Baseline	2.45±3.08 ^a	3.96±5.51 ^a	1.54±4.24 ^b	< 0.001	0.468	0.919	0.405
	Endpoint	3.62±4.87	6.21±9.91	2.47±4.69				
<i>Lactobacillus</i>	Baseline	1.95±4.98	1.93±3.42	1.52±3.48	0.146	0.423	0.947	0.381
	Endpoint	2.44±6.83	1.32±3.64	2.04±5.00				
<i>Collinsella</i>	Baseline	1.39±3.92	1.87±6.66	1.44±5.72	0.331	0.773	0.280	0.193
	Endpoint	2.18±4.28	2.87±8.03	0.97±3.75				
<i>unclassified_f_Enterobacteriaceae</i>	Baseline	2.18±9.02 ^a	1.34±3.27 ^{ab}	0.99±3.25 ^b	0.015	0.276	0.102	0.676
	Endpoint	0.47±2.13	0.86±3.61	0.95±3.21				
<i>Blautia</i>	Baseline	2.56±6.70	0.59±2.29	0.39±2.69	0.055	0.002	0.048	0.156
	Endpoint	0.78±1.68	1.96±6.51	0.38±2.45				
<i>Veillonella</i>	Baseline	1.23±4.01	1.11±4.50	1.01±4.89	0.769	0.392	0.873	0.470
	Endpoint	2.67±10.42	1.03±3.74	2.18±7.15				

Data were expressed as mean±SD. p_{Baseline} indicated baseline comparisons among the three groups using the Kruskal-Wallis test. Genera with significant differences were further analyzed by Bonferroni-corrected post hoc pairwise comparisons; values with different superscript letters (a, b) indicated significant differences at baseline. Linear mixed-effect models were used to examine the changes of genera during the two-month intervention, with fixed effects including time × group, gender, mode of delivery, gestational age, maternal education, and change in $Z_{\text{w/a}}$, with subjects included as random effects. p -values indicated the significant differences of the time × group interaction between the IG and CG (p^1), IG and BG (p^2), and CG and BG (p^3).

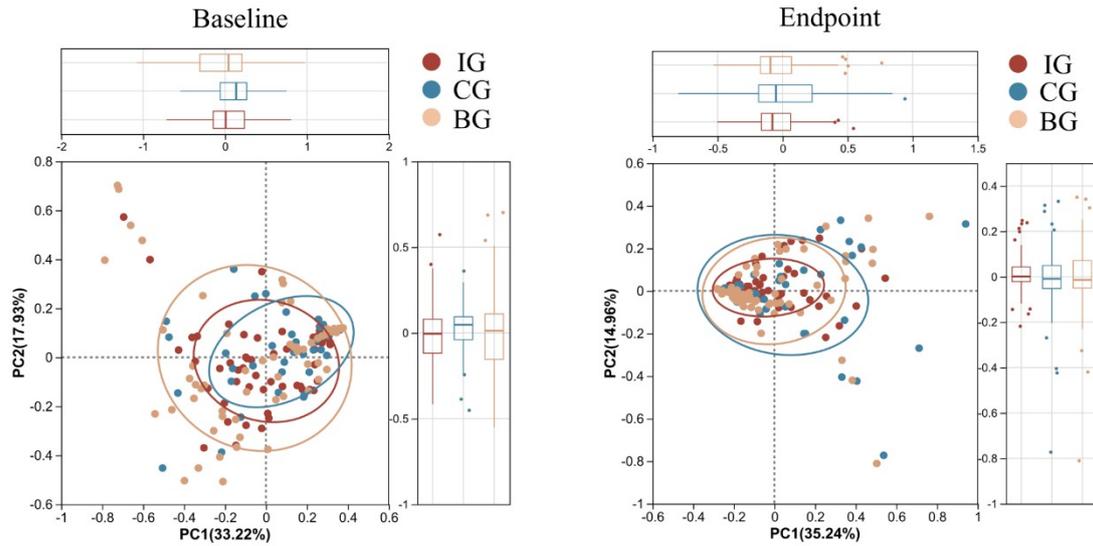


Figure S1. Beta diversity of the gut microbiome at the genus level. PCoA plots showed the microbial communities based on weighted UniFrac distance across groups. Box plots on the axes represented the distribution of values for PC1 and PC2. Differences in beta diversity between groups were assessed using the Adonis test and the results indicated no significant differences among the three groups at both baseline ($p = 0.077$) and endpoint ($p = 0.139$).