Case Report

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Six-week Diet Correction for Body Weight Reduction and Its Subsequent Changes of Gut Microbiota: A Case Report

Bumjo Oh*, Jong Seung Kim1, Meera Kweon2, Bong-Soo Kim3, In Sil Huh4

1Department of Family Medicine, Seoul National University Boramae Hospital, Seoul 07061, Korea
2Nutrition Department, Seoul National University Boramae Hospital, Seoul 07061, Korea
3Department of Life Sciences, Hallym University, Chuncheon 24252, Korea
4Graduate School of Public Health, Seoul National University, Seoul 08826, Korea

Impact of intestinal microbes on obesity and health is a new topic recently started to get attention. Comparing to the global concern and research trends, there are few research on the association between intestinal bacteria and lifestyle disease in Korean. One voluntary case (female) was reported to show the change in gut microbiota and weight by diet intervention. She was overweight (BMI 23.2 kg/m²) and has abnormal liver function, and the causes of overweight were frequent drinking and meat consumption at the late evening hours. For 47 days, she was administered an improved diet on breakfast and dinner with reduction of meat consumption frequency by 50%. Alcohol consumption was reduced to once a week. As a result, she lost 3 kilograms of body weight. Her fecal sample was collected before and after the intervention, and gut microbiota change was compared using a high-throughput sequencing technique. After diet correction, the shift of gut microbiota was clearly observed with decreased proportion of Firmicutes (from 75.7% to 47.3% in total microbiota) but increased proportion of Bacteroidetes up to 47.7%. After incorporating the diet intervention, it is meaningful to confirm the changes in dominant gut microbiota and weight loss.

Key Words: Weight reduction, Gut microbiota, Dietary intervention, Alcohol consumption, Caloric restriction, Overweight, Diet

Introduction

Obesity is not only a problem of appearance, but also a main cause of various lifestyle diseases such as hyperlipidemia, hypertension, cardiovascular diseases, and diabetes. Although obesity has emerged as a cause of these complications, in reality, there are lack of efforts to prevent and manage it, thus, the prevalence of obesity is steadily increasing [1].

Obesity is mainly caused by lack of regular physical exercise, westernized diet, genetic factors, and excessive and unbalanced nutrition. Especially, the workers have high vulnerability to obesity and lifestyle diseases by unhealthy life habits such as lack of physical exercise due to busy work, stress, eating-out, and alcohol consumption. Therefore, countermeasures are needed to prevent obesity [2,3].

Impact of intestinal microbes on obesity and health is a new topic started to get attention recently [4]. In 2009, an
experimental research was introduced to prove the hypothesis, "Gastrointestinal bacteria contribute to obesity." Four atypical human twins (pair of one lean and one obese twin) were recruited. Their gut microbiota was extracted and transplanted into sterile mice. The observed results have shown that mice received gastrointestinal bacteria from the lean twin maintained their weight whereas mice transplanted with gut microbiota from obese twin showed a rapid weight gain [5]. After the researchers confirmed "the effects of gastrointestinal bacterial transplantation on obesity", they assessed two mice received bacteria from one pair of twins by an additional experiment to verify the exchange of two different bacteria. It confirmed the unidirectionality of bacterial survival, in which the gut microbiota from obese mice ceases to survive in the gastrointestinal tract of lean mice whereas bacteria from lean mice survived in the obese mouse. Bacteria in the gastrointestinal tract of lean mice did not tolerate high proportion of saturated fat ‘human’ diet with less fruits and vegetables but transformed into the bacteria in the fat twin. Follow-up studies have confirmed that diet determines the composition of a bacteria, and gastrointestinal bacteria has an impact on obesity [6,7].

Considering to the global concern and research trends, there are few research on the association between intestinal bacteria and body weight change in Korean. In this report, one voluntary case (female) will be introduced to show the change in gut microbiota and weight by diet intervention.

Case
Dietary assessment and intervention
The 27-year-old Korean woman with no underlying disease or medical history volunteered in this research to improve obesity. She visited outpatient department of family medicine on October 1, 2013. According to anthropometric measurements, she was 1.73 meters tall with body mass index (BMI) of 23.2 kg/m² with weight of 69.5 kilograms. The WHO Regional Office for the Asia Pacific Region recommends defines obesity by a BMI ≥ 25 kg/m² and overweight by BMI above 23 kg/m² and below 25 in Asians. The Korean Society for the Study of Obesity also adopted the WHO-recommended definition to study the cutoff of BMI for obesity-related disease [8]. Now, Korean government organizations officially use this definition when defining and implementing health policies regarding obesity in Korea.

After verifying obesity test results, she was explained about the research in detail and asked to compose a meal record for three days prior to the test. The result from the hospital’s dietician showed the causes of overweight are frequent drinking and meat consumption at late evening hours.

Nutrition Consultation Results
To determine the diet responsible for the weight loss, change in the intakes of total calorie, carbohydrate, and fat (especially animal fat) were analyzed based on the diet history of the case. The result showed that overall intakes of total calories, carbohydrate, and protein were evenly decreased after the diet intervention, and the reduction in large amount of animal fat was observed by cutting down the consumption of fish and meat.

She regularly participated in hazardous drinking twice a week. The amount of alcohol she consumes at once is approximately total of 95 grams; from 30 grams in 4.5 glasses of Soju and 65 grams in 9 cups of beer. Blood and urine tests were performed to determine whether comorbid conditions associated with drinking habit. The results confirmed normal except for mild anemia (Hemoglobin, Hb 11.6g/dL) and mild increases in aspartate aminotransferase (AST; 58IU/L).

In detail, frequent intake of carbohydrate= and excessive consumption of fish, meat, bread, ice cream, etc. at late evening hours was noticed. However, after the diet intervention, she put efforts to reduce food intake and select vegetable side dishes and fruit as a replacement for snacks, thus, average consumption of calories, carbohydrates, protein, and animal fat decreased (Table 1).

Additionally, decrease in frequency of food intake with high in sodium such as soup or stew was observed. Changed behavior in alcohol consumption cannot be determined by the short study period; however, a consultation confirmed that she managed to reduce her alcohol intake by approximately 50 percent.

Table 1. Nutrition intake comparison between before and after the diet intervention

|                    | Before   | After   |
|--------------------|----------|---------|
| Calories, kcal/day | 2953 ± 768 | 2100 ± 50 |
| Carbohydrates, g/day | 341 ± 54 | 238 ± 62 |
| Protein, g/day   | 102 ± 16  | 45 ± 2  |
| Vegetable fat, g/day | 58 ± 18  | 45 ± 26 |
| Animal fat, g/day | 45 ± 43   | 10 ± 1  |

Analysis Program: CANpro 3.0, The Korean Nutrition Society.
Gut Microbiota Change after Diet Intervention

For 47 days, she was administered an improved diet with breakfast and dinner with less meat and reduction of alcohol consumption and frequency by 50% and once a week, respectively. At the second follow-up, her weight has reduced to 66.5 kilograms. Compared to the initial weight at the first visit, she lost 3 kilograms.

Gut microbiota: DNA extraction and Pyrosequencing

The request on case’s fecal sample analysis was submitted to Chunlab, Inc. prior to participating in diet improvement research. After the 47 days of research, the case’s fecal sample was again collected on November 19, 2013 to compare the distribution of intestinal micro flora with the first sample. The Seoul National University-Boramae Hospital Institutional Review Board approved this study.

Bacterial DNA was extracted from the fecal samples of subjects before and after the treatment using a FastDNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA). Gut microbiota were compared before and after the treatment using the high-throughput sequencing technique. Obtained sequences from the pyrosequencer were analyzed according to previous descriptions [9]. The shift of gut microbiota was clearly observed after the diet correction. The compositions of phyla and genera were compared between before and after the diet correction. Firmicutes was predominant phylum (75.7% of total microbiota) before the diet correction, whereas Firmicutes (47.3%) and Bacteroidetes (47.7%) were dominant phyla after the diet correction. In sample collected before the diet correction, genera of Faecalibacterium (14.9% of total microbiota), Roseburia (14.8%), Blautia (9.5%) and Lactobacillus (9.1%) within Firmicutes, and Prevotella (15.0%) within Bacteroidetes were dominant members of gut microbiota. After the diet correction, the relative abundance of Prevotella (42.1% of total microbiota) and Megasphaera (10.7%) within Firmicutes was...
observed, while those of Faecalibacterium (8.6%), Roseburia (1.2%), Blautia (3.0%), and Lactobacillus (1.7%) were decreased. The proportion of Bacteroides (4.4%) within Bacteroidetes was also increased after sample (Figure 1).

Discussion
This observation, confirmed the association between the changes in body weight, diet and gut microbiota. In particular, 3 kilograms of body weight was reduced by diet correction, which includes limiting the overall intakes of total calories, fat, protein, and carbohydrate, as well as reducing salt intake and alcohol consumption frequency. Considering previous studies with various method of short-term dietary interventions, it is observed that a person with diet intervention showed 2-4% additional weight loss than general attempts [10].

During the weight loss, the gut microbiota was changed by diet correction. The proportion of Firmicutes was decreased while Bacteroidetes was increased after the weight loss. This change was consistent with previous studies which determined that the ratio of Firmicutes/Bacteroidetes was higher in an obese person than a lean person [11].

Although the impact on gut microbiota depends on genetic, and environmental factors, and obesity, obese people showed a change of microbiota on the phylum levels and rapid decrease of microbiota diversity compared to healthy people.

Conclusions
It is meaningful to confirm the changes in gut microbiota and weight loss through the diet intervention. Further study on this subject is needed to investigate the effect of dietary intervention on body weight loss and gut microbiota change.

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