Asian Journal of Medicine and Biomedicine
eISNN: 2600-8173
https://journal.unisza.edu.my/ajmb

PENERBIT Universiti Sultan Zainal Abidin

Asian Journal of Medicine and Biomedicine, Vol 6:1.

Open Access

A Comparative Study on Faecal Short-Chain Fatty Acids Concentration in Lean, Overweight and Obese School Children

Nurul Ain Saipudin, Nornazira Suhairom, Nur Husna Abd Wahid
Department of Technical and Vocational Education, School of Education, Faculty of Social Science and Humanities, Universiti Teknologi Malaysia 81310 Skudai, JohorBahru, Johor, Malaysia
**nurulain.s@graduate.utm.my*

Abstract

The relationship between body weight and faecal short-chain acids (SCFAs) concentration may be interrelated and contribute to the development of childhood obesity and other metabolic diseases. Aim & Objectives: This study aims to compare the faecal SCFAs concentration produced in lean, overweight and obese children aged 7 to 10 years old. Malay ethnic, healthy, lean, overweight and obese, aged 7 to 10 years old from the selected primary school participated in this study. We assessed the socio-demographic and nutritional status of lean, overweight and obese children. A faecal sample was collected and analysed for faecal SCFAs concentration (acetic acid, butyric acid and propionic acid) by using the high-performance liquid chromatography (HPLC) method. A total of 42 overweight and obese children (n=22) and lean children (n=20) with mean age = 8.73 ± 1.03 years old; BMI = 33.51± 20.82 kg/m^2; 50 % boys participated in the study. Intake of macronutrients was distributed evenly, but a substantial number of the participants did not achieve the RNI for energy and Malaysian Dietary Guidelines for Children and Adolescents (MDG) for carbohydrates and fibre intake. There was no significant difference between the group on dietary intakes ($p>0.05$). The fecal SCFAs concentration of propionic acid (Mean=43.08 µmol/g; SD= 37.14) was the highest and followed by acetic acid (Mean= 29.73 µmol/g; SD= 23.30) and butyric acid (Mean= 23.19 µmol/g; SD= 17.75) with the overweight and obese children reported a higher concentration of butyrate (52%) and propionate (38%) as compared to lean children ($p>0.05$). This data on faecal SCFAs concentration of overweight and obese among Malay school children had revealed the total faecal SCFAs being produced was 106.60 ± 63.83 µmol/g which is 34% higher than lean children. Conclusion: Despite the difference in trend found in faecal SCFAs concentration between lean, overweight and obese school children, this study had found the insignificant. Therefore, the underlying mechanisms that increased the concentration of SCFAs concentration in overweight and obese children warrant future investigation in combating childhood obesity.

Keywords: childhood obesity; school children; fibre; faecal analysis; short-chain fatty acids

*Author for Correspondence

Cite as: Nurul Ain, S., Nornazira, S. & Nur Husna, A.W. (2022). A Comparative Study on Faecal Short-Chain Fatty Acids Concentration in Lean, Overweight and Obese School Children. Asian Journal of Medicine and Biomedicine, 6(1), 77- 83.

DOI: https://doi.org/10.37231/ajmb.2022.6.1.465

Introduction

Childhood obesity is seen as a universal health problem, particularly in developing countries with the highest prevalence reported in the Middle East (89.6%) and Eastern Europe (48.3%) [1, 2]. Within Asia pacific regions, 7.9% of urban school children of age 7 to 9 years were categorized as overweight in the north-eastern part of Thailand [3]. While in Indonesia, the urban areas record a higher prevalence of overweight children among 8 to 10 years, if compared to rural areas. This number comprised 15.3% among girls and 17.8% among boys [4].

In line with the urbanization and increased rate of economic status in the developing countries, the prevalence of childhood obesity is rapidly increasing[1, 2]. Similarly, in Malaysia, the trend of obesity among school children is worrying. A recent study revealed about 30% of 8 to 12 years old school children in Kuala Lumpur and Selangor were obese [5]. Among the consequence of childhood obesity is the possible and greater chances to grow up as overweight and obese adults. In addition, obese children have a higher potential to develop heart disease, diabetes and other chronic diseases during adulthood [6, 7].

The major factor of childhood obesity is an imbalance between energy intake and energy expenditure. But lately, the factor of the short-chain fatty acids (SCFAs) produced by the gut microbiota of different bodyweight individuals has been increasingly upfront [8]. Studies found the higher and lower of certain microbes namely *Bacteroidetes* and *Firmicutes* in the gut microbiota have influenced the total energy harvesting in the gut which then may affect the individual and total SCFAs concentration [9, 10]. For a better understanding of obesity pathophysiology, the release of microbiota-induced fermentation products such as SCFAs and gram-negative lipopolysaccharides (LPS) must be clarified [11].

SCFAs, which are mostly composed of acetic, propionic, and butyric acids, are the most common SCFAs in the intestinal lumen, accounting for 90 to 95 percent of total SCFAs content [12, 13]. SCFA synthesis is advantageous because a more acidic environment and a lower luminal pH may limit pathogenic microbe colonisation and boost food absorption. For instance, acetic acid is connected with Bifidobacteria's ability to suppress enteropathogens. Additionally, butyrate stimulates the formation of mucin, altering the bacterial adhesion potential. Despite the fact that all three of these SCFAs are critical in diet-induced obesity, butyrate and propionate have been proven to have larger impacts than acetate [14]. Surprisingly, the combination of butyric and propionic acids results in the formation of the gut hormones peptide tyrosine (PYY), glucagon-like peptide-1 (GLP 1), and ghrelin [15]. By contrast, acetate interacts with the central nervous system to suppress hunger, hence enhancing satiety [16]. Therefore, by considering the difference in faecal SCFAs concentration might be connected with different body weights and contributed to childhood obesity development, the principal aim of this study was to compare the faecal SCFAs concentration produced in lean, overweight and obese children aged 7 to 10 years old from the selected primary school in Selangor.

Materials and Methods

Study Design and Samples

This was preliminary data for a community-based intervention, randomized cross-over trial design study. The inclusion criteria were healthy Malay and registered school children aged 7 to 10 years old with overweight/obese body weight (z-scores for BMI-for-age were more than +1 SD and more than +2 SD) and lean body weight (z-scores for BMI-for-age between -1 SD and +1 SD). Among other criteria were children who did not take any supplements, antibiotics, or vaccination for the past 2 weeks before recruiting. By using multistage clusters and purposive sampling, this study was conducted at the primary school in Serdang district, Selangor. About 469 children were screened to participate in the study, but only 42 participants were eligible, responded and enrolled. This study protocol was approved by the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (JKEUPM), Malaysia [FPSK_November (13) 03], Ministry of Education, Putrajaya [KP(BPPDP)603/5/JLD.16(1 5 4)] and Department of Education of Selangor, Shah Alam [JPNS.PPN 600-1/4 JLD.32(32)].

Measures

Data on the demographic of the participants which included demographic and socioeconomic were gathered and used as background information for the participants. The questionnaire was conducted in the Malay language and the questions were related to the parents of participants, including their gender, age and monthly income.

Nutritional status included assessment of body weight status and dietary intake of the participants. Bodyweight was assessed using the digital weighing machine to the nearest 100 g or ¼ lb. Height has been evaluated using a stadiometer to the nearest 0.1 cm or 1/8 in. and with the eye level parallel to the headboard to prevent parallax error [17,18].

Measurements were repeated at least three times and the average value was measured. BMI was calculated and categorized according to WHO child growth standards; z-scores. The growth indicators of participants were classified as BMI-for-age which was> +1.0 SD for overweight and > +2.0 SD for obese.

Mean dietary intake was assessed using 3 days diet record. The portion sizes were estimated using a standard household measurement. All foods are converted into grammes based on the standard household measurements. All nutrients were analyzed, averaged and reported as a daily consumption by using the Nutritionist Pro® Software Version 4.0 (Axxya System LLC., Stafford, Texas, USA) [19] and the adequacy of the intake was compared with the Malaysian Recommended Nutrient Intakes (RNI) [20].

Faecal samples were collected in a sterile container and transferred into sterile 1.5 ml microtubes at the exact weight of 0.3 g. These aliquots were placed at – 6 °C immediately for further analysis. The SCFAs (acetic acid, propionic acid and butyric acid) of faecal samples were extracted with few modifications by the HPLC method [21].

Statistical Analyses

Data were analysed using SPSS version 22.0 [22] and a statistical level of $p < 0.05$ was considered significant. Descriptive statistics including means and standard deviations (SD) used to report demographic information, nutritional status (anthropometry and dietary intake) and SCFAs concentration. The mean differences between the groups on anthropometry measurement and dietary intakes were determined using the Independent t-test.

Results

A total of 42 overweight/obese and lean Malay school children with a mean age of 8.73 ± 1.03 years old ranging from 7 to 10 years old with 50% males participating in the study. Most of the participants lived with monthly household incomes ranging from RM 1,501 – to RM 3,500 and RM 5,501 – to RM 7,500 (36.4%) (Table 1).

On average, participants had a mean height of 156.90 ± 9.29 cm; a weight of 38.21 ± 13.94 kg and BMI of 33.51± 20.82 kg/m^2. Twenty participants were overweight/obese (> +2.0 SD) and the other twenty participants were lean with the mean z-score of BMI-for-age 2.59 ± 0.99 SD and 0.03 ± 1.22 SD respectively. Overweight/obese children had significantly higher weight and height as compared to lean children ($p<0.05$) (Table 2).

The majority of the participants consumed relatively higher dietary protein and fat. A substantial number of the participants did not achieve the RNI for energy and MDG for carbohydrates and fibre intake. No significant difference was found between groups on all mean dietary intakes ($p>0.05$) (Table 3).

The mean faecal SCFAs concentration of overweight/obese participants was 29.27 ± 26.67 µmol/g for acetate, 27.70 ± 19.00 µmol/g for butyrate, 49.63 ± 42.03 µmol/g for propionate and 106.60 ± 63.83 µmol/g for total SCFAs. While the mean faecal SCFAs concentration of lean participants was 25.17 ± 19.39 µmol/g for acetate, 18.22 ± 15.22 µmol/g for butyrate, 35.88 ± 30.32 µmol/g for propionate and 79.28 ± 106.60 µmol/g for total SCFAs. The SCFAs ratio of 9:6:5 was observed in all participants and the concentration of propionic acid (45%) was the highest followed by acetic acid (31%) and butyric acid (24%).

The overweight/obese participants reported a higher concentration of butyrate (52%) and propionate (38%) as compared to lean participants. However, no significant difference was found between the group ($p>0.05$) (Table 2).

Table 1: Socio-Demographic Characteristics of the Participants (N=42)

Parameters	Overweight/Obese Children (n = 22)	Lean Children (n = 20)	Total (n=42)
Mean age (years)			8.7 ± 1.0
7	4 (18.2)	4 (20.0)	8 (19.0)
8	3 (13.6)	5 (25.0)	8 (19.0)
9	10 (45.5)	6 (30.0)	16 (38.1)
10	5 (22.7)	5 (25.0)	10 (23.8)
Sex			
Boy	12 (54.5)	9 (45.0)	21 (50.0)
Girls	10 (45.5)	11 (55.0)	21 (50.0)
Monthly Household Income (Ringgit Malaysia)			
Less than 1500	2 (9.1)	4 (20.0)	6 (14.3)

1501 – 3500	6 (27.3)	7 (35.0)	13 (31.0)
3501 – 5500	5 (22.7)	2 (10.0)	7 (16.7)
5501 – 7500	8 (36.4)	5 (25.0)	13 (31.0)
Above 7500	1 (4.5)	2 (10.0)	3 (7.0)

Table 2: Anthropometric Data, Body Weight Status and SCFAs Concentration of The Participants (N=42)

Parameters	Overweight/Obese Children (n = 22)	Lean Children (n = 20)	Total (n=42)	P value
Anthropometric data				
Weight (kg)	47.76 ± 12.09	27.71 ± 6.01	38.21 ± 13.94	p<0.05
Height (cm)	140.87 ± 8.71	131.05 ± 7.02	156.90 ± 9.29	p<0.05
BMI (kg/m^2)	24.73 ± 3.91	16.62 ± 2.48	33.51± 20.82	p<0.05
BMI-for-age Z-score	2.59 ± 0.99	0.03 ± 1.22	4.86 ± 1.37	p<0.05
Bodyweight status				
Overweight			2	
Obesity			20	
Lean			20	
SCFAs Concentration (µmol/g)				
Acetic acid	29.27 ± 26.67	25.17 ± 19.39	29.73 ± 23.30	ns
Butyric acid	27.70 ± 19.00	18.22 ± 15.22	23.19 ± 17.75	ns
Propionic acid	49.63 ± 42.03	35.88 ± 30.32	43.08 ± 37.14	ns
Total SCFA	106.60 ± 63.83	79.28 ± 106.60	96.00 ± 57.74	ns

Values were stated as mean ± SD; p<0.05 = significantly different based on Independent Samples T-Test; ns=no significant

Table 1: The Mean Dietary Intakes of the Participants (n=42)

Dietary Intakes	Overweight/Obese Children (n = 22)	Lean Children (n = 20)	Total (n=42)	p-value
Energy (kcal/d)	1462 ± 533	1364 ± 506	1415 ± 516	ns
RNI Adequacy (%)	82	78	80	ns
Carbohydrate (g)	201 ± 85	173 ± 69	187 ± 78	ns
Intake (%)	54	51	53	ns
Protein (g)	52 ± 21	54 ± 23	53 ± 22	ns
Intake (%)	15	16	15	ns
RNI adequacy (%)	152	151	156	ns
Fat (g)	50 ± 19	51± 23	51 ± 21	ns
Intake (%)	31	33	32	ns
Dietary fibre	1.64 ± 0.80	1.50 ± 0.98	1.57 ± 0.88	ns
Dietary fibre/1000kcal	1.38 ± 0.75	1.19 ± 0.87	1.29 ± 0.80	ns

Values were stated as mean ± SD; ns = no significant differences based on the Independent Samples T-Test

Discussion

As compared with many other Malaysian dietary studies, 7 to 10 years old children involved in this study were not meeting the RNI for energy and MDG recommendation for carbohydrates and dietary fibre intake [5, 23, 24]. The habits of skipping the intake of fruits, vegetables and sweetened foods and beverages during diet records were observed among the participant. The mean dietary fibre intakes of the participants were reported lower than MDG guidelines which about 20-30g intakes per day [25]. The majority of the participants did not consume fruits, vegetables, legumes and dairy products every day. The recommendation for 3 serving sizes of vegetables and 2 serving sizes of fruits every day was not fulfilled [26]. The main reason for low dietary fibre intake among the participant may be due to environmental influences either in-home or at school [24]. There were various types of highly refined sugar beverages and processed foods and snacks from the vending machine and food stalls that were available within and outside the school premises. This study suggested further nutrition intervention should be done in the future to encourage healthier food choices among the children, especially during schooling.

This study had determined the total faecal SCFAs being produced in the overweight and obese Malay children were 106.60 ± 63.83 µmol/g in which the highest SCFAs found were propionic acid (47%) followed by acetic acid and butyric acid. Noteworthy, Malaysia still had no preliminary data on faecal profiles for overweight and obese children except in a 2010 publication on SCFAs differences among healthy and inflammatory bowel disease (IBD) adults that found 78 –160µmol/g SCFAs per day were being produced among healthy adults [21].

As to be compared with global reports, faecal acetate was highlighted as a major product of the total SCFAs, followed by propionate, and butyrate with a molar ratio of 60:20:20 [10]. While in this current study, the molar ratio of faecal SCFAs concentration produced among the participants was 45:30:25. Indeed the rate, molar ratio and SCFAs production may ranging from 40:40:20, 60:25:15 to 75:15:10 [26]. The differences are highly dependable on the complex interaction between different patterns of dietary factors (especially fatty food and non-digestible carbohydrates) in different regions and countries which thus may lead to different microbiome diversity and activity and gut transit time [10, 26]. These differences require to be studied separately.

No significant difference was found in the faecal SCFAs between overweight, obese and lean participants in the study ($p>0.05$). However, overweight and obese participants reported a higher trend of faecal SCFAs concentration as compared to lean participants. This current study had suggested the tendency of Malaysian obese gut microbiota in producing higher SCFAs concentrations especially butyrate and propionate from the faecal samples. The phenotype of the obese itself is perceived to be linked with a higher SCFAs production in the faecal sample than the lean phenotype. These results were consistent with another cohort study among Swiss children; 15 obese and 15 normal-weight Swiss children aged 8 to 14 years old, in which faecal butyrate and propionate were significantly higher in obese than normal-weight children ($p=0.01$) [27]. Nevertheless, their acetate concentrations remained the same in each group [27].

In contrast, higher faecal SCFAs production was found among Japanese and Mexican lean children than their obese children [23,24]. In a study among Mexican children aged 9 to 11 years old, butyric acid and propionic acid were lower in overweight and obese children than in normal-weight children [24]. Other SCFAs such as acetic acid remained no difference across body weight status [28, 29]. The altered faecal propionic and butyric concentration may be related to gut microbiota dysbiosis which then caused to reduction in the concentration of SCFAs production and mucosal absorption in the children [28]. Some other factors that could be proposed are differences in genetics, environment and diet practices of the children in another country than Malaysia.

No significant difference was observed in dietary intake among the overweight, obese and lean children. The energy harvest in overweight and obese participants may be higher than in lean children which subsequently extracted more of their energy intake even though the dietary intakes were similar. Nevertheless, there was a study that found no significant association between the gut microbiota with diet intake throughout childhood life [26].

In the study, the cause and consequence of the higher concentration of SCFAs in overweight and obese versus lean children and its relationship to obesity remain unclear. The higher concentrations of butyric acid and propionic acid in overweight and obese children in this current study could be suggested by the higher presence of butyrate and propionate-producing species like *Eubacterium hallii* and *Anaerostipes caccaein* in obese children [27]. The obese individual also may have a lower relative abundance of *Bacteroidetes* as well as a higher relative abundance of *Firmicutes* [27]. This may contribute to increasing energy harvest or in other words, increase the energy extraction from the diet. As a consequence, the obese gut tends to store the energy as fat leading to promoting weight gain [8,10, 30].

There are limitations in this preliminary study on nutritional status and faecal profiles. The comprehensive components of nutritional status; biochemical status and faecal profiles such as gut microbiota composition and faecal pH should be included in the future for further investigation.

Conclusion

This current study revealed the total faecal SCFAs being produced in overweight and obese children were 106.60 ± 63.83 µmol/g which is 34% higher than in lean children. Despite these differences in trends that have been found, this study also revealed no significant difference between faecal SCFAs concentration in lean, overweight and obese children. Therefore, the underlying mechanisms that increased the concentration of SCFAs concentration in overweight and obese children, warrant future investigation in combating childhood obesity.

Acknowledgement

We thank the Research Management Center of Universiti Teknologi Malaysia (UTM) as well as teachers, parents and participants for their contribution and cooperation.

Conflict of Interest Statement

This research was held at the author's cost and there was no relevant conflict of interest in this research.

References

1. De Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *American Journal of. Clinical Nutrition*. 2010;92(5):1257-1264. https://doi.org/10.3945/ajcn.2010.29786
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study. *Lancet*. 2014: *384*: 766-781. http://.doi.org/10.1016/S0140-6736(14)60460-8
3. Langendijk G, Wellings S, van Wyk M, Thompson SJ, McComb J, Chusilp K. The prevalence of childhood obesity in primary school children in urban Khon Kaen, northeast Thailand. *Asia Pacific Journal of Clinical Nutrition*. 2003;12(1):66-72.
4. Jus'at I, Jahari AB. Regional Study of Nutritional Status of Urban Primary Schoolchildren. 2. West Jakarta and Bogor, Indonesia. *Food and Nutrition Bulletin*. 2002;23(1):31-40. https://doi.org/10.1177/156482650202300105
5. Yang WY, Burrows T, Macdonald-wicks L, Williams LT, Collins CE, Siew W, Colyvas K. Body Weight Status and Dietary Intakes of Urban Malay Primary School Children: Evidence from the Family Diet Study. *Children*, 2017. 1–16. http://doi.org/10.3390/children4010005
6. Freedman DS, Mei Z, Srinivasan SR, Berenson GS. & Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents the Bogalusa Heart Study. *Journal of Pediatric*, 2007;*150*: 62-72. https://doi.org/10.1016/j.jpeds.2006.08.042
7. Bingham MO, Harrell JS, Takada H, Washino K, Bradley C & Berry D. Obesity and cholesterol in Japanese, French, and U.S. children. *Journal of Pediatric Nursing*, 2009; 24: 314-22. https://doi.org/10.1016/j.pedn.2008.01.002
8. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444(7122):1022-1023. https://doi.org/10.1038/4441022a
9. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, Comelli EM. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutrition Diabetes*. 2014;4(6):e121. Published 2014 Jun 30. https://doi.org/10.1038/nutd.2014.23
10. Schwiertz A, Taras D, Schäfer K, et al. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)*. 2010;18(1):190-195. https://doi.org/10.1038/oby.2009.167
11. Joseph N, Vasodavan K, Saipudin NA, Yusof BNM, Kumar S, & Nordin SA. Gut microbiota and short-chain fatty acids (SCFAs) profiles of normal and overweight school children in Selangor after probiotics administration. *Journal of Functional Foods*, 2019; 57, 103–111. https://doi.org/10.1016/j.jff.2019.03.042
12. Huazano-García A, & López M. Metabolism of short chain fatty acids in the colon and faeces of mice after a supplementation of diets with agave fructans. *Intech* 2013; pp. 163–182. https://doi.org/10.5772/51248
13. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, & Salazar N. Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology*, 2016; 7(185), https://doi.org/10.3389/fmicb2016.00185
14. Lin HV, Frassetto A, Kowalik EJ, Nawrocki AR, Lu MM, Kosinski JR, Marsh DJ. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS ONE*, 2012; 7(4), 1–9. https://doi.org/10.1371/journal.pone.0035240
15. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, & Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, 2013; 54, 2325–2340. https://doi.org/10.1194/jlr.R036012
16. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Bell JD. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, 2014; 5, 1–11. https://doi.org/10.1038/ncomms4611
17. World Health Organization 2006 WHO Child Growth Standards. Geneva: WHO. http://www.who.int/childgrowth (accessed July 2020).
18. World Health Organization 2007 Growth Chart for Children 5–19 years. Geneva: WHO. http://www.who.int/growthref/ en/ (accessed July 2020).
19. First Data Bank. Nutritionist Pro. San Bruno, 2005; CA 94066.
20. Ministry of Health Malaysia. A Report of the Technical Working Group on Nutritional Guidelines National Coordinating Committee on Food and Nutrition Ministry of Health Malaysia Putrajaya 2005 RNI Recommended Nutrient Intakes for Malaysia. 2017; Putrajaya.
21. Huda-Faujan N, Abdulamir AS, Fatimah AB, et al. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. *Open Biochem J*. 2010;4:53-58. Published 2010 May 13. https://doi.org/10.2174/1874091X01004010053
22. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
23. Woon FC, Chin YS, Kaartina S, Fara Wahida R, Hiew CC, Mohd Nasir MT. Association between Home Environment, Dietary Practice, and Physical Activity among Primary School Children in Selangor, Malaysia. *Malaysia Journal of Nutrition*,

2014; *20*, 1–14.
24. Poh, BK, Ng BK, Din M, Haslinda S, Shanita SN, Wong JE, Budin SB, Ruzita AT, Ng LO, Khouw I & Norimah AK. Nutritional Status and Dietary Intakes of Children Aged 6 Months to 12 Years: Findings of the Nutrition Survey of Malaysian Children (SEANUTS Malaysia). *British Journal Of Nutrition* 2013; *110* :21–35. https://doi.org/10.1017/S0007114513002092
25. National Coordinating Committee on Food and Nutrition (NCCFN). *Malaysian Dietary Guidelines for Children and Adolescents*—Summary. 2013; Ministry of Health, Putrajaya, Malaysia.
26. Bervoets L, Van Hoorenbeeck K, Kortleven I, et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathog*. 2013;5(1):10. Published 2013 Apr 30. https://doi.org/10.1186/1757-4749-5-10
27. Payne AN, Chassard C, Zimmermann M, Müller P, Stinca S, & Lacroix C. The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutrition & Diabetes*, *1*, 2011; e12. https://doi.org/10.1038/nutd.2011.8
28. Nagata, S., Chiba, Y., Wang, C., & Yamashiro, Y. (2017). The effects of the Lactobacillus casei strain on obesity in children: A pilot study. Beneficial Microbes, 8(4), 535–543. https://doi.org/10.3920/BM2016.0170
29. Murugesan S, Ulloa-Martínez M, Martínez-Rojano H, Galván-Rodríguez FM, Miranda BC, Romano MC. Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. Eur *J Clin Microbiol Infect Dis*.; 2015; 34:1337–1346. https://doi.org/10.1007/s10096-015-2355-4
30. Koleva PT, Sarah LB, & Anita LK. The Infant Gut Microbiome: Evidence for Obesity Risk and Dietary Intervention. *Nutrients.* 2015; *7*: 2237–2260. https://doi.org/10.3390/nu7042237