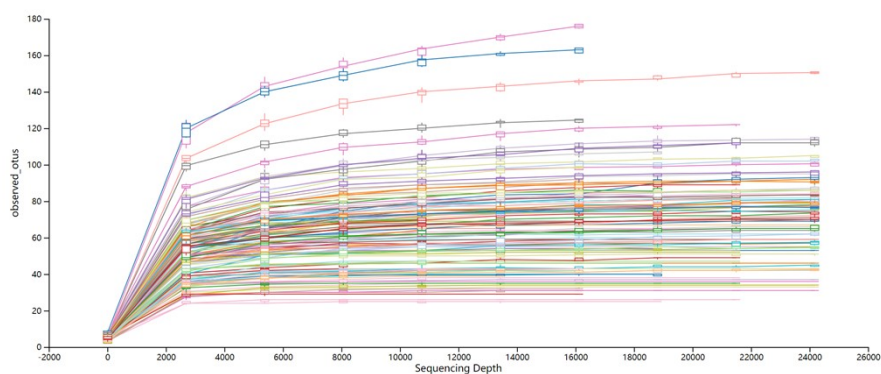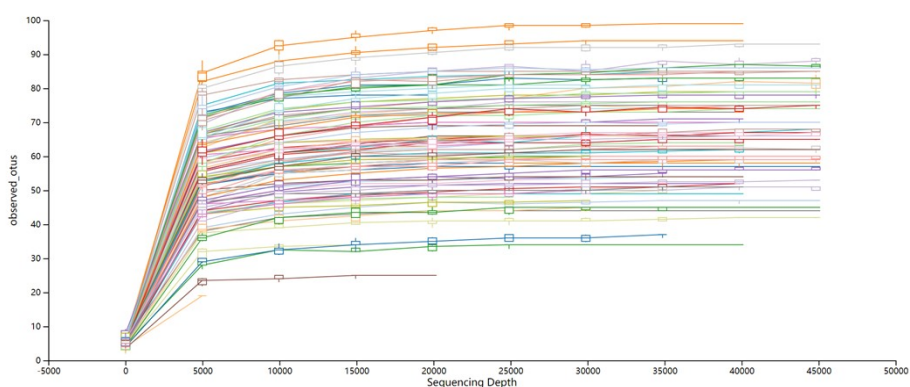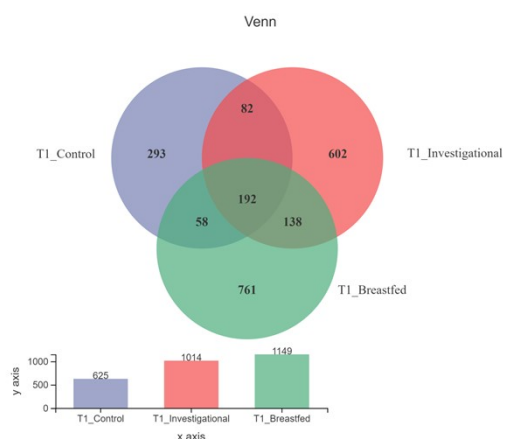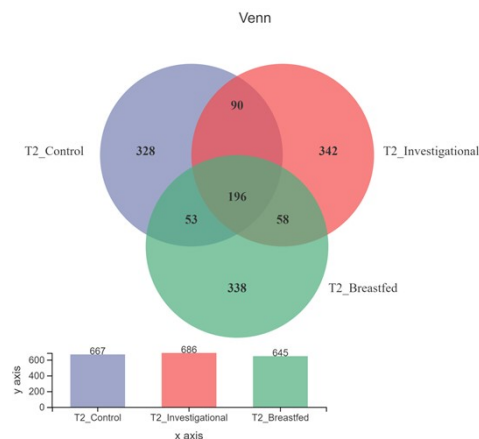# Supplementary material

Fig.S1

A

B

Fig.S1. (A) The rarefaction curves of sequencing samples at 8 weeks. (B) The rarefaction curves of sequencing samples at 16 weeks.

Fig.S2

A   **B**

Fig.S2. (A) The Venn diagram of OTU numbers at 8 weeks. (B) The Venn diagram of OTU numbers at 16 weeks.

Fig.S3

A
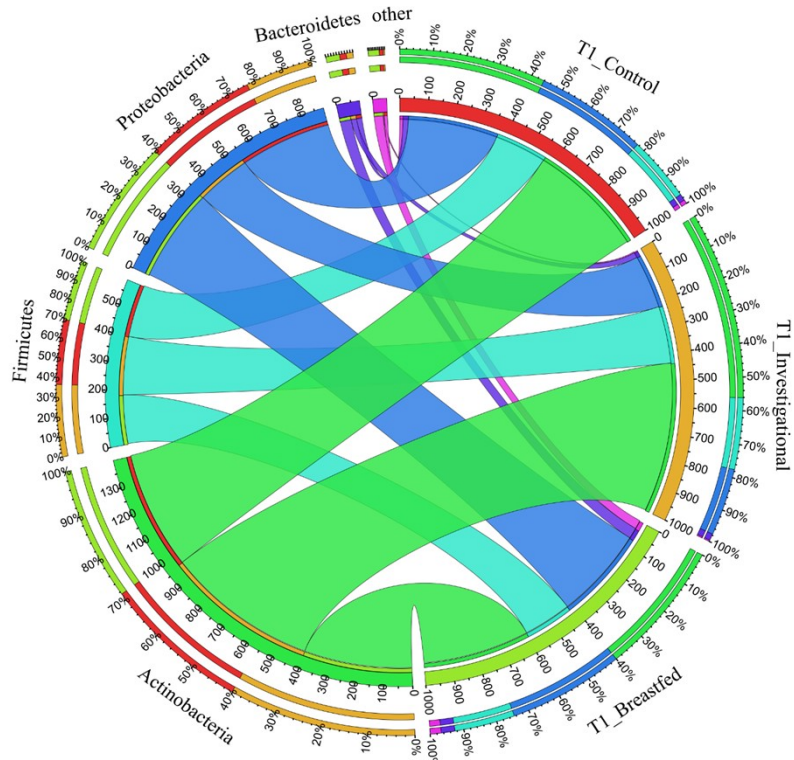
B
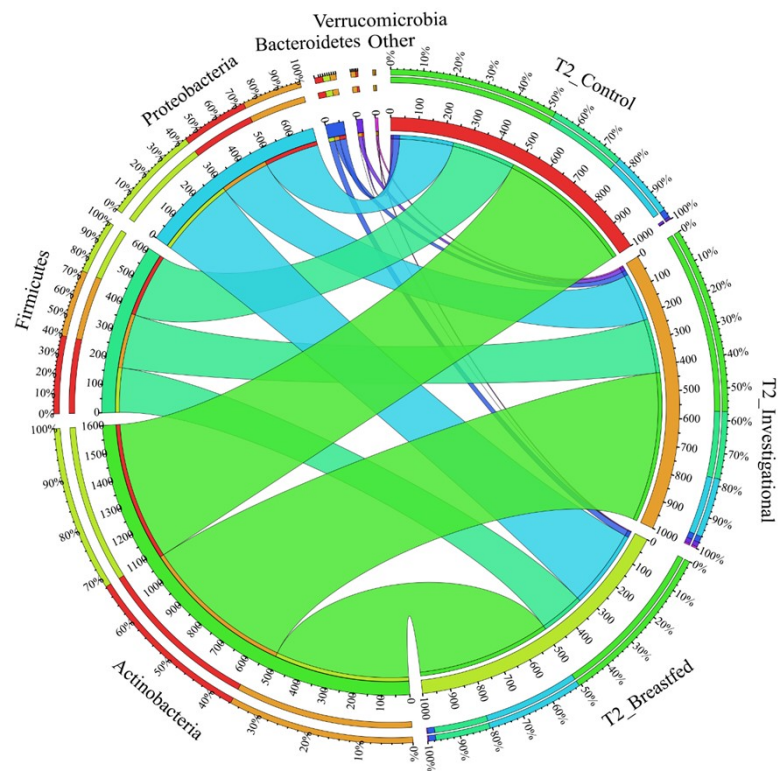
Fig.S3. (A) A circos plot of gut microbiota structure at the phylum level at 8 weeks; (B) A circos plot of gut microbiota structure at the phylum level at 16 weeks.

Fig.S4
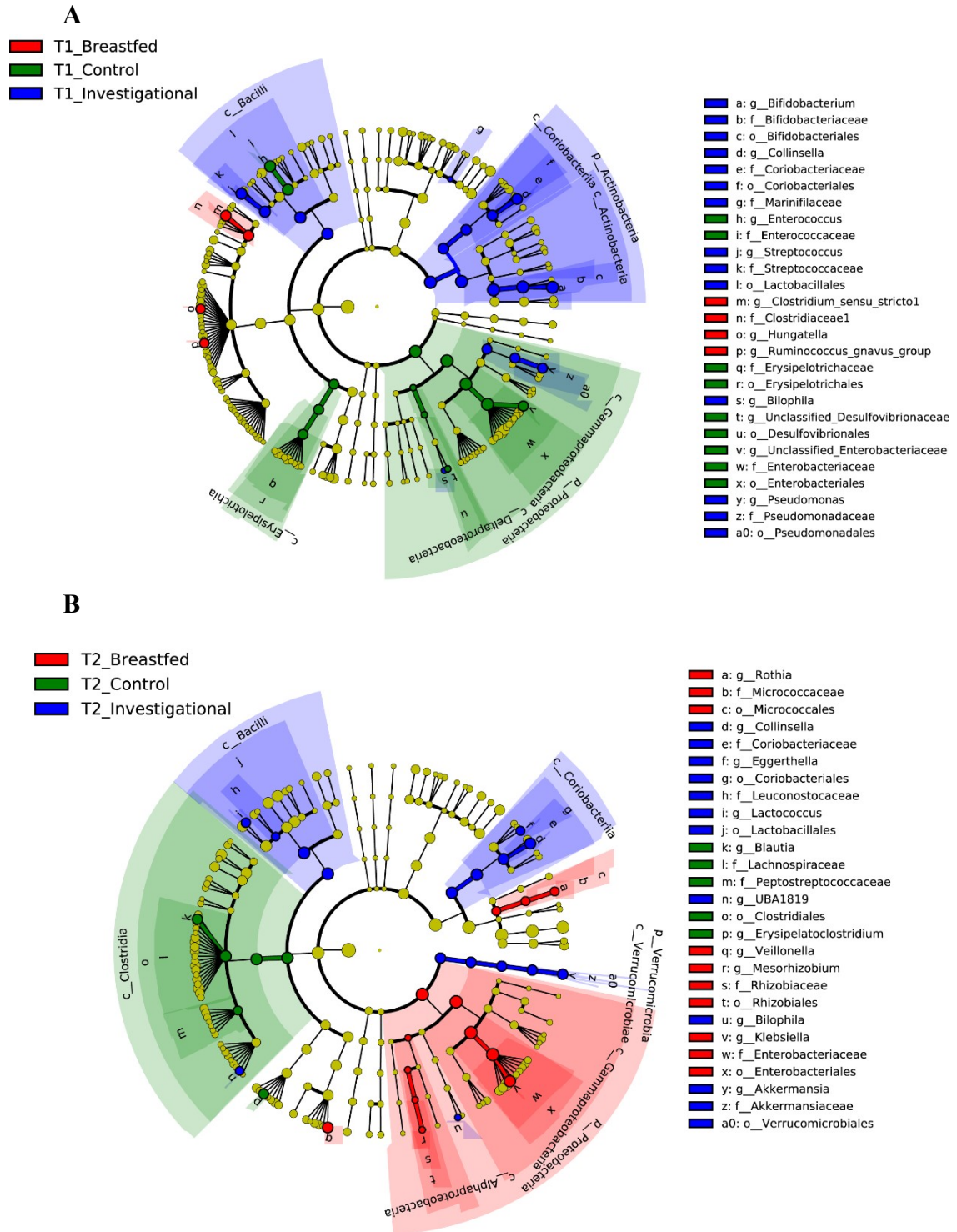
Fig.S4. (A) LEfSe analysis at the genus level at 8 weeks, LDA = 3.0, α = 0.05; (B) LEfSe analysis at the genus level at 16 weeks, LDA = 2.0, α = 0.05.

Fig.S5

A
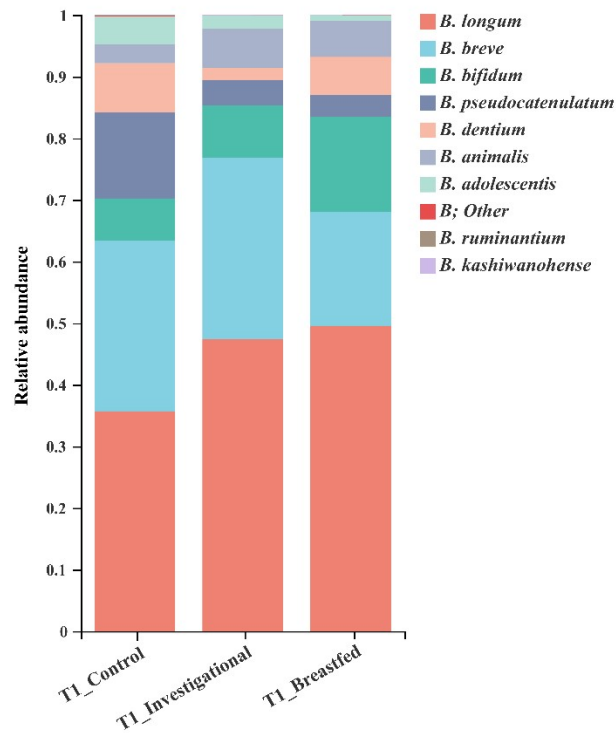
B
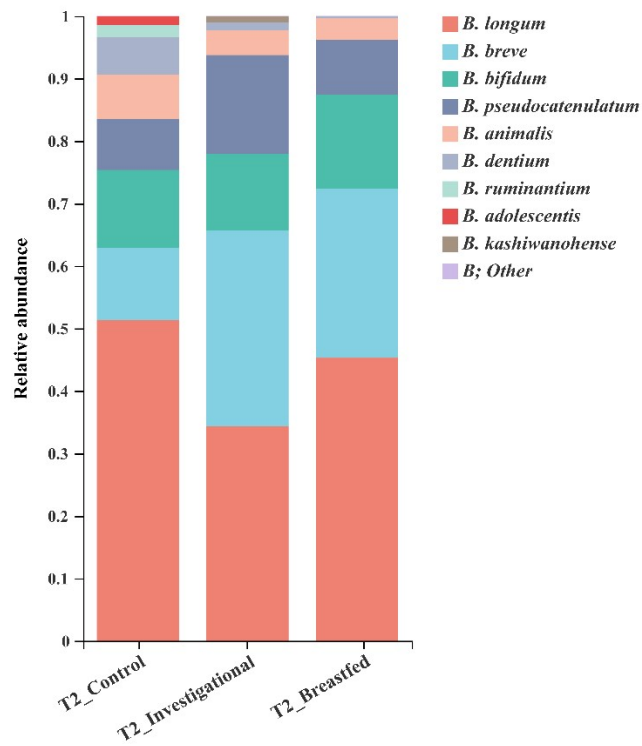
Fig.S5. (A) The species composition of Bifidobacterium in infant feces at 8 weeks; (B) The species composition of Bifidobacterium in infant feces at 16 weeks.

Fig.S6
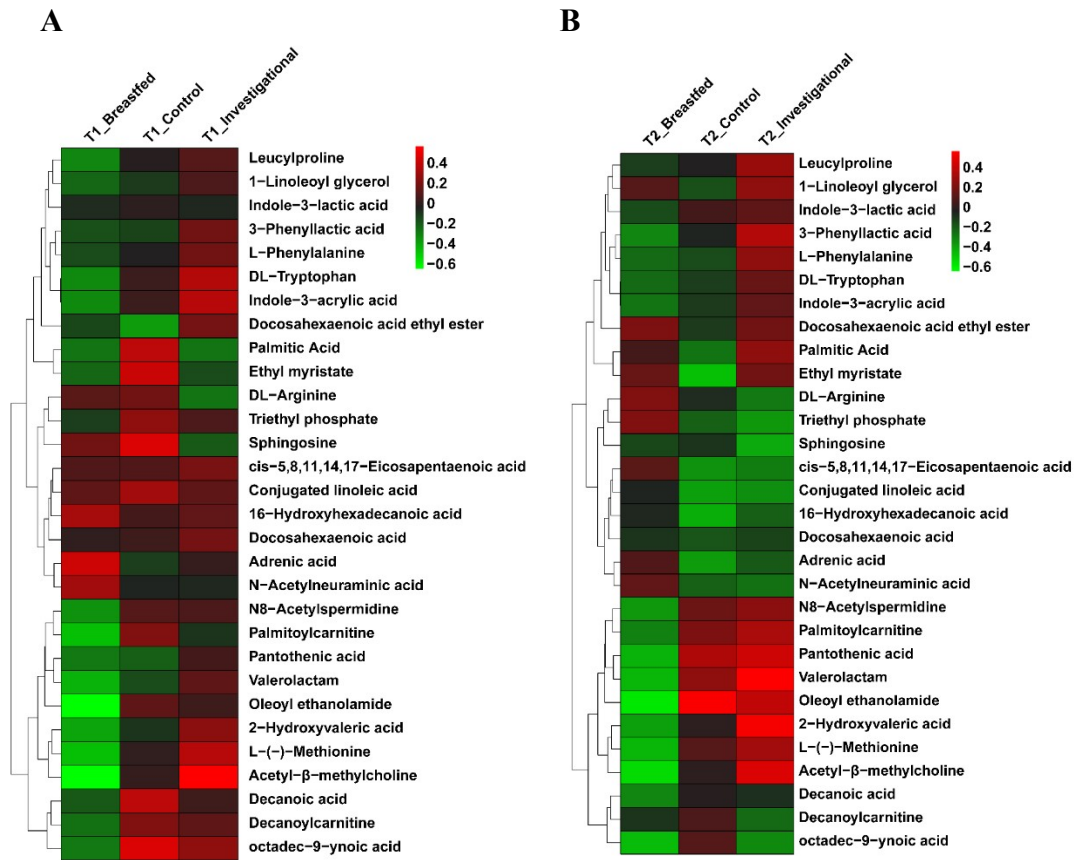
Fig.S6. (A) Clustering heatmap of the top 30 most abundant metabolites and three groups at 8 weeks; (B) Clustering heatmap of the top 30 most abundant metabolites and three groups at 16 weeks.

Fig.S7

Fig.S7. (A) The top 17 of the enriched pathways are shown in the figure by using over-representation analysis (ORA) at 8 weeks; (B) The top 20 of the enriched pathways are shown in the figure by using over-representation analysis (ORA) at 16 weeks.

Table S1. Baseline Characteristics by Study Group

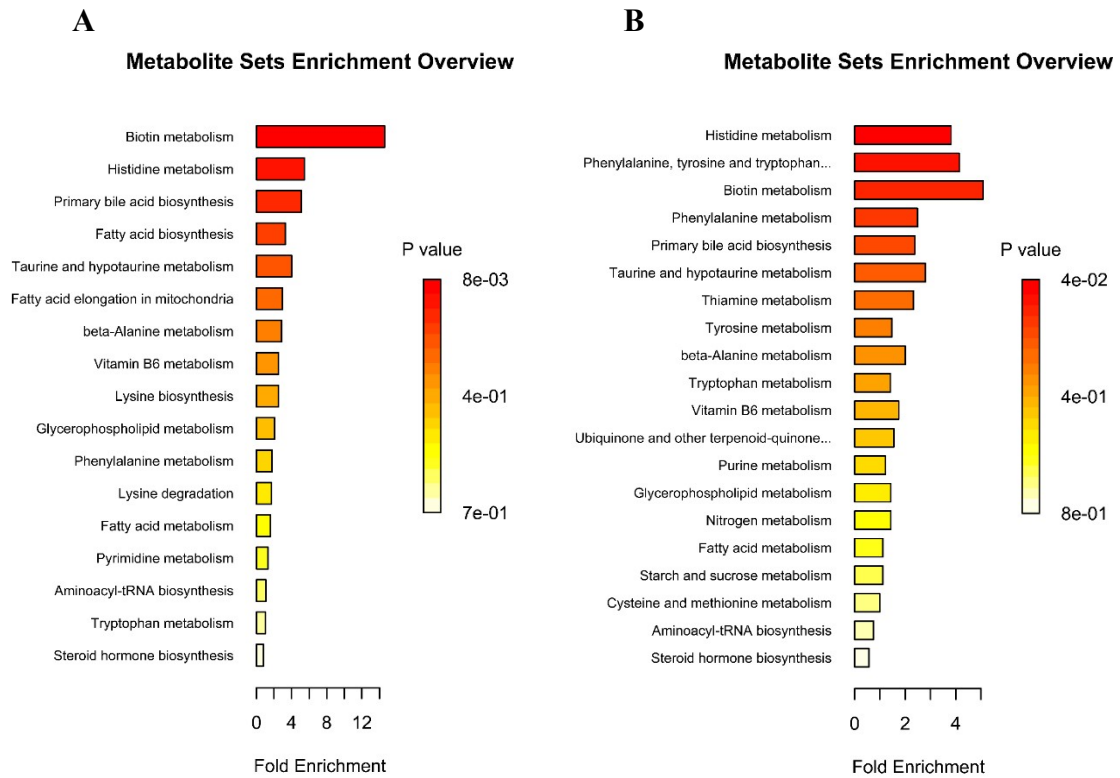| Variable	Investigational (n=150)	Control (n=150)	Breastfed (n=150)	Group Difference p-value
Birth History:				
Weight at birth, g	3308.3±370.6	3325.3±341.5	3277.5±345.0	0.494
Length at birth, mm	499.1±6.8	498.6±7.2	498.7±5.5	0.817
Demographics:				
Infant male sex, %	75 (50.0)	75 (50.0)	75 (50.0)	NA
Infant gestational age, day	39.3±1.0	39.3±0.9	39.3±0.9	0.655
Mother's age, year	29.2±4.6	29.0±4.0	28.5±4.0	0.349
Mother's marital status, %				0.378
Single	1 (0.7)	4 (2.7)	1 (0.7)	
Married	149 (99.3)	146 (97.3)	149 (99.3)	
Mother's smoking status during pregnancy, %	0 (0.0)	0 (0.0)	0 (0.0)	NA
Mother's alcohol intakes during pregnancy, %	0 (0.0)	0 (0.0)	0 (0.0)	NA
Number of previous live births to the infant's mother	0 (0-3)	0 (0-2)	0 (0-2)	0.707
0	76 (50.7)	80 (53.3)	78 (52.0)	
1	71 (47.3)	69 (46.0)	60 (40.0)	
2	2 (1.3)	1 (0.7)	12 (8.0)	
3	1 (0.7)	0 (0.0)	0 (0.0)	
Infant insurance, %	150 (100.0)	150 (100.0)	150 (100.0)	NA
Anthropometrics at baseline:				
Infant age, days	13.3±1.0	13.3±1.0	13.1±1.2	0.361
Weight, g	3758.2±417.4	3746.4±380.9	3682.6±342.7	0.183
Length, mm	521.9±10.6	520.7±10.8	519.8±10.0	0.174
Head circumference, mm	354.4±8.9	355.3±8.1	353.4±7.9	0.121
Weight-for-age z-score	0.21±0.75	0.19±0.67	0.09±0.68	0.258
Length-for-age z-score	0.21±0.57	0.14±0.52	0.10±0.56	0.250
Weight-for-length z-score	-0.27±0.86	-0.21±0.84	-0.33±0.86	0.476
Head circumference-for-age z-score	-0.03±0.80	0.05±0.68	-0.10±0.69	0.169
Socioeconomic status:				
Number of family members	3 (2-6)	3 (2-6)	3 (2-7)	0.095
Household size, %				0.303
<60 m^2	19 (12.7)	21 (14.0)	26 (17.3)	
60-90 m^2	48 (32.0)	50 (33.3)	57 (38.0)	
90-120 m^2	66 (44.0)	60 (40.0)	59 (39.3)	
>120 m^2	17 (11.3)	19 (12.7)	8 (5.3)	
Mother's educational level, %				0.203
Primary school	3 (2.0)	4 (2.7)	10 (6.7)	
Junior school	39 (26.0)	41 (27.3)	46 (30.7)	
High school/ Technology school	95 (63.3)	94 (62.7)	89 (59.3)	
Bachelor	12 (8.0)	11 (7.3)	5 (3.3)	

Master and above	1 (0.7)	0 (0.0)	0 (0.0)	
Father's educational level, %				0.079
Primary school	2 (1.3)	0 (0.0)	4 (2.7)	
Junior school	39 (26.0)	43 (28.7)	44 (29.3)	
High school/ Technology school	94 (62.7)	98 (65.3)	98 (65.3)	
Bachelor	15 (10.0)	9 (6.0)	4 (2.7)	
Master and above	0 (0.0)	0 (0.0)	0 (0.0)	
Mother currently employed, %	81 (54.0)	73 (48.7)	63 (42.0)	0.114
Father currently employed, %	149 (99.3)	150 (100.0)	148 (98.7)	0.776

Data presented are mean ± standard deviation for continuous variables and frequency (%) for categorical variables. Group difference was evaluated using one-way ANOVA for continuous variables and chi-square/Fisher's exact test for categorical variables.

Table S2. The differential metabolites of three groups at 8 weeks

Differential metabolites	VIP	-log10 (p)
N,N-Diphenylguanidine	1.836358292	13.06505
4-Pyridoxic acid	2.073043887	10.87769
18-beta-Glycyrrhetinic acid	1.169071328	9.547585
Corticosterone	2.400458032	8.682961
Propylparaben	2.284840684	8.514932
Dodecyltrimethylammonium	2.364755085	8.091692
Acetyl-beta-methylcholine	2.122043356	7.588801
Ethyl paraben	1.640881928	7.434778
Prostaglandin K2	1.700598765	7.414517
Oleoyl ethanolamide	1.782186534	5.165687
Carnosine	2.057929639	4.907069
Senkyunolide H	1.99376272	4.594961
1-Methylhistidine	2.141056401	4.468764
Monobutyl phthalate	1.708659865	3.833836
Isophorone	1.491348949	3.789441
Tetrahydrocortisone	2.002909784	3.41679
Diethyl phosphate	1.395174597	3.297328
Glycochenodeoxycholic Acid	1.924712427	3.265865
Pulegone	1.529418699	3.210292
Adrenic acid	1.022865672	3.037484
Taurocholic acid	1.926436567	2.770882
2,4-Quinolinediol	1.552118347	2.770882
N-Acetylhistamine	1.015502659	2.698427
Kynurenic acid	1.291145004	2.662521
DL-Arginine	1.546215797	2.560115
2,4-Xylidine	1.286099008	2.560115
Valpromide	1.249759476	2.417596

2,4-di-tert-Butylphenol	1.305969641	2.313873
1,5-Isoquinoline diol	1.383267061	2.261497
C-8 Ceramide-1-phosphate	1.339164282	2.261497
Triethyl phosphate	1.268245577	2.261497
2-(Methylthio)benzothiazole	1.105032791	2.212008
Phenylacetic acid	1.469764495	2.115851
Taurochenodeoxycholic Acid	1.642154441	2.019329
4-Phenolsulfonic acid	1.504756438	1.8882
Taurodeoxycholic Acid	1.584375816	1.680249
DL-Carnitine	1.12103632	1.671682
Valerolactam	1.428591975	1.549428
Nicotinuric acid	1.571222579	1.50941
12-oxo Phytodienoic Acid	1.147298788	1.477673
L-Lysine	1.18950412	1.377589
Biotin	1.213578476	1.376823
3-Indoxyl sulphate	1.269212498	1.347773

Table S3. The differential metabolites of three groups at 16 weeks

Differential metabolites	VIP	-log10 (p)
Ethyl paraben	2.50338062	12.5391
Corticosterone	2.249073696	9.302771
4-Pyridoxic acid	1.932754207	8.357535
Isophorone	2.16447135	7.058986
N,N-Diphenylguanidine	1.665398929	6.821023
Propylparaben	1.984599204	6.417937
Diethyl phosphate	2.09408764	6.289037
Valpromide	2.068422118	6.184422
Triethyl phosphate	2.034957044	6.184422
Monobutyl phthalate	1.884839336	6.184422
12-Hydroxydodecanoic acid	1.389977726	6.184422
Decanamide	1.952674992	5.480172
Carnosine	1.91794478	5.452225
Acetyl-beta-methylcholine	1.872901202	5.452225
C-8 Ceramide-1-phosphate	1.573021268	4.692504
Valerolactam	1.612952661	4.621602
Oleoyl ethanolamide	1.520246488	4.447332
2,4-Quinolinediol	1.847375514	4.432974
1-Methylhistidine	1.702556474	4.195861
Tetrahydrocortisone	1.663548504	4.053057
Dodecyltrimethylammonium	1.579490881	4.053057
Erucamide	1.28110365	3.90389
Sphingosine _d18_1_	1.637361382	3.802968

Differential metabolites	log2(FC)	-log10 (p)
DEET	1.637150687	3.802968
12-Aminododecanoic acid	1.514601772	3.802968
1-Palmitoylglycerol	1.471418293	3.802968
4-Hydroxybenzaldehyde	1.442423517	3.639956
N-Acetylneuraminic acid	1.415842823	3.635055
N-Acetylhistamine	1.028969515	3.635055
Docosahexaenoic acid ethyl ester	1.290940854	3.003138
Pulegone	1.216195025	2.981757
2,4-Xylidine	1.311590884	2.940702
1-Methylxanthine	1.174684801	2.844482
2-(Methylthio)benzothiazole	1.490703943	2.755031
1-Linoleoyl glycerol	1.096346078	2.66615
1-Tetradecylamine	1.42093395	2.468176
DL-Arginine	1.38319731	2.443143
N8-Acetylspermidine	1.143663368	2.441052
12-oxo Phytodienoic Acid	1.433633763	2.375677
Tyrosol	1.03118906	2.364165
4-Hydroxyphenylpyruvic acid	1.282142817	2.234026
N,N-dimethyl-9H-purin-6-amine	1.372143058	2.186786
Testosterone enanthate	1.02019852	2.186786
Senkyunolide H	1.248506725	2.117231
2-Hydroxyvaleric acid	1.244841192	2.116736
Prostaglandin K2	1.319770216	2.077991
Kynurenic acid	1.257829486	2.04761
Decanoylcarnitine	1.037321097	1.996453
Aspartylphenylalanine	1.23066507	1.889579
1,5-Isoquinoline diol	1.229941927	1.814373
Myristic acid alkyne	1.015838168	1.758006
11-Deoxy prostaglandin F1	1.241984316	1.73781
Tridecylic acid	1.095490374	1.582628
Indole-3-acrylic acid	1.006026767	1.531239
Dodecyl sulfate	1.024037321	1.520468
Acetanilide	1.041120733	1.49853
Acetylcholine	1.084232004	1.463303
12-dihydroxyheptadec-16-yn-4-yl acetate	1.006286011	1.43903
2-Aminooctanedioic acid	1.011714436	1.405386
2,4-di-tert-Butylphenol	1.124799801	1.347976
Biopterin	1.080451219	1.347976

Table S4. The differential metabolites between the investigational and the control groups at 8 weeks

Differential metabolites	log2(FC)	-log10 (p)
N,N-Diphenylguanidine	1.1033	2.34106373

Acetylcholine	1.0395	2.672273348
Palmitic Acid	-1.0409	2.119443632
Taurocholic acid	-1.1729	2.160069667
2-(Methylthio)benzothiazole	-1.21	2.169905566
18-beta-Glycyrrhetinic acid	-1.2626	4.246838419
Glycochenodeoxycholic Acid	-1.4995	2.291953213
Erucamide	-2.1067	3.296424918
Propylparaben	-2.8018	2.617154804
Taurochenodeoxycholic Acid	-2.9165	2.165255127
Stearoyl Ethanolamide	-4.2756	3.693832408

Table S5. The differential metabolites between the investigational and the control groups at 16 weeks

Differential metabolites	log2(FC)	-log10(p)
Monoolein	3.0693	1.371365891
2,4-Quinolinediol	2.0916	2.527228928
N-Acetylhistamine	2.0424	4.279361169
Corticosterone	1.2634	1.496222931
2-Aminooctanedioic acid	1.0802	1.727601368
Panthenol	-1.1598	1.33048787
4-Hydroxybenzaldehyde	-1.2181	3.340692429
4-Hydroxyphenylpyruvic acid	-1.2729	1.581765018
N-Acetylsphingosine	-1.685	1.371703048
Taurocholic acid	-2.3172	1.788078916
Glycochenodeoxycholic Acid	-3.4309	2.670764884
Taurodeoxycholic Acid	-3.4967	1.879360272