

Supplemental Digital Content 1, Supplementary Methods

Food Frequency Questionnaire

Data on typical diet were collected using a quantitative Food Frequency Questionnaire (FFQ) that asked parents to report their child's frequency of consumption and typical portion size of 128 items, over the past month. The FFQ was specifically designed for this research study to include items commonly consumed by Australian infants, including items uniquely associated with infant's diet such as breast milk and teething rusks. In addition, items of particular relevance to the gut microbiota such as fermented foods were included. A template was created in Foodworks 8 (Xyris Software, Australia) to convert frequency of consumption and typical portion size data obtained from the FFQ into daily serve intake of food groups and sub-groups, using standard adult sized serves. The food groups in Foodworks 8 are designed to correspond to the food groups in the Australian Dietary Guidelines [1] and include legumes in both the vegetable and protein food groups and milk alternatives within the dairy food group. To make the food groups mutually exclusive a vegetarian protein food group was manually created to include the sub-groups for nuts and seeds, legumes, soy products, and milk alternatives, resulting in a total of 6 food groups and 26 sub-groups being calculated from the FFQ data. Due to the rapidly changing nature of dietary intake during the period of complementary feeding food group intake was converted into Z scores within 2 age groups (0.5 – 0.99; and 1.00 - 2.00 years), using SPSS (IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp).

Body Composition Assessment

Hand to foot Bioelectrical Impedance Analysis (BIA) was used to partition the body into fat (BIA FM) and fat free mass (BIA FFM) using a Bodystat Ltd 1500. Total Body Water (TBW) was computed from body weight, length and 50 Hz impedance using the Fjeld equation [2], which was developed in children aged 3 to 30 months of age in Peru. TBW was then converted to fat free mass using age and gender specific hydration constants [3] and fat mass was calculated as the difference between weight and fat mass.

Dual energy X-ray Absorptiometry (DXA) was used to partition the body into fat mass (DXA FM), lean mass (DXA LM) and bone mineral content (DXA BMC) using a GE Lunar Prodigy. One whole body scan was performed by a trained technician while the researcher and parent applied light restraint to the areas of the child's body not currently being scanned to attempt to restrict movement during scanning. All scan were provided with a quality score by a trained technician and scans with a score below 5/10 were not used for further analysis.

All measures of body composition were adjusted for length to control for natural variation in body composition by age, with length being raised to the power of the slope of the regression line relating log (body composition in kg) to log (length in metre) [4]. This resulted in the creation of the following measures of body composition: BIA Fat Mass Index (BIA FMI = BIA FM (kg)/ length (m)^{2.3}); BIA Fat Free Mass Index (BIA FFMI = BIA FFM (kg)/ length(m)^{1.6}); DXA Fat Mass Index (DXA FMI = DXA FM (kg)/ length(m)^{1.4}); DXA Lean Mass Index (DXA LMI = DXA LM (Kg)/ length(m)^{1.9}); DXA Bone Mineral Content Index (DXA BMCI = DXA BMC (kg)/ length (m)^{3.0}).

References

1. National Health and Medical Research Council, *Australian Dietary Guidelines*. 2013, NHMRC: Canberra.
- 2.. Fjeld, C.R., J. Freundt-Thurne, and D.A. Schoeller, *Total body water measured by 18-O dilution and bioelectrical impedance in well and malnourished children*. *Pediatr Res*, 1990. **27**(1): p. 98-102.
3. Fomon, S.J., et al., *Body composition of reference children from birth to age 10 years*. *Am J Clin Nutr*, 1982. **35**(5 Suppl): p. 1169-75.
4. Hill, R.J., et al., *Resting energy expenditure in children with inflammatory bowel disease*. *J Pediatr Gastroenterol Nutr*, 2007. **45**(3): p. 342-6.

Supplemental Digital Content 2, Table: Serve intake of food groups and sub-groups by age group, as assessed using a Food Frequency Questionnaire.

	Age Group 1 (0.5 – 1 year) n = 18			Age Group 2 (1 – 2 years) n = 30		
	Median	Min	Max	Median	Min	Max
Grains	0.68	0.00	2.49	2.90	1.17	6.29
Refined	0.25	0.00	2.47	1.34	0.71	3.03
Wholegrains	0.22	0.00	0.76	1.26	0.43	3.72
Fruit	0.68	0.06	3.40	1.53	0.40	4.79
Citrus melons berries	0.03	0.00	0.29	0.22	0.00	0.85
Other fruit	0.44	0.05	3.38	1.24	0.26	3.94
Fruit juice	0.00	0.00	0.02	0.00	0.00	0.08
Vegetables	1.33	0.13	3.19	1.82	0.00	8.12
Dark green vegetables	0.06	0.00	0.42	0.17	0.01	0.73
Red orange vegetables	0.61	0.02	1.48	0.56	0.02	3.10
Tomatoes	0.01	0.00	0.12	0.09	0.00	1.63
Other red orange veg	0.59	0.02	1.47	0.40	0.00	2.30
Starchy vegetables	0.26	0.03	1.05	0.39	0.01	1.65
Potatoes	0.11	0.00	0.63	0.21	0.00	0.91
Other starchy veg	0.10	0.01	0.51	0.14	0.00	1.04
Other vegetables	0.17	0.02	1.18	0.62	0.03	3.17
Animal protein foods	0.18	0.00	1.07	0.60	0.00	2.34
Red meats	0.02	0.00	0.39	0.15	0.00	1.23
Poultry	0.04	0.00	0.36	0.16	0.00	0.87
Eggs	0.01	0.00	0.42	0.18	0.00	0.77
Processed meats	0.00	0.00	0.09	0.02	0.00	0.56
Organ meats	0.00	0.00	0.00	0.00	0.00	0.02
Seafood high LC N3	0.00	0.00	0.23	0.03	0.00	0.55
Seafood low LC N3	0.00	0.00	0.38	0.02	0.00	0.18
Dairy	0.65	0.00	2.49	1.57	0.26	4.03
Infant formula	0.00	0.00	1.94	0.00	0.00	2.76
Cow's milk	0.07	0.00	0.34	0.43	0.00	2.19
Cheese	0.05	0.00	0.84	0.43	0.00	1.49
Yoghurt	0.12	0.00	0.68	0.24	0.00	1.02
Vegetarian protein	0.02	0.00	0.34	0.28	0.00	3.45
Nuts seeds	0.01	0.00	0.33	0.16	0.00	1.09
Soy products	0.00	0.00	0.06	0.00	0.00	2.86
Legumes	0.00	0.00	0.08	0.07	0.00	1.27
Milk alternatives	0.00	0.00	0.13	0.00	0.00	0.65
Breast feeds	5.25	0.00	12.0	3.00	0.00	9.00

Supplemental Digital Content 3, Table: Associations between participant characteristics and microbiota β -diversity (Adonis of weighted UniFrac) and α -diversity (Shannon Index). Spearman correlation used for continuous variables and Mann Whitney U test used for categorical variables.

	Microbiota Composition				
	β -diversity (weighted UniFrac)		α -diversity (Shannon Index)		
	R ²	p	rho	p	FDR
Age	0.20	<0.001**	0.75	<0.001**	0.006
Income	0.01	0.684	0.005	0.973	0.973
Gestation length	0.02	0.470	-0.07	0.615	0.840
Birth weight	0.03	0.165	0.06	0.700	0.840
Mother's pre-pregnancy BMI	0.01	0.936	-0.08	0.582	0.840
Age started complementary foods	0.01	0.640	0.14	0.351	0.840
	R ²	p	Z	p	
Gender	0.01	0.900	-1.17	0.244	0.465
Ethnicity	0.03	0.259	-0.25	0.819	0.983
Delivery mode	0.005	0.967	-0.14	0.887	0.983
Exclusively breast fed at 4 months	0.02	0.288	-1.325	0.193	0.465
Currently breast fed	0.03	0.246	-1.09	0.277	0.465
Out of home child care	0.02	0.463	-1.72	0.086	0.465
Siblings	0.025	0.298	-1.08	0.279	0.465
Pets	0.01	0.879	-1.12	0.263	0.465
Antibiotics before 12 months age	0.01	0.917	-0.02	0.983	0.983
Antibiotics before 1 month age	0.02	0.458	-0.67	0.502	0.717

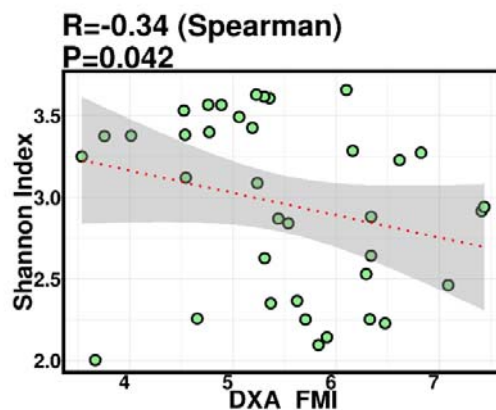


Supplemental Digital Content 4, Table: Associations between body composition and microbiota

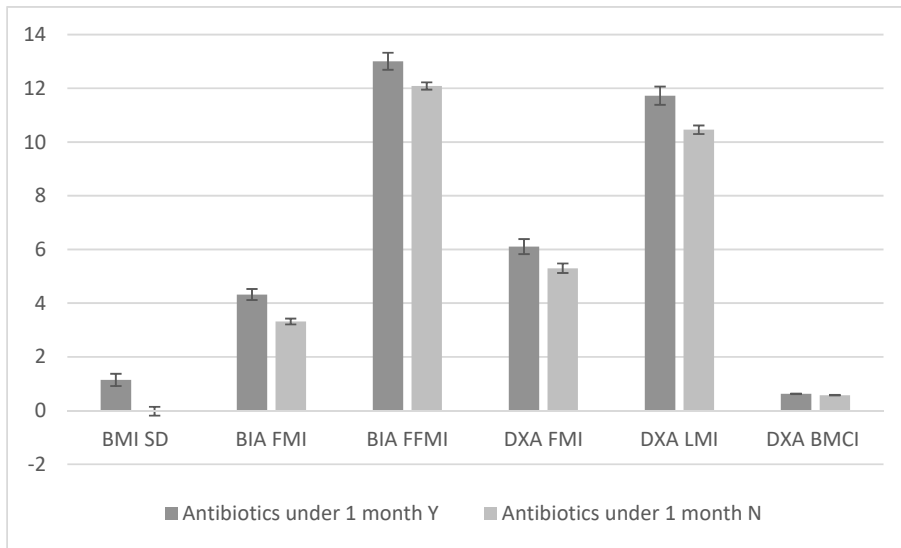
composition

	Microbiota Composition					
	β-diversity (weighted UniFrac)			α-diversity (Shannon Index)		
	R ²	p	pFDR	rho	p	pFDR
BMI SD	0.01	0.897	0.917	-0.135	0.362	0.411
BIA FMI	0.02	0.322	0.880	-0.27	0.066	0.198
BIA FFMI	0.02	0.440	0.880	-0.19	0.203	0.304
DXA FMI	0.05	0.099	0.594	-0.34	0.042*	0.198
DXA LMI	0.01	0.917	0.917	-0.25	0.139	0.278
DXA BMCI	0.01	0.912	0.917	-0.14	0.411	0.411

Associations between microbiota β-diversity (weighted UniFrac) and α-diversity (Shannon Index) and body composition (Body Mass Index – BMI; Fat Mass Index – FMI; Fat Free Mass Index – FFMI; Lean Mass Index –LMI ; Bone Mineral Content Index –BMCI), as assessed using BIA (Bioelectrical Impedance Analysis) and DXA (Dual energy X-ray Absorptiometry). Adonis used for microbiota β-diversity (weighted UniFrac) and Spearman correlation used for α-diversity (Shannon Index).

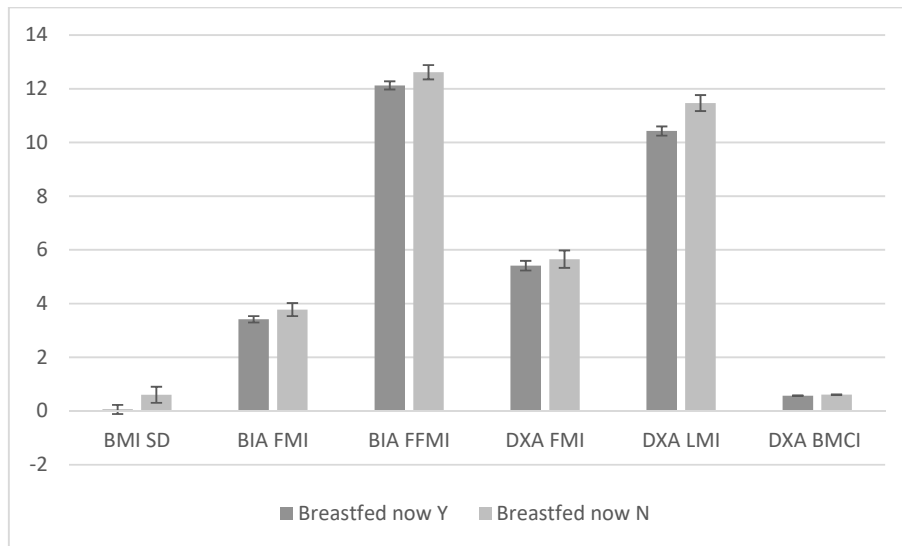


Supplemental Digital Content 5, Figure: Body size and composition by antibiotic exposure in the first month of life.



Body size (Body Mass Index – BMI SD) and body composition (Fat Mass Index – FMI; Fat Free Mass Index – FFMI; Lean Mass Index -LMI; Bone Mineral Content Index –BMCI), as assessed using BIA (Bioelectrical Impedance Analysis) and DXA (Dual energy X-ray Absorptiometry) by antibiotic exposure in the first month of life (Y = 11, N = 39).

Supplemental Digital Content 6, Figure: Body composition by current breastfeeding status.



Body size (Body Mass Index – BMI SD) and body composition (Fat Mass Index – FMI; Fat Free Mass Index – FFMI; Lean Mass Index -LMI; Bone Mineral Content Index –BMCI), as assessed using BIA (Bioelectrical Impedance Analysis) and DXA (Dual energy X-ray Absorptiometry) by current breastfeeding status (Y = 33, N = 16).

Supplemental Digital Content 7, Figure: Scatterplot of DXA LMI (Lean Mass Index as assessed using Dual energy X-ray Absorptiometry) by dairy serve intake Z score, as assessed using Food Frequency Questionnaire.

