Original Article

Dietary Fiber Intake and Its Relationships with Lipid Profiles and Gut Microbiota in Obese Thai Children: A Pilot Study

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Objective: To assess the dietary fiber intake in obese Thai children and its relationships with lipid profiles and gut microbiota.

Materials and Methods: A cross-sectional study of obese Thai children aged 7 to 15 years was conducted between October 2017 and April 2018. The dietary fiber intake, body composition, and plasma lipid profiles of the participants were evaluated. Stool samples were analyzed for gut microbiota by 16S rRNA sequencing.

Results: There were 43 participants in the present study of which 27 participants (63%) were boy. The median (interquartile range, IQR) age was 10.0 (9.0 to 12.0) years. The median (IQR) BMI z-score was 3.0 (2.6 to 3.5). The mean±SD body fat percentage was 42.2±4.6%. The median (IQR) daily fiber intake was 2.3 (0.9 to 3.5) grams per 1,000 kcal. The prevalence of hypercholesterolemia, elevated LDL, and hypertriglyceridemia was 26%, 37%, and 14%, respectively. The total dietary fiber intake was negatively associated with the plasma total cholesterol (r=–0.379, p=0.01). Twenty-nine stool samples were analyzed. The most abundant bacterial groups belonged to the phyla Bacteroidetes (46%) and Firmicutes (39%). The dietary fiber intake tended to be positively associated with Lactobacillales (r=0.313, p=0.09) but it was not associated with Bifidobacteriales (r=0.109, p=0.57).

Conclusion: Obese Thai children consumed inadequate fiber. The higher dietary fiber intake was associated with a lower total cholesterol level and the more favorable gut microbiota composition. Further interventional study of dietary fiber supplement in this population is warranted.

Keywords: Dietary fiber, Gut microbiota, Lipid profiles, Body composition, Obesity

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The prevalence of childhood obesity has increased worldwide[1]. The complications of childhood obesity include type 2 diabetes, hypertension, dyslipidemia, cancer, and cardiovascular disease[2,3]. In Thailand, the prevalence of childhood obesity among school children (6 to 11 years) increased from 3.5% in 2009 to 6.8% in 2014[4,5]. Moreover, the prevalence in older children (12 to 14 years) is nearly 9%[5].

Obesity is defined as body mass index (BMI) greater than two standard deviations (SD) for age and sex above the World Health Organization (WHO) growth

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reference median for children and adolescents[3,6]. The major contributing factors of obesity consist of genetics and lifestyle behaviors. Healthy eating habit, especially adequate dietary fiber intake, may play an important role in management of obesity. Dietary fiber is non-digestible carbohydrates and lignin that are intrinsic and intact in plants[7]. The proposed mechanisms of cholesterol-lowering effects of dietary fiber are fecal loss of bile acids, up-regulation of hepatic low-density lipoprotein (LDL) receptor, and modification of the activity of enzymes regulating in cholesterol homeostasis[8,9]. In adults, several studies reported the inverse associations between the dietary fiber and lipid profiles including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG)[10-12]. However, there are few studies in overweight and obese children.

The human intestine harbors a complex bacterial community called the gut microbiota[2]. The related mechanisms between gut microbiota and obesity are dysbiosis concept, increased energy harvest and storage, appetite regulation, and metabolic endotoxemia[2,13,14]. Dietary fiber interventional studies in humans reveal favorable outcomes on modulating the intestinal microbiota such as increasing specific bacteria (*Bifidobacteria* and *Lactobacilli*) and increasing microbial diversity[15-17].

There are few studies of the relationships between dietary fiber intake, lipid profiles, and gut microbiota in obese children and there has been no previous study in Southeast Asia. Therefore, the aims of the present study were to assess the dietary fiber intake in obese Thai children and to assess the relationships of the dietary fiber with lipid profiles and gut microbiota.

Materials and Methods

Study design and participants

The present cross-sectional study was conducted between October 2017 and April 2018. Obese children were recruited from the Pediatric Obesity Clinic and the Nutrition Clinic at the King Chulalongkorn Memorial Hospital. The inclusion criteria were 1) children aged 7 to 15 years with obesity, the diagnosis of obesity was defined as BMI greater than 2 SDs above the WHO Growth Reference median[18], and 2) no recent use of antibiotics in the previous four weeks. Children with obesity that were caused by genetic obesity or endocrine problems, had used drugs that affect weight or BMI such as steroids, or attended other weight reduction programs were excluded. Written informed consents were obtained from all participants and their parents or guardians. The study protocol was approved by the Medical Ethics Committee of the Research Affairs, Faculty of Medicine, Chulalongkorn University (IRB No.240/60).

Anthropometry and body composition

Trained personnel performed the anthropometric measurements of the participants including body weight (kg), height (cm), and waist circumference (cm). The BMI (kg/m^2) was calculated. The body composition was measured by bioelectrical impedance analysis using the InBody 770 (InBody Co., Ltd., Chungcheongnam-do, Korea).

Dietary assessment

The dietary intake was assessed by one dietitian during an interview using a 24-hour dietary recall. The daily energy and nutrient intake were calculated by using the Institute of Nutrition, Mahidol University Calculation-Nutrients Version 2.

Biochemical analysis

Blood samples were collected from antecubital vein after a 12-hour overnight fasting. The blood samples were analyzed with an Architect c16000 (Abbott Laboratories, IL, USA). The fasting plasma glucose was measured using the hexokinase/G-6-PDH method. The TC, LDL-C, and high-density lipoprotein cholesterol (HDL-C) were measured using the enzymatic essay, the measured liquid selective detergent, and the accelerator selective detergent methods, respectively. The TG was analyzed by using glycerol phosphate oxidase method. Dyslipidemia was defined as either TC of 200 mg per dL or greater, LDL-C of 130 mg per dL or greater, HDL-C of less than 40 mg per dL, TG of 150 mg per dL or greater, or combination of them.

Gut microbiota analysis

Fecal sample collection: Each participant was given an instruction to collect stool samples at home[19]. A 25 ml-fresh stool was placed into a sterile container and immediately kept in the home freezer (–18°C). The sample was shipped to the laboratory within 24 hours and was subsequently stored in the laboratory freezer at –80°C for DNA extraction.

DNA extraction: Stool sample was removed from the –80°C freezer and thawed on ice. An approximately 180 to 220 mg stool sample was suspended in 1 ml of InhibitEx buffer and incubated at 70°C for five minutes, followed by centrifugation at 20,000×g for one minute. Two hundred microliters of aqueous phase were collected. The bacterial DNA

was extracted from the 200 µl using the QIAamp fast DNA stool mini kit (QIAGEN) according to the manufacturer's instruction. The DNA concentration was measured by using the DeNovix dsDNA High Sensitivity kit (DeNoVix Inc., Delaware, USA).

16S rRNA amplification and sequencing: The DNA amplicon of bacterial 16S rRNA gene (V3-V4) was polymerase chain reaction (PCR) amplified using a specific primer that was linked with the Illumina's adaptor. The PCR products were cleaned up using magnetic beads, the Agencourt AMPure XP beads (Beckman Coulter Inc.). The Illumina's indices were added to both ends of the PCR products to allow the multiplexing of samples. The indexed PCR products (DNA libraries) were then cleaned up using the magnetic beads. The concentration of the DNA library was quantitated using DeNovix dsDNA High Sensitivity kit (DenoVix Inc., USA). The size of DNA library was checked by QIAxcel capillary electrophoresis (QIAgen). Forty libraries were pooled and sequenced using paired-end, 2×301 base pair (bp) on Illumina MiSeq with the Illumina reagent kit version 3 (600 cycles).

Amplicon sequencing data processing: The USEARCH[20] software suite was used to process the amplicon sequencing data. Unless specified otherwise, the default settings were used. First, paired-end reads were merged using the *fastq_mergepairs* command with either the default setting that permitted up to five mismatches in the alignment, or a more relaxed setting that permitted up to 50 mismatches but no more than 20% of the alignment length. The relaxed setting was selected in the present study because the paired-end reads had long overlap of more than 140 bp on average. This setting allowed high number of mismatches to be tolerated during the paired-end read merging. Overall, both settings yielded similar mapped microbial taxonomic profiles. However, the relaxed setting consistently produced 62% more reads per taxon. Hence, the results obtained by the relaxed read merging parameters were selected for further analyses. Following the read merging, the *fastq_filter* command was used to trim the first 17 bp of each read and remove the low-quality reads with expected error of more than 1 bp. The quality-filtered reads from all samples were merged. The duplicates were removed to create a non-redundant list of representative sequences. Next, the *cluster_otus* command that implemented the UPARSE[21] algorithm was used to cluster similar reads with at least 97% identity. The chimeric sequences were removed using the *uchime2_ref* command that implemented the UCHIME2[22] algorithm, with SILVA non-redundant 16s rRNA sequence database as reference[23].

Taxonomic assignment: The Ribosomal Database Project (RDP) Classifier[24] was used to assign the representative sequences to the microbial taxon, with the default confidence threshold of 0.8. Overall, out of 949 representative sequences, 762 representative sequences were assigned to a bacterial phylum, 735 to a class, 729 to an order, 667 to a family, and 470 to a genus. To estimate the abundance of each taxon in each sample, the *otutab* command of the USEARCH was used to map the reads in each sample to the representative sequences at 97% sequence identity threshold. The number of mapped reads for each taxon was directly taken as the abundance value of that taxon in subsequent analyses.

Statistical analysis

Descriptive data and correlations were performed using the IBM SPSS Statistics version 23 (SPSS Inc., Chicago, IL, USA). The normality of data was tested by using the Shapiro-Wilk Test. Normally distributed data were presented as means with SDs, while others were reported as medians with interquartile ranges (IQR). The associations between the dietary fiber and metabolic profiles were assessed using Spearman correlation. Gut microbiota sequences were evaluated by using bioinformatics. A p-value of less than 0.05 was considered as statistical significance.

Results

Participant characteristics

Forty-three obese Thai children participated in the present study of which, 27 participants (63%) were male. The general characteristics and body composition of the participants are shown in Table 1.

Dietary assessment

The mean±SD energy intake was 1,826.9±658.8 kcal per day. The proportions of energy intake from carbohydrate, protein, and fat were 51%, 15%, and 34%, respectively. The mean±SD protein intake was 1.2±0.4 grams per kg per day. The median (IQR) dietary fiber intake was 3.1 (2.0 to 7.1) grams per day. After adjusting for energy intake, the median (IQR) dietary fiber intake was 2.3 (0.9 to 3.5) grams per 1,000 kcal energy per day.

Metabolic profiles

The results of blood glucose and lipid profiles are shown in Table 1. The prevalence of hyper-cholesterolemia, high LDL-C, low HDL-C, and

Table 1. Demographic data and metabolic profiles of study participants

	Results (n=43)
General characteristics	
Age (years)†	10.0 (9.0 to 12.0)
Body weight (kg)†	57.7 (49.7 to 73.3)
Height (cm)‡	147.4±11.3
% weight for height (%)‡	158.0±18.7
BMI (kg/m^2)†	27.5 (25.4 to 29.8)
BMI z-score†	3.0 (2.6 to 3.5)
Waist circumference (cm)‡	91.1±10.2
Body composition	
FM (kg)†	24.1 (19.6 to 32.3)
FFM (kg)†	33.3 (29.4 to 39.4)
FMI (kg/m^2)‡	11.9±2.7
FFMI (kg/m^2)†	15.6 (14.8 to 16.9)
Body fat percentage (%)‡	42.2±4.6
Metabolic profiles	
Fasting plasma glucose (mg/dL)†	84.0 (78.0 to 85.0)
Total cholesterol (mg/dL)‡	186.1±25.4
LDL-C (mg/dL)‡	121.2±24.5
HDL-C (mg/dL)†	50.0 (44.0 to 58.0)
Triglyceride (mg/dL)†	96.0 (66.0 to 132.0)
ALT (U/L)†	28.0 (19.0 to 41.0)

BMI=body mass index; FM=fat mass; FFM=fat free mass; FMI=fat mass index; FFMI=fat free mass index; LDL-C=low density lipoprotein cholesterol; HDL-C=high density lipoprotein cholesterol; ALT=alanine aminotransferase

† Median (interquartile range), ‡ Mean ± standard deviation

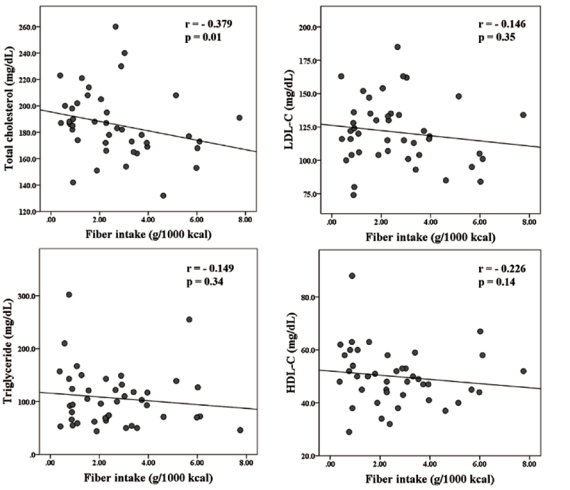

Figure 1. Spearman correlations were performed on forty-three obese Thai children.

LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol

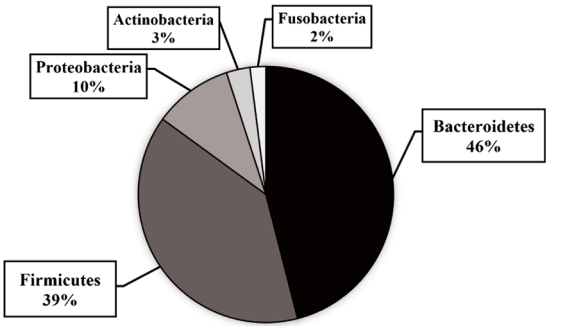

Figure 2. Composition and relative abundance of fecal bacteria of the twenty-nine obese Thai children.

hypertriglyceridemia was 26%, 37%, 14%, and 14%, respectively. The overall prevalence of dyslipidemia in the present study was 49%.

Association between dietary fiber intake and lipid profiles

The dietary fiber intake was negatively associated with the TC (r=–0.379, p=0.01) but there was no association with the LDL-C, HDL-C, and TG levels (Figure 1).

Intestinal microbiota composition and the association with dietary fiber intake

Of the 43 participants, 29 stool samples were collected and underwent 16S rRNA sequencing and analyses. The most abundant bacterial groups belonged to the phyla Bacteroidetes (46%) and Firmicutes (39%). Other bacterial compositions in phylum level are shown in Figure 2. The total dietary fiber intake tended to be positively associated with the Lactobacillales (r=0.313, p=0.09), but it was not associated with the Bifidobacteriales (r=0.109, p=0.57) assessed by Spearman correlation. No significant association between dietary fiber intake and bacterial composition at phylum-level was found.

Discussion

Dietary fiber intake provides many potential health benefits and is inversely related to obesity, dyslipidemia, and cardiovascular disease (CVD)[9,25]. The present study demonstrated that higher dietary fiber intake was associated with lower TC and higher

Lactobacillales in obese Thai children. However, they consumed inadequate daily dietary fiber at 2.3 g per 1,000 kcal. According to the Institute of Medicine, the adequate intake level of 14 g of dietary fiber per 1,000 kcal of energy is recommended for protection against CVD[7]. The U.S. National intake data indicate that the dietary fiber intake is inadequate in most American children as well[26].

The results from the present study showed the prevalence of hypercholesterolemia was nearly 30% and about half of the participants had abnormal lipid profiles. The dietary fiber was inversely associated with the TC level, which was similar to the previous studies[10,27]. However, the relationships between the fiber intake and LDL-C and TG were not found.

The most abundant intestinal microbiota was the phyla Bacteroidetes, followed by the Firmicutes. Similarly, a study in Korean children reported by Hu et al showed that the Bacteroidetes and Firmicutes were the two major bacterial taxa (more than 90%) and the Bacteroidetes was the greatest[28]. Another study in adults reported by Schwiertz et al revealed that the proportion of the phyla Bacteroidetes versus Firmicutes in overweight and obese subjects were nearly equal at 46.8% versus 47.7% in overweight group and 45% versus 51% in obese groups[29]. In contrast, the studies in Italy and Japan found that the Firmicutes was the predominated phyla in obese children[30,31]. These inconsistent results may be due to the differences in genetic, environmental, and dietary factors.

The present study found that the dietary fiber intake tended to be positively associated with Lactobacillales. An in vitro study investigated the bile salt hydrolase (BSH) enzyme activities of human-derived lactic acid bacteria and *Bifidobacteria*[32]. The BSH catalyzes the hydrolysis of glycine- or taurine-conjugated bile salts, resulting in the deconjugated bile salts that are less soluble and less efficiently reabsorbed from the intestinal lumen. Thus, the free bile acids are excreted. The bile salt pool is decreased and then the cholesterol is removed from the plasma. The authors hypothesized that the amount of dietary fiber intake, which was positively associated with Lactobacillales, related inversely with serum cholesterol via this mechanism. There is limited evidence on the intersection of dietary fiber, gut microbiota, and lipid profiles. Further interventional and metabolomics studies are needed.

To the authors' knowledge, the present study is the first study in Southeast Asia that examined dietary fiber intake status in obese children together with its relationships with lipid profiles and gut microbiota. There are some limitations in the present study including (i) the small sample size and (ii) using the 24-hour dietary recall that may not reflect the habitual dietary pattern as dietary assessment tool.

Conclusion

None of the study participants consumed dietary fiber above the recommended intake and half of them had at least one plasma lipid abnormality. The dietary fiber intake was negatively associated with the plasma TC level while positively associated with Lactobacillales. Future directions of new interventions to increase dietary fiber intake in obese children could potentially provide additional health benefits.

What is already known on this topic?

Childhood obesity is one of the most serious health problems and lead to many complications including dyslipidemia.

The inverse relationships between dietary fiber intake and lipid profiles were widely reported in adult studies but not in children.

The composition of gut microbiota in obese children was investigated in many countries, but the results are still inconclusive.

What this study adds?

Obese Thai children in the present study consumed inadequate dietary fiber.

Dietary fiber intake was negatively associated with TC in obese Thai children.

According to gut microbiota composition in obese Thai children, the most abundant intestinal microbiota was the phyla Bacteroidetes and followed by the Firmicutes.

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Potential conflicts of interest

The authors declare no conflict of interest.

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