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HUBUNGAN MIKROBIOTA SALURAN CERNA DAN OBESITAS PADA ANAK DI LOMBOK BARAT, NUSA TENGGARA BARAT, INDONESIA

The Relationship between Gut Microbiota and Obesity among Children in West Lombok, West Nusa Tenggara, Indonesia

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ABSTRACT

Background*. Obesity in children makes the higher risk of micronutrients deficiency, increase profile lipid, and promote cell inflammation. Some studies report that gut microbiota may have roles in body metabolism include obesity.* ***Objective.*** *Our study aimed to compare the number of Lactobacillus, Bifidobacteria, Escherichia coli, and Enterobacter between obese, normal, and wasted children.* ***Method****. The study was performed in 115 healthy children in West Lombok, West Nusa Tenggara, Indonesia. The total number of bacteria was counted using a culture technique with selective media and total plate count method. Dietary intake assessed to all subjects using a semi-quantitative food frequency questionnaire. Data were analyzed using one-way ANOVA between three groups.* ***Results****. The results showed a significant difference in the number of Escherichia coli between obese, normal, and wasted children (p=0.02), meanwhile there were no significant differences of dietary intake and the number of Lactobacillus, Enterobacter, and Bifidobacteria between the three groups. A potential mechanism by which dysbiosis may cause obesity is its ability to produce short-chain fatty acid (SCFA) by fermentation in the colon. It may increase gut permeability, ghrelin secretion, or bind to toll-like-receptor which leads to enhancement of free fatty acid, cholesterol, and adipose tissue synthesis.* ***Conclusion****. Dysbiosis often happened in obese children. Obese children tend to have an imbalance of gut microbiota. However, it needs further study to assess the effects of certain gut microbiota on dietary intake and their effects on obesity cases among children.*

Keywords*: children, dysbiosis, gut microbiota, obesity*

ABSTRAK

Latar Belakang. Obesitas pada anak-anak dapat meningkatkan risiko defisiensi zat gizi mikro, meningkatkan profil lipid, dan mendorong inflamasi sel. Ketidakseimbangan antara asupan makanan dan aktivitas fisik dapat menyebabkan disbiosis. Beberapa penelitian melaporkan bahwa mikrobiota usus berperan dalam metabolisme tubuh termasuk obesitas. **Tujuan.** Penelitian bertujuan membandingkan jumlah Lactobacillus, Bifidobacteria, *Escherichia coli*,

dan Enterobacter antara anak-anak dengan obesitas, normal, dan kurus. **Metode**. Penelitian *cross-sectional* dilaksanakan pada 115 anak-anak sehat di Lombok Barat, Nusa Tenggara Barat Indonesia. Total bakteri dihitung menggunakan teknik kultur dengan metode *selective media* dan *total plate count*. Asupan makan dianalisis menggunakan kuesioner *semi-quantitative food frequency.* **Hasil**. Terdapat perbedaan yang signifikan pada total *Escherichia coli* antara anak-anak dengan obesitas, normal, dan kurus (p=0,02). Tidak terdapat perbedaan yang signifikan pada asupan makan dan total Lactobacillus, Enterobacter, dan Bifidobacteria antara ketiga kelompok. Mekanisme potensial disbiosis yang dapat menyebabkan obesitas yaitu kemampuan mikrobiota untuk menghasilkan asam lemak rantai pendek melalui fermentasi di kolon. Hal ini dapat memicu peningkatan permeabilitas usus, sekresi *ghrelin*, atau ikatan dengan *toll-like-receptor* yang mendorong pembentukan asam lemak bebas, kolesterol, dan sintesis jaringan adiposa. **Kesimpulan**. Disbiosis sering dialami oleh anak dengan obesitas. Anak-anak dengan obesitas cenderung memiliki mikrobiota usus yang tidak seimbang. Akan tetapi, diperlukan penelitian lebih lanjut untuk memastikan korelasi antara bakteri tertentu dan asupan makan terhadap kejadian obesitas pada anak.

Kata kunci: anak-anak, disbiosis, mikrobiota saluran cerna, obesitas

INTRODUCTION

As a developing country, Indonesia has a double burden of malnutrition phenomenon. Nutrition problems are not only about under-nutrition but also the increasing prevalence of overnutrition in adults and children. In 2013, the national prevalence of wasted children aged 5-12 reach 11.2%. Some provinces in east Indonesia, in which the prevalence of under-nutrition often high, the condition of over-nutrition can increase the burden of these provinces. According to Health Profile 2013 of West Nusa Tenggara,[1] the prevalence of wasted children was 7.49% while severely wasted 2.84%. Meanwhile, in the same year, the prevalence of obese children aged 5-12 reach 18.8% and 10% in West Nusa Tenggara.[2]

Obesity in children brings great effects such as a higher risk of micronutrients deficiency (Zinc, Fe, Vitamin A, C, and E), increase profile lipid, promote cell inflammation, and promote insulin resistance. Obesity is also a potential to develop hypertension, cardiovascular, diabetes, and metabolic syndrome.[3] Children's period is the time when gut microbiota developed. The existence of gut microbiota is often be ignored even though it has an important role in children's nutritional status, including obesity. Several studies propose that alteration of the gut microbiome could increase the risk of childhood obesity.[4–6] There were several mechanisms that link the presence of gut microbiota to body metabolisms such as the excretion short-chain fatty acids (SCFAs), conjugated linoleic acids (CLAs), lipopolysaccharide (LPS), and methane (H_2S). These molecules modify metabolism regulation, change gene expression, and create metabolic endotoxemia which leads to obesity, insulin resistance, and others.[7] Machado and Cortez-Pinto have reviewed some experiments on human and animal proved that gut microbiota has a role on obesity.[8] The risk of children being overweight could be predicted for six years old by the number of Bifidobacterium dan *Staphylococcus aureus*. Besides affecting the

nutritional status of the children, gut microbiota helps strengthen mineral bone, modulate the immune system, improve cell development and proliferation, and protect the body from the pathogen.[9]

The development of gut microbiota is affected especially by food intake and antibiotic usage.[6] Several micronutrients are known to have a negative impact on gut microbiota growth. Paganini *et al*,[10] mentioned that consumption of high-dose iron increases the number of *Escherichia coli* and reduces the number of beneficial bacteria such as Bifidobacteria and Lactobacilli. Considering the potential effects of gut microbiota, not much research in Indonesia has been done to investigate the differences and effects of gut microbiota according to body composition. Therefore this study intended to investigate the difference of gut microbiota between body conditions.

Our study aimed to compare the number of Lactobacillus, Bifidobacteria, *Escherichia coli*, and Enterobacter between obese, normal and wasted children. This study is important to comprehend the state of gut microbiota of obese children in West Nusa Tenggara, Indonesia. Understanding either positive or negative relation between gut microbiota and nutritional status potentially drive to novel intervention, involving gut microbiota modification, to improve the nutritional status of the children.

METHODS

Subjects

The study was approved by the Institutional Review Board of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia no. KE/FK/049/EC. The subjects were chosen by inclusion criteria: (1) the children should be 9-12 years old and (2) the children receive permission from the parents or guardian to join the study. The sample size was determined according to the sample size calculation for the master research according to Sastroasmoro and Ismael.[11] We used a cross-sectional study design micronutrient performed among 115 children in West Lombok, West Nusa Tenggara, Indonesia. The researchers were helped by trained enumerators.

Feces Collection

Feces sample collected from all subjects. For stool collection, we employed stool collectors who are local people trained in stool collecting. Once children were going to the toilet, they were asked to call a stool collector. The stool collector then came to the children's home. The stool collector collected the stool correctly and placed stool into a sterile container with a lid and stick label (child's name. school and date). Before defecate, the subjects were asked to pee so that the stool would not be mixed with urine. The stool was collected at 2-5 g. The collected stool was placed in a cool box containing ice gel then was carried to a laboratory. Materials and equipment needed for stool collection were samples (feces), disposable petri dish, laminary airflow, 37° C

incubator, autoclave, water bath. Quebec Colony counter, refrigerator, vortex, glassware, blue tip, micropipettes and centrifuge tube.

Data Measurements

Anthropometric data were measured using microtome 0.1 cm accuracy to measured height and a digital scale with 0.1 kg accuracy to measured weight. Software WHO AnthroPlus was used for determining children's nutritional status then categorized the nutritional status into obese, wasted, and normal (non-obese) children. This categorization was made according to the body mass index for age (BMI/U) z-score category. Subjects who have BMI/U z-score ranged between +3 to -2 standard deviation (SD) considered as a normal while those who have lower than -2 SD were wasted and higher than +3 SD were obese. Dietary intake was collected from the subjects by semi-quantitative food frequency questionnaire and then converted into nutrient amount using software Nutrisurvey.

Fecal bacteria numbers were analyzed in the Science Faculty Laboratory of Universitas Mataram, West Nusa Tenggara. We used traditional culture techniques with selective media and total plate count method to count the total number of Lactobacillus, Bifidobacteria, *Escherichia coli*, and Enterobacter. Lactobacillus was determined by MRS medium agar, Bifidobacteria with Bifidobacterium agar, Enterobacteria with MacConkey agar, and *Escherichia coli* with TBX (Tryptone Bile X-Glucuronide) agar. The total number of microbiota data presented in Log CFU/g. These bacteria were chosen as a representative of beneficial and pathogenic bacteria. Lactobacillus and Bifidobacteria were well known for their good effects while *E. coli* and Enterobacter vice versa.

After data collection completed, a statistical analysis was conducted to identify the difference in nutrients intake and the number of gut microbiota between the three groups of BMI. The data were analyzed by One-Way ANOVA using the SPSS software.

RESULTS

Characteristics of the Subjects

The study was performed among 115 students aged 9-11 years old in class 3-5 in West Lombok, West Nusa Tenggara, Indonesia. Fifty-four percent of all the subjects are female. The subjects were classified within three groups of body mass index (BMI). There were 98 children (85%) have a normal body mass index, followed by 11 children (9.56%) categorized as wasted, and the rest were obese. We also identified parents' education and occupation. Mainly, father's education varied between primary, junior, and senior high school while almost 50% of mother's education was a primary school. Approximately 79 fathers (69%) work as a labor and more than half of mothers were a housewife (Table 1).

Table 1. Subjects' Characteristics

Characteristics	Freq. n (%)
Class	
Three	32 (27.82%)
Four	48 (41.73%)
Five	35 (30.43%)
Sex	
Female	62 (53.91%)
Male	53 (46.08)
Father's education	
College	4 (3.47%)
Primary school	37 (32.17%)
Senior high school	27 (23.47%)
Junior high school	29 (25.21%)
Did not attend school	18 (15.65%)
Father's occupation	
Did not have occupancy	1 (0.86%)
Labor	79 (68.69%)
Honorer	2 (1.73%)
Farmer	10 (8.69%)
Civil servant	2 (1.73%)
Private	2 (1.73%)
Household assistant	1 (0.86%)
Entrepreneur	18 (15.65%)
Body mass index (BMI) categories	
Obese	6 (5.21%)
Wasted	11 (9.56%)
Normal	98 (85.21%)
Mother's education	
College	2 (1.73%)
Primary school	49 (42.60%)
Senior high school	19 (16.52%)
Junior high school	26 (22.60%)
Did not attend school	18 (15.65%)
Mother's occupation	
Labor	33 (28.69%)
Housewife	59 (51.30%)
Farmer	3 (2.60%)
Civil servant	1 (0.86%)
Private	3 (2.60%)
Entrepreneur	15 (13.04%)

Body Mass Index and Dietary Intake

The study shows there is no significant mean differences between body mass index (BMI) categories and energy, protein, fat, fiber, zinc, and iron intake (p>0.05). However, children with normal BMI tend to have energy, protein, fat, fiber, and zinc intake higher than obese and wasted children while obese children tend to have higher iron intake than wasted and normal children (Table 2). If compared to nutritional adequacy rate 2013 (Angka Kecukupan Gizi or AKG), the intake of all nutrients in all BMI group are below the standard. For example, the adequacy rate of energy, fat, protein, and fiber intake for 9 years old children is 1850 kcal, 72 g, 49 g, and 26 g, respectively. The same condition applies to micronutrient intake. The adequacy of iron and zinc daily intake for 9 years old children is 10 mg and 11 mg. It can be happened due to the methodology to collect the data. Semi-quantitative food frequency questionnaire only gathers information about food intake habits of the children in the last three months and could not identify the real daily food intake of the subjects.

Table 2. Energy, Protein, Fat, Fiber, Zinc, and Iron Intake according to Body Mass Index (BMI)

Variable	Body Mass Index			*p*
	Obese	Wasted	Normal	
Energy (kcal)	1353.43 ± 581.68	1141.66 ± 445.77	1406.86 ± 392.27	0.126
Protein (gram)	33.00 ± 10.88	33.16 ± 21.45	39.94 ± 15.91	0.284
Fat (gram)	36.67 ± 23.22	43.18 ± 30.12	50.32 ± 20.83	0.224
Fiber (gram)	3.82 ± 1.04	3.17 ± 2.55	4.71 ± 2.92	0.196
Iron (mg)	3.74 ± 2.25	3.55 ± 2.17	6.43 ± 8.66	0.418
Zinc (mg)	4.09 ± 1.21	3.32 ± 1.88	4.31 ± 1.82	0.233

One-Way Anova, significant if p<0.05

Body Mass Index and Gut Microbiota

The counts of Lactobacillus, Bifidobacteria, Enterobacter, and *Escherichia coli* show different results. The statistical analysis only shows a significant mean difference in the number of *Escherichia coli* between the three BMI categories (p=0.02). Obese children tend to have a higher composition of the four types of bacteria. Lactobacillus and Bifidobacteria were found the lowest in wasted children meanwhile Enterobacter and *Escherichia coli* found the lowest in normal children (Table 3).

Table 3. The Relation between Body Mass Index (BMI) and Total Number of Lactobacillus, Bifidobacteria, *Escherichia coli*, and Enterobacter (log CFU/g)

Variable	Body Mass Index			*p*
	Obese	Wasted	Normal	
Lactobacillus	7.80 ± 0.78	6.86 ± 1.01	7.11 ± 0.97	0.154
Bifidobacteria	8.40 ± 0.84	8.11 ± 0.90	8.20 ± 0.74	0.756
Escherichia coli	8.12 ± 0.42	7.21 ± 1.31	6.91 ± 1.03	0.02
Enterobacter	8.14 ± 0.45	8.02 ± 0.82	7.73 ± 0.73	0.229

One-Way Anova, significant if p<0.05

DISCUSSION

This study gave the results that obese children have a higher number of Lactobacillus, Enterobacter, and *E. coli* compared to wasted and normal children. It should be noted that the number of *E. coli* was significantly higher than wasted and normal children. On the other hand, obese children also have the highest Bifidobacteria number although it related to lower LPS concentration. The study by Cani *et al,* shows that lower LPS level improves gut barrier leads to lower fat mass.[34] Probably, it is because Bifidobacteria has several strains that have an agonist effect than common effects we know. Antagonistic effects of the same strain bacteria have also happened in Lactobacillus strain.[12] *L. paracasei* and *L. plantarum* were associated with lower body fat mass, meanwhile *L. reuteri* exists in high number among obese individuals.

Children obesity is a phenomenon that may result from a variety of causes. Factors like excessive food intake and inactivity often considered as the main causes. However, not all the case of obesity is a reflection state of over nutrition. Most obese individuals are consuming either the same or fewer calories than people with normal weight. It may explain why in this study, obese children almost have the same amount of energy intake with normal children. Million *et al*, stated that besides dietary habits and inactivity, genetics also play a great role.[13]

There is 10^{13}-10^{14} million microbiota in the gut. The main gut microbiota was classified as four phyla; Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. One example of Bacteroidetes species is *Bacteroides* sp. while Proteobacteria includes *Escherichia*, *Salmonella*, and *Helicobacter* species.[14] Bifidobacteria is one example of Actinobacteria species and Lactobacillus belongs to Firmicutes. Gut microbiota has the main role to ferment undigested fiber and saccharide in the colon. This process produces short-chain fatty acid (SCFA) and other intermediate metabolites. SCFA can provide 10% energy the body needs.[15]

The composition of gut microbiota could be affected by dietary intake and physical activity.[14] An imbalance between the two may cause dysbiosis. Dysbiosis is a change of quality and quantity of gut microbiota, its activities, and distributions in the gut. Chronic dysbiosis may develop metabolic and degenerative diseases. It could affect the fermentation process in the colon lead to disturbance of whole-body metabolism such as the development of adipose tissue.[16] The composition of gut microbiota could be a weight change predictor. The existence of *E. coli* and *M. smithii* indicates weight reduction.[17] However, this study indicates the higher number of *E. coli* in obese children. It may be still a controversy, but the study by Gao *et al,*[18] mentioned that obese children have higher *E. coli* than normal children. Furthermore, obese children have smaller Bifidobacteria/ *E. coli* (B/E) ratios compared to normal children. Besides *E. coli*, obese people tend to have a higher number of Firmicutes but fewer Bacteroidetes*, Clostridium leptum,* and *Enterobacteriaceae* than normal people.[19] According to Kang *et al,*[20] obese children have a higher number of *Lactobacillus* spp. and *Staphylococcus* spp. in the gut.

Dietary analysis of this study showed that none of the dietary intakes, include energy, protein, fat, fiber, iron and zinc, meet the daily adequacy for Indonesian children aged 9 years old. It could not be concluded either the subjects had micro- and macronutrients deficiency or it just the effects of the data collection methodology which was considered as the limitation of this study. Micronutrient deficiency may lead to fat deposition and chronic inflammation which causes obesity.[3,21] Zinc concentration on serum, plasma, and erythrocyte of obese people are tending to be deficient. It causes disturbance of body metabolism and appetite control besides

disruption of insulin synthesis and storage which leads to insulin resistance and diabetes mellitus.[3]

Obesity may cause anemia by hampering iron absorption in duodenum due to fat accumulation. In a state of severe anemia, hepcidin serum level will increase and manifest to two things. First, pro-inflammation cytokine will increase. It affects the erythropoietin and leads to iron deficiency anemia. Conversely, increasing the level of serum hepcidin in people with metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) will increase the number of iron storage (transferrin saturation and hyperferritinemia).[22] Obesity is a less favorable condition for both gut microbiota balance and iron metabolism in the body. For an obese person with iron deficiency anemia, the consumption of iron supplements can have a lower effect than that in non-obese people. This is supported by Cepeda-Lopez *et al*,[23] who mentioned that although the person consumed ascorbic acid to enhance the iron absorption, the effect was just one-half. Meanwhile, as proposed by several studies, the intake of high-dose iron potentially increased the number of pathogenic bacteria in the gut, include *Escherichia coli.*[10,24,25]

Some studies stated that probiotic and prebiotic consumption decrease fat storage in the body.[26–28] According to Sanchez *et al,*[29] prebiotic and probiotic improve gut microbiota balance and increase hormones production like glucagon-like-peptide-1 (GLP-1), peptide tyrosin tyrosin (PYY), and ghrelin. These hormones reduce appetite and increase satiety leads to lower calorie intake and promote weight loss.

Prebiotic supports probiotic growth in the human gut. Consuming prebiotic could fix dysbiosis by improving body composition, and lower the health risk related to obesity. It increases the number of Firmicutes, Bifidobacteria, and Lactobacillus and reduces *Bacteroides* number in the gut. Prebiotics derived from the groups of dietary fiber such as inulin, fructo-oligosaccharides, oligofructose, and others.[30] In this study, fiber consumption did not differ significantly between all groups of BMI even though the highest amount of fiber intake was in the normal group. Alongside with probiotic, prebiotic intake also has a positive impact of micronutrient absorption.

There are four mechanisms that may be the reason why the addition of prebiotics in food could increase iron absorption. First, gut microbiota produces SCFA which could lower gut acidity or pH so that the iron complex could break easily. SCFA also promotes gut cell proliferation which helps to expand the iron absorption area of each cell of the gut. The addition of prebiotics enhances the environment where iron could be changed into an easily absorbed form (from ferric to ferrous form). Prebiotics also acts as a regulator signal from genes that control transporter and iron receptor.[21]

Short-chain fatty acids or SCFA is one of the factors that create adipose tissue.[29] A study of feces samples from obese children proves that it has a higher concentration of SCFA than lean children.[31] Mainly, SCFA from microbiota is classified to acetate, propionate, and butyrate. Acetate and propionate are produced by Bacteroidetes, while butyrate by Firmicutes.[8] The proportion of acetate could be improved by lower carbohydrate intake. It also decreases butyrate proportion but could not affect propionate proportion.[32]

Butyrate and propionate are anti-obesogenic compounds. The two may increase leptin secretion and lower cholesterol synthesis. Butyrate acts as a main substrate of colonocyte, improve gut health, and lower gut permeability.[33] On the other hand, acetate traits as an obesogenic compound. Acetate may increase adipose tissue, cholesterol synthesis in the liver, and improve lipogenesis.[34] SCFAs could act as a signal receptor. Acetate and propionate bind to G-protein-coupled receptors (GPR41 and GPR43). GPR41 stimulates Peptide YY (PYY)

and lower the rate of gastric emptying. GPR41 increases leptin secretion which lowers the fat mass. Furthermore, GPR43 triggers lipolysis, lower lipid synthesis and improve glucose metabolism.[14]

Diet has a rapid and reversible effect on gut microbiota composition.[35] Consumption of food high in calories, fat, and carbohydrates leads to dysbiosis. The high-fat meal may enhance the number of Firmicutes, Proteobacteria, and on. A study by Cani *et al,* found that the number of Firmicutes is higher in obese than the lean mice.[36] Escobedo *et al,*[14] reviewed some studies on obese mice which showed an increasing number of Firmicutes and Proteobacteria. Other studies showed an increasing number of Actinobacteria, and lower Bacteroidetes. However, research by Duncan *et al,*[32] in humans found that there were no significant differences between the proportion of Firmicutes and Bacteroidetes in an obese and normal subject.

Some bacteria like Firmicutes may produce metabolites that could enhance gut permeability, leads to a higher number of free fatty acid (FFA) and ghrelin secretion.[37] Gut permeability increased by the changes of tight junction in gut epithelia (ZO-1 and occludin).[14,38] The metabolites bind to Toll-like-receptors 2 (TLR2) react to lipoteichoic acid from Gram-positive bacteria.[23] Higher gut permeability causes increase Lipopolisaccharide (LPS) levels in blood leads to a higher level of FFA and adiposity.[14,39] Increase LPS level may cause metabolic syndrome, inflammation, insulin resistance, and obesity.[40]

A little information about SCFA limited *in vivo* studies due to its inaccessibility.[39] A study showed that microbiota transfer from lean to obese mice improves insulin sensitivity and lower metabolic syndrome symptoms include obesity.[41] Schwiertz *et al,*[15] assumed SCFA has more effect on obesity than the proportion or composition of gut microbiota. Obese people have lower microbiota diversity but higher in SCFA production and fast to form adipose tissue.[15,39]

This study has several limitations. Firstly, it could not explain how the consumption of macro- and micronutrients affect the composition of gut microbiota and its relevance to the nutritional status of the children. Second, this study could not determine the real average amount of dietary intake of the children. However, this study could give a depiction of how gut microbiota differs between obese, wasted, and normal children. From the discussion above, it can be known that gut microbiota has the ability to affect the nutritional status and it also has a relation with micronutrient intake. The pathway of SCFA, prebiotic, and iron metabolism is complex and maybe contradicts from research to another. It is a chance to do further research about gut microbiota composition among Indonesian tribes and ethnics, also its correlation to local dietary habits. The findings of this study support the urgency to reckon the role of gut microbiota in the state of human health, especially childhood obesity.

CONCLUSIONS

Dysbiosis often happened in obese children. It was characterized by a higher number of *Escherichia coli* and Enterobacter than normal children. Further study is needed to explore the effect of certain bacteria and nutrients which may cause obesity among the children.

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