https://doi.org/10.46389/rjd-2020-1018
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2020; volume 27, issue 2, pages 104-111

Original Article

Supplementing Obese Subjects with a High Fiber and Antioxidant-Rich Snack from Local Indonesian Yam Leads to Increased Bifidobacterium Spp. and Clostridium coccoides/ Eubacterium Rectale Groups

Abrory Agus Cahya Pramana [1]*, Dianandha Septiana Rubi [1], Sunarti [1]

[1] **Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia**

*Corresponding Author: Abrory Agus Cahya Pramana, Department of Biochemistry, 6th floor, Radiopoetro Building, Jl. Farmako Skip Utara, Yogyakarta, Indonesia, 55281. E-mail: abrory.agus.c.p@ugm.ac.id, Phone: +6281-5794-2326

Received: December 21st, 2019 / Accepted: April 16th, 2020

Abstract

Introduction: The gut microbiome has been known to affect the immune, gastrointestinal, nervous, and cardiovascular systems, and it can also alter the host metabolism and trigger metabolic syndrome, resulting in obesity. The two major phyla of the gut microbiome, Bacteriodetes and Firmicutes, are known to be altered in obese individuals compared with the healthy ones. Local Indonesian yam was identified to contain high insoluble and soluble fiber that is fermented by the microbiome in the gut of the host and is a specific nutrient for the gut microbiome. Therefore, we aimed to compare the gut microbiome composition in obese individuals supplemented with local Indonesian yam as the tested snack with obese individuals supplemented by wheat fluor as the standard snack. **Material and Methods:** A high-throughput screening method by real-time quantitative polymerase chain reaction (qPCR) was used to observe the gut microbiome composition, including Bacteriodetes and Firmicutes phyla, Bacteriodetes-Prevotella-Porphyromonas groups, C. coccoides-Eubacterium rectale groups, Lactobacillus spp., and Bifidobacterium spp. Cq values were further normalized by all bacteria as a reference. We compared ΔCq before and after the intervention and used the paired sample t-test to analyze the significant differences. **Results:** Our results found that obese individuals supplemented with the tested snack that contained local Indonesian yam showed a significant increase in Bifidobacterium spp. and Clostridium coccoides-Eubacterium rectale groups ($p<0.05$). **Conclusions:** Finally, we suspect that local Indonesian yam could have a specific prebiotic function to modulate specific gut microbiome rand improve gut microbiota composition in obese individuals.

Keywords: Local Indonesian Yam, Gut Microbiome, Obese, Bifidobacterium spp., C. coccoides-E. rectale

Introduction

Obesity is a non-communicable disease defined as the accumulation of excessive fat impairing human health and is the fifth global health problem with a high risk of death [1]. Unbalancing energy and shifting diet patterns with the domination of snacks and high hydrogenated fats with a low fiber diet has led to increased obesity incidence, a serious problem [2]. The rising of obesity prevalence has been a health concern because it can provoke several diseases, most notably cardiovascular disease, diabetes, and cancers [3].

The problem of obesity is faced around the world, including developing countries or developed countries [4], since the global prevalence is estimated to be around 2.1 billion people in 2013 [5]. The trends of obesity increase radically, and high-income countries (several European countries), and several low-income countries (i.e., Mexico, Egypt, and South Africa) have equitably high rates of obesity among women. Meanwhile, in large countries (i.e., China), the rate of obesity involves more than 20% of women and men [2]. Further, reports suggest that by 2025, obesity will be a major cause of death among the population of Europa North America, Australia and New Zealand, East Asia,

and South Asia [6].

The gut microbiome has emerged as a popular research topic due to its implication on the health and diseases of its host. Recently, advanced technologies reported that the human gut microbiome affected the immune, gastrointestinal, nervous and cardiovascular systems [7]; the gut microbiome could also alter the metabolism and degrade several metabolic compounds of their host, which could impact the metabolism of the host [8]. The connection of gut microbiome with energy homeostasis and inflammation, contributing to disease-related to obesity, insulin resistance, and diabetes pathogenesis, is surprisingly observed when using an animal model. The mechanisms included enhanced energy harvest, altered fatty acid metabolism and composition of the adipose tissue and liver, induction of peptide YY and glucagon-like-peptide (GLP)-1 secretion, activation of the lipopolysaccharide toll-like receptor-4, and modulation of the intestinal barrier integrity by GLP-2 [9].

The human gut microbiome is composed of 5 dominated Phyla, including Bacteriodetes, Firmicutes, Actinobacteria, Proteobacteria, and Cerrucomicrobia [10, 11]. Bacteriodetes and Firmicutes are two main phyla that constitute more than 90% of the bacteria in the human gut microbiome [11]. The presence of these two dominant phyla could be rapidly altered as a response in diet changes, in which animal-based diets altered the gut microbiome composition more rapidly compared with plant-based diets [12]. The condition caused by unbalanced gut microbiota composition is known as dysbiosis. The situation is mainly associated with multiple disease-related gut microbiome dysbioses, such as inflammatory bowel disease, *Clostridium difficile* infection, autoimmune disorders, and even obesity [13]. In obese subjects, a higher ratio of Firmicutes compared to Bacteriodetes, and *Lactobacillus* genus bacteria was reported [14].

Diet has an important function in determining human colon function, and the gut microbiome [15]. Consumption of specific prebiotics could modulate the growth and composition of gut microbiota [16] as they use the prebiotic as a food source to grow. The presence of nondigestible carbohydrates, for instance, fructans, lactulose, galactooligosaccharides, fructooligosaccharides, soybean oligosaccharides, are already known to modulate the growth of *Bifidobacterium* spp. [15, 17, 18]. Likewise, the probiotic consumption will also alter the fermentation product as a response since the gut microbiome is modulated by a specific prebiotic.

In our study, the supplementation was given by snack consumption to obese participants, which were divided into two groups, the standard snack intervention and tested snack intervention. The standard snack was composed of common wheat flour, while the tested snack was made by local Indonesian yam. The qPCR method is a fast, reliable, and low-cost method [14] that was used to evaluate the gut microbiota composition before and after the intervention with the standard and tested snack. Bacteriodetes and Firmicutes phyla and also *Bacteriodetes-Prevotella-Porphyromonas*, *Clostridium coccoides-Eubacterium rectale*, *Lactobacillus* spp., *Bifidobacterium* spp. groups were followed in the gut microbiome. In this present study, we evaluate the effect of a high-fiber and antioxidant-rich snack supplementation composed of local Indonesian yam on the gut microbiome composition in obese participants.

Material and Methods

Study subject and collection of samples

Sixty-nine obese subjects (men and women) (body mass index ≥ 25 kg/m3) between the ages of 25 and 56 years were voluntarily recruited from Universitas Gadjah Mada, Indonesia, between May and November 2018. Fifty-seven out of the 69 participants collected a fecal sample. Further, we screened for antibiotic and probiotic usage using the interview method. We excluded the participants who used antibiotics and probiotic drinks up to one month before the first intervention day and during the period of intervention (six weeks). Finally, 10 subjects, five of whom received the standard snack and five received the tested snack, were included in the analysis. The Faculty of Medicine, Public Health, and Nursing provided ethical approval for this study, and written informed consent was obtained from each participant.

Genomic DNA isolation from fecal samples

Immediately, before DNA isolation, fecal samples were stored at -20°C until the DNA isolation process. 200 mg of feces were used to isolate genomic DNA.

Genomic DNA was extracted from the fecal samples by using the Stool Kit Isolation (Favorgen, Taiwan). The protocol isolation was conducted according to manufacturer instructions with a modification that involved adding lysozyme as an additional enzyme during the first incubation at the isolation stage. The isolated DNA concentration was measured by Nanodrop (Maestro, Taiwan).

High-throughput screening of gut microbiome by real-time quantitative PCR (qPCR)

Real-time qPCR was performed using the C1000 Thermal Cycler (Bio-Rad) and controlled by CFX Manager version 3.1 (Bio-Rad). The sequence primers used in this study are listed in Table 1.

Table 1: Sequence primers for real-time PCR.

No.	Targeted		Sequence (5'→3')	Ref.
1.	**All Bacteria**	F	CGGCAACGAGCGCAACCC	[19]
		R	CCATTGTAGCACGTGTGTAGCC	
2.	**Bacteriodetes**	F	CATGTGGTTTAATTCGATGAT	[20]
		R	AGCTGACGACAACCATGCAG	
3.	**Firmicutes**	F	ATGTGGTTTAATTCGAAGCA	[20]
		R	AGCTGACGACAACCATGCAC	
4.	**Bacteriodes-Prevotella-Porphyromonas groups**	F	GGTGTCGGCTTAAGTGCCAT	[21]
		R	CGGAYGTAAGGGCCGTGC	
5.	**C. coccoides-E. rectale groups**	F	CGGTACCTGACTAAGAAGC	[21]
		R	AGTTTYATTCTTGCGAACG	
6.	**Bifidobacterium spp.**	F	TCGCGTCYGGTGTGAAAG	[21]
		R	CCACATCCAGCRTCCAC	
7.	**Lactobacillus spp.**	F	AGCAGTAGGGAATCTTCCA	[21]
		R	CACCGCTACACATGGAG	

Amplifications were performed in a Thermal Cycler 1000 (Bio-Rad) and the total volume used was 10 µL (5 µL SYBR Green, 3 µL DNA template, 1 µL for each primer). The program was as follows: 1 cycle at 95°C for 5 min, 39 cycles at 95°C for 1 min, with the following primers' annealing temperatures (All Bacteria 60.1°C, Bacteroidetes 60°C, Firmicutes 66.4°C, *Bacteriodes-Prevotella-Porphyromonas* groups 67.5°C, *C. coccoides-E. rectale* groups 60°C, *Bifidobacterium* spp. 60°C, and *Lactobacillus* spp. 60°C) for 1 min, and 72°C for 30 s with signal acquisition. A melting curve was prepared to confirm the specificity of the targeted amplicons.

Normalization of qPCR data

The qPCR data were normalized by subtracting the value obtained from each targeted bacteria group into the "All bacteria" group.

Statistical analysis

The data was visualized by the graph of the individual value type as mean. Statistical analyses of each targeted groups' bacteria were conducted in each group at pre- and post-intervention. The normality of data was analyzed by using Shapiro-Walk. A paired student's t-test was used for comparing pre- and post-intervention in each group. A Mann-Whitney U test was used to compare each group if the data were not normally distributed. A p-value of ≤ 0.05 was considered statistically significant.

Results

Subjects characteristics

Sixty-seven subjects were initially included in this study, and screening the use of antibiotic treatment for one month before the study and during the study since the antibiotic usage before and during the study would change the gut microbiome composition.

https://doi.org/10.46389/rjd-2020-1018

Table 2: Subjects' baseline characteristics.

	Standard Snack	Standard Snack	p-value
Age	44.00 (31-50)	28.00 (21-55)	0.15
Weight	70.30 (66.30-92.40)	86.60 (58.9-110.6)	0.22
BMI	27.60 (26.60-37.20)	35.80 (25.5-38.7)	0.84
Sex			
Male	1	1	
Female	4	4	

Note: Values are presented as median (min-max). p-value was considered significant at ≤ 0.05 according to the Mann-Whitney U test. BMI: Body Mass Index.

Further, 24 participants were excluded, and the remaining 23 subjects needed to be screened. The second screening was conducted to exclude the remaining subjects that consumed probiotic foods and drinks, such as yogurt, during the research study. Therefore, we obtained 5 subjects in the standard snack group and five subjects in the tested snack group.

The median age of subjects in the standard snack group was 44.00 (31-50) years and 28.00 (21-55) years in the tested snack group. The median weight of subjects in the standard snack group was 70.30 (66.30-92.40) kg and 86.60 (58.9-110.6) kg in the tested snack group. The median body mass index (BMI) of subjects in the standard snack group was 27.60 (26.60-37.20) kg/m2. The age, weight, and BMI in both groups were not significantly different. The baseline characteristics of subjects in both groups are shown in Table 2.

Gut Microbiota Composition

We compared the gut microbiota composition both in subjects supplemented with the standard and tested snack. The standard snack was composed of 100% wheat flour; meanwhile, the tested snack was composed of mixed local Indonesian yam, composed of yellow squash, arrowroot, and sweet potato. To obtain the gut microbiota composition, we applied high throughput screening by qPCR since this method is acceptable for microbiota composition analysis [22]; other reports also showed similar results when comparing qPCR analysis with other methods such as pyrosequencing [23]. Here, we showed the ratio of the two dominant phyla in gut microbiota composition, Bacteriodes to Firmicutes (B/F) ratio (Figure 1). Also, bacterial genus-level group compositions, including *Bacteriodes-Prevotella-Porphyromonas* groups, *Clostridium coccoides-Eubacterium rectale* groups, *Lactobacillus* spp., and *Bifidobacterium* spp. were shown in Figures 2-5.

In the standard snack, the B/F ratio showed a slight decline after the intervention, but it was not significantly different. Meanwhile, the tested snack showed an increase in the B/F ratio after the intervention, but it was also not significantly different. In the *Bacteriodes-PrevotellaPorphyromonas* group, the standard and tested snacks showed an increase in gut microbiome composition after the intervention, but it was not significantly different. In the *C. coccoides-E. rectale* group, the standard snack showed a decrease that was not significantly different. However, in the tested snack, the gut microbiota composition in this genus-group level was increased, and the difference was significant (p=0.019). Interestingly, regarding the *Lactobacillus* groups, both standard and tested snacks showed a decrease in this genus microbiome level, yet, it was not significantly different. Then, in the B*ifidobacterium* group, the standard snack showed no significant decrease in this bacteria group, yet, the tested snack showed a significant increase in *Bifidobacterium* (p=0.0149).

Discussion

Firmicutes and Bacteriodetes are two dominant phyla of the gut microbiome residing in the healthy human gut. Dysbiosis of the gut microbiome was thought to lead to obesity since it unbalances energy absorption in the host metabolism [24]. In our study, we found that obese individuals treated with the tested snack showed an increasing trend of the Bacte-

Figure 1: Bacteriodetes/Firmicutes (B/F) ratio in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack) (grey square). B/F ratio increased in the tested snack, and decreased in the standard snack. However, no significant difference was observed in any of the groups. Values are expressed as mean.

Figure 2: ΔCq values of the *Bacteriodes-Prevotella-Porphyromonas* group in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.

Figure 3: ΔCq values of *Lactobacillus* spp. in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.

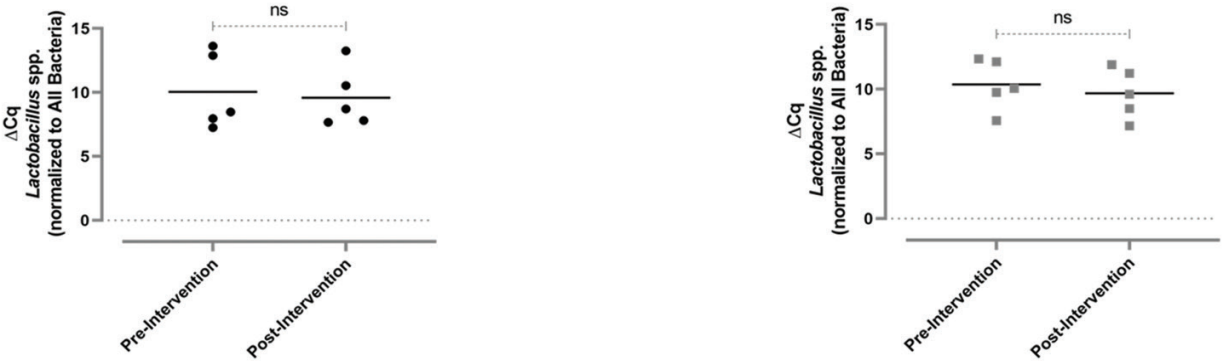
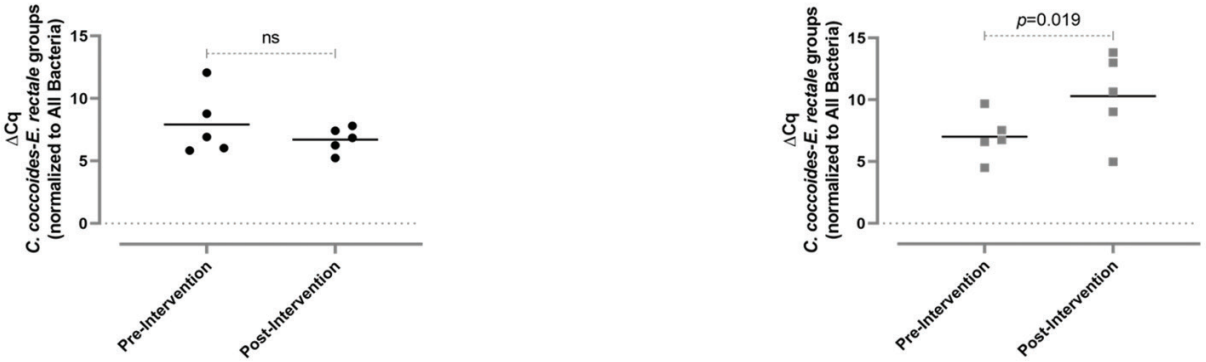
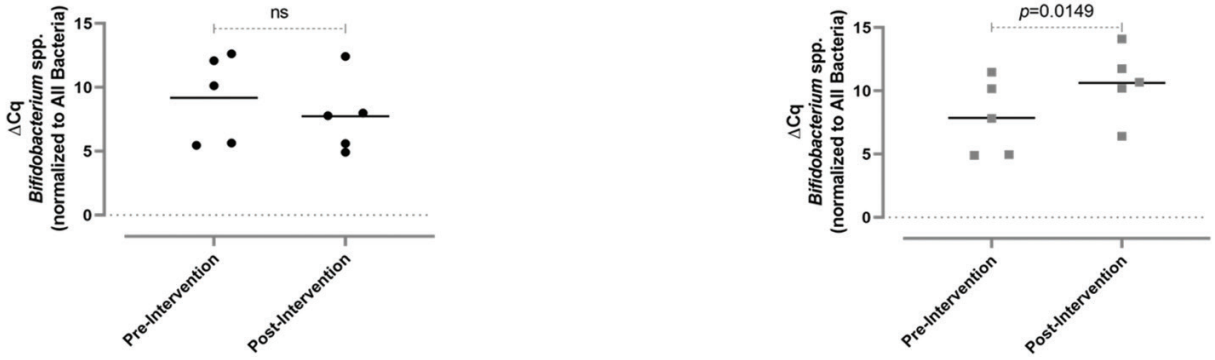


Figure 4: ΔCq values of the *C. coccoides-E. rectale* group in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values are expressed as mean.



https://doi.org/10.46389/rjd-2020-1018

Figure 5: ΔCq values of *Bifidobacterium* spp. in obese participants treated with the standard snack (black round) and tested snack (high fiber and antioxidant-rich snack - grey square). Values were expressed as mean.



riodetes/Firmicutes ratio. We showed that supplementation with the tested snack (high in fibers and antioxidants) could modulate and alter the population of gut microbiome phyla. The Bacteriodetes phyla were more increased than the Firmicutes phyla in obese participants supplemented with the tested snack, but the difference was not significant. Previous studies revealed that obese individuals have decreased Bacteriodetes and increased Firmicutes phyla, which was also the prominent phyla in the gut microbiome of obese individuals [25–27]. The increase of this phyla is assumed to accelerate the fermentation of indigestible carbohydrates leading to acetate formation, altering the energy absorption [28, 29]. A study conducted by Perry et al. [30] using a rat model showed increased acetate formation from Firmicutes phyla metabolism, which led to the activation of the parasympathetic nervous system. This condition stimulates the β-cells of the pancreas to promote insulin secretion, which then leads to chronic hyperinsulinemia. Rats receiving intragastric acetate perfusion exhibited an increased weight gain and double calory intake and increased ghrelin level concentration [30].

Prebiotics, including fiber and other non-digestible carbohydrates, are known to modulate the gut microbiome composition and are an essential dietary component for our gut microbiome necessities [31]. Arrowroot (*Maranta arundinacea L.*), for example, was reported to contain 2.37% (db) soluble dietary fiber and 12.49% (db) insoluble fiber, and also a high amount of raffinose and lactulose, and a low amount of stachyose [18]. Yellow squash from the *Cucurbita* genus is known to contain 4% soluble dietary, 15.68% insoluble fiber, and 2.67 mg of β-carotene as an antioxidant [32]. Meanwhile, sweet potatoes (from the *Ipomea* genus) are reported to contain, on average, 5.30% and 5.43% of soluble and insoluble fiber, respectively [33]. The presence of high soluble and insoluble fiber in the tested snack could affect and support the growth of the "good" microbiome and affect the host physiology and metabolism.

Therefore, we also tried to observe gut microbiome composition into the bacteria-groups genus level. First, we looked at the genus-group level of *Bacteriodes-Prevotella-Porphyromonas* groups and *Lactobacillus* spp. We observed an increase in the *Bacteroides-Prevotella-Porphyromonas* group and a decrease in *Lactobacillus* spp. both in subjects treated with the standard and tested snacks, but the differences were not significantly different. Two studies by Paturi et al. [34, 35] showed that one prebiotic, inulin, exhibits the ability to increase the *Bacteroides-Prevotella-Porphyromonas* group and *Lactobacillus* spp. in a rat model. However, we observed a different pattern modulation both in the standard and tested snacks. We suggest that the dietary components of standard and tested snacks have similar substances that modulate the *Bacteriodes-Prevotella-Porphyromonas* groups; on the other hand, they also inhibit the growth of *Lactobacillus* spp. We also found significantly increased *Bifidobacterium* spp. in obese individuals treated using the tested snack. A study on prebiotics found that some of the fiber and non-digestible carbohydrates could modulate *Bifidobacterium* spp. and are known as bifidogenic, such as fructooligosaccharides, lactulose, raffinose, stachyose, and inulin [18]. Our results showed that the existence of fiber from arrowroot could modulate and enhance the growth of *Bifidobacterium* spp. Harmayani et al. [18] showed increased *Bifidobacterium* spp. in a rat model, similar to our results. The presence of a prebiotic such as lactulose, raffinose, and stachyose could be the primary component that affects the growth of this bacteria. *Bifidobacterium* spp. are known to improve the function of the intestinal epithelial barrier and also

promote immunomodulatory substances [36].

Interestingly, we obtained a significant increase in the level of *C. coccoides-E. rectale* group in the tested snack compared with the standard snack. *E. rectale* is a useful bacteria in the gut system that can produce butyrate as a product of fermentation [37]. This is the first study to report an increased level of *C. coccoides-E. rectale* by using a high fiber and antioxidant-rich snack obtained from mixed yellow squash, arrowroot, and sweet potato. A previous study conducted by Gomez et al. [37] showed that pectin from lemon and sugar beet could enhance the growth of *C. coccoides-E. rectale* groups. We observed the pattern similarities and showed that mixed yellow squash, arrowroot, and sweet potato also could modulate the bacteria from this genus level. Pectins can be found easily in the form of vegetables and fruits, which have a benefit in delaying gastric emptying and glucose tolerance [38]. Moreover, a study on a specific dietary component in the tested snack compared to the standard snack should be conducted to know what is the specific prebiotic contained in both snacks that will differently modulate the *Bacteriodes-PrevotellaPorphyromonas* groups, *Lactobacillus* spp., and also *C. coccoides-E. rectale* groups.

In this research, we tried to use a combination of fiber and antioxidant sources to compose the tested snack. The tested snack was composed of mixed yellow squash, arrowroot, and sweet potato. Meanwhile, the standard snack was made from wheat flour as the standard flour used in the food industry. Yellow squash, arrowroot, and sweet potato are the main local foods in Indonesia, but they are little utilized. Nowadays, the necessity of non-digestible carbohydrates in functional food is significantly increased [18]. Changing diet patterns to a diet high in glucose and low in fiber leads to obesity, which has been on the rise for several decades. Therefore, choosing a diet high in fiber and non-digestible carbohydrates is a vital point in the case of obese individuals that can repair the gut microbiome composition. This study provided evidence that a combination of fiber and antioxidants from yellow squash, arrowroot, and sweet potato can modulate the gut microbiota composition. Here, we observe that the combination can increase or decrease several bacteria from the gut microbiome composition. However, given the limited access to what specific prebiotic substances are contained in the tested snack since they can modulate the gut microbiota composition in obese volunteers, especially *C. coccoides-E. rectale* groups should be studied further in order to increase the growth of butyrogenic gut microbiota, and increase the health of the gut system to prevent low-grade inflammation in obese individuals. Furthermore, research on the metabolomic of the compound-derived gut microbiome should be conducted to elucidate how *C. coccoides-E. rectale* groups can be modulated by high fiber and antioxidant-rich snacks from local Indonesian yam.

Conclusions

We obtained a significant difference in altering gut microbiome composition in obese individuals treated with a snack high in fiber and antioxidants. Here, we showed a significant increase in *Bifidobacterium* spp. compared with obese individuals treated with a standard snack made of wheat flour. Interestingly, we also found increased levels of *C. coccoides-E. rectale* groups, a pattern that was not found in obese subjects treated with the standard snack.

Acknowledgments

We would like to thank Universitas Gadjah Mada who offered a research grant to author Abrory Agus Cahya Pramana (Young Lecturer Grant, No. 3943/UN1/DITLIT/DIT-LIT/LT/2019). We would also like to thank Mrs. Tri Purwanti (laboratory technician at the Graduate School of Biotechnology, Universitas Gadjah Mada) for her help during technical assistance in this research.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Ellulu M, Abed Y, Rahmat A, Ranneh Y, Ali F. Epidemiology of obesity in developing countries: challenges and prevention. Glob Epidemic Obes. 2:2, 2014.
2. Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J Clin Nutr. 84:289–98, 2006.
3. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M.

 https://doi.org/10.46389/rjd-2020-1018

Health and economic burden of the projected obesity trends in the USA and the UK. Lancet. 378:815–25, 2011.
4. World Health Organization. Fact sheets: Obesity and overweight. 2016. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. [Accessed 8th August 2019].
5. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. 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 2013. Lancet. 384:766–81, 2014.
6. Di Angelantonio E, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet. 388:776–86, 2016.
7. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND, et al. The Effects of Vegetarian and Vegan Diets on Gut Microbiota. Front Nutr.6(47):1-10, 2019.
8. Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. Curr Obes Rep. 8(3):317-332, 2019.
9. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: The hygiene hypothesis expanded? Diabetes Care. 33:2277–84, 2010.
10. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Circ Res. 120:1183–96, 2017.
11. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 464:59–65, 2010.
12. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 505:559–63, 2014.
13. Tseng CH, Wu CY. The gut microbiome in obesity. J Formos Med Assoc. 118:S3–9, 2019.
14. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PLoS One. 4:1–8, 2009.
15. Cumming JH, Bingham SA. Dietary fibre, fermentation and large bowel cancer. Cancer Surv. 6:601–21, 1987.
16. Christensen EG, Licht TR, Leser TD, Bahl MI. Dietary Xylo-oligosaccharide stimulates intestinal bifidobacteria and lactobacilli but has limited effect on intestinal integrity in rats. BMC Res Notes. 7:1–14, 2014.
17. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourié B, et al. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. Am J Clin Nutr. 80:1658–64, 2004.
18. Harmayani E, Kumalasari DI, Marsono Y. Effect of arrowroot (Maranta arundinacea L.) diet on the selected bacterial population and chemical properties of caecal digesta of Sprague Dawley rats. Int Res J Microbiol. 2:278–84, 2011.
19. Bahl MI, Bergström A, Licht TR. Freezing fecal samples prior to DNA extraction affects the Firmicutes to Bacteroidetes ratio determined by downstream quantitative PCR analysis. FEMS Microbiol Lett. 329:193–7, 2012.
20. Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: A case-control study. BMC Med. 11:1–12, 2013.
21. Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 97:1166–77, 2004.
22. Bai G, Ni K, Tsuruta T, Nishino N. Dietary Casein and Soy Protein Isolate Modulate the Effects of Raffinose and Fructooligosaccharides on the Composition and Fermentation of Gut Microbiota in Rats. J Food Sci. 81:H2093–8, 2016.
23. Oh HY, Kim BS, Seo SS, Kong JS, Lee JK, Park SY, et al. The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. Clin Microbiol Infect. 21:674.e1-674.e9, 2015.
24. Sivamaruthi BS, Kesika P, Suganthy N, Chaiyasut C. A Review on Role of Microbiome in Obesity and Antiobesity Properties of Probiotic Supplements. Biomed Res Int. 2019:1-20, 2019.
25. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human Gut Microbes Associated with Obesity. Br Commun. 444:1022–3, 2006.
26. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 457:480–4, 2009.
27. Santacruz A, Collado MC, García-Valdés L, Segura MT, Marítn-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 104:83–92, 2010.
28. Ismail NA, Ragab SH, ElBaky AA, Shoeib ARS, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. Arch Med Sci. 7:501–7, 2011.
29. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA. 106:2365–70, 2009.
30. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Nature. 534:213–7, 2016.
31. Ercolini D, Fogliano V. Food Design to Feed the Human Gut Microbiota. J Agric Food Chem. 66:3754–8, 2018.
32. Jacobo-Valenzuela N, Zazueta-Morales J de J, Gallegos-Infante JA, Aguilar-Gutierrez F, Camacho-Hernández IL, Rocha-Guzman NE, et al. Chemical and physicochemical characterization of winter squash (Cucurbita moschata D.). Not Bot Horti Agrobot Cluj-Napoca. 39:34–40, 2011.
33. Mullin WJ, Rosa N, Reynolds LB. Dietary fibre in sweet potatoes. Food Res Int. 27:563–5, 1994.
34. Paturi G, Butts CA, Monro JA, Hedderley D. Effects of Blackcurrant and Dietary Fibers on Large Intestinal Health Biomarkers in Rats. Plant Foods Hum Nutr. 73:54–60, 2018.
35. Paturi G, Butts CA, Stoklosinski H, Ansell J. Effects of early dietary intervention with a fermentable fibre on colonic microbiota activity and mucin gene expression in newly weaned rats. J Funct Foods. 4:520–30, 2012.
36. Paturi G, Nyanhanda T, Butts CA, Herath TD, Monro JA, Ansell J. Effects of Potato Fiber and Potato-Resistant Starch on Biomarkers of Colonic Health in Rats Fed Diets Containing Red Meat. J Food Sci. 77:216–23, 2012.
37. Gómez B, Gullón B, Yáñez R, Schols H, Alonso JL. Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. J Funct Foods. 20:108–21, 2016.
38. Larsen N, De Souza CB, Krych L, Cahú TB, Wiese M, Kot W, et al. Potential of pectins to beneficially modulate the gut microbiota depends on their structural properties. Front Microbiol. 10(223):1–13, 2019.

