보건학석사 학위 논문

Comparison of Intestinal Microbial Isolates from Obese and Normal Twins

정상인과 비만인 쌍둥이에서 분리한 장내 미생물 균주의 비교 연구

2013년 02월

서울대학교 보건대학원
환경보건학과 환경보건학 전공
홍 성 호

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지도교수 고 광 표
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서울대학교 보건대학원
환경보건학과 환경보건학 전공
홍 성 호

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위 원 장 정 해 원 (인)

부위원장 최 경 호 (인)

위 원 고 광 표 (인)

Abstract

Comparison of Intestinal Microbial Isolates from Obese and Normal Twins

Sung-Ho Hong

Dept. of Environmental Health

The Graduate School of Public Health

Seoul National University

The aim of the present study was to evaluate the differences between Korean monozygotic obese and normal twins. Firstly we isolated various intestinal bacteria from obese and normal twins. Furthermore, we observed the secretion of inflammatory responses in intestinal epithelium cell line, Caco-2. The results showed isolates from obesity have the potential pro-inflammatory response (IL-6, TNF-α) in Caco-2 cells. C57BL/6 forty-eight male mice were divided into six groups depending on feeding high fat diet and oral administration of *Bifidobacterium* isolated from twins in order to investigate their effect against obesity and

metabolic change. Feeding *Bifidobacterium* groups showed significantly lower body weight than high fat diet groups. In addition, *Bifidobacterium* group was lower in fasting blood glucose and oral glucose tolerance test. Taken together, *Bifidobacterium* showed a significant anti-obesity effect in high fat diet induced obese mice, and improved glucose tolerance, but intestinal microbial isolates were no significant difference between obese and normal twins except pro-inflammatory response in isolates from obese twins.

Key word : Microbiome, Obese, Twin, Probiotics, Mouse model

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Ⅰ. Introduction

The human gut harbors 10^{14} bacteria and colonized at birth by bacteria from the mother but it is changed by several environment factor. Nutritional value of food and status may change human gut microbial diversity by different life style (13, 34). Some studies have indicated that gut microbiota is associated with several diseases such as obesity, intestinal disorder. Obesity has increased in developed and developing countries. Obesity caused by imbalance between energy intake and energy expenditure, it results in several metabolic syndrome including heart disease, cancer, hypertension and type 2 diabetes by insulin resistance (26, 34). Therefore, the prevention and treatment of obesity are warranted for a human healthy life. Recent studies have shown significantly differences in gut microbial communities between obese and lean humans (4, 18). Recent studies in mouse implicated that diet induced obese mice harbor a dominant gut microbiota in the phylum Firmicutes but depleted in Bacteroidetes (17, 35).

Probiotics are defined as a live organism and may exert a health benefit to the host. Furthermore, probiotics have demonstrated anti-obesity effect and reduce of gut inflammatory, risk of type 2 diabetes mellitus and insulin resistance (1, 5, 12). *Bifidobacterium* which is one of probiotics

strains is anaerobic bacteria that naturally colonize the human intestinal tract and is believed to be beneficial to human health. The number of *Bifidobacterium* in gut can be increased by food consumption with probiotics or prebiotics. In one study, *Bifidobacterium longum* was demonstrated that a more significant effect in lowering total cholesterol than *Streptococcus thermophilus* and *Lactobaciilus delbrueckii* both in human and animal models (37). Another study shows that a strain of *Bifidobacterium* would be effective in reducing the risk of obesity (14). Indeed, some strains appear to exert anti-inflammatory effects in mouse models. For example, *Lactobacillus* and *Bifidobacterium* produced a reduction of inflammation in the colon of the pro-inflammatory cytokines IFN-γ, TNF-α, and IL-12 but levels of the anti-inflammatory cytokine were maintained (23). A previous study showed *Bifidobacterium* can be soluble factors and reduce the inflammatory response within the epithelium (9). However, the difference of effect of *Bifidobacterium* according to host was not found within metabolic change and microbiome. Furthermore, previous study used *Bifidobacterium* isolated from breast milk or food (14, 20, 25). This is the first study used intestinal microbial isolates from Korean monozygotic twins in cell line tests and mouse models.

This study aimed at assessing whether differences between intestinal microbial isolates from obese and normal monozygotic twins. In the

present study, several types of bacteria in intestinal microbiota were isolated from Korean monozygotic twins. Targeted microorganisms for isolation were *Lactobacillus, Bifidobacterium, Bacteroides* and *Fusobacterium*. Several types of bacteria can use variety of experiment for investigating cause of obesity. To demonstrate differences between intestinal microbial isolates from obesity and normal twins, we observed secretion of inflammatory cytokine levels in enterocyte cell line Caco-2 and isolated bacteria using specific enzyme-linked immunosorbent assay (ELISA). Furthermore, the comparison of probiotics isolated from discordant twins was examined in a mouse model with high-fat diet. In this mouse model, we can observe body weight and metabolite change by fasting blood glucose concentration and oral glucose tolerance test.

Ⅱ. Materials and Methods

1. Selection of discordant twin and culturing of fecal microbiota

The flow of the present study design is shown in Figure 1. First, twenty Korean healthy individuals who was a set of 10 monozygotic twin pairs were selected by body mass index (BMI) greater than 10% difference. The information of participants is shown in Table 1. To find comparison of mother and twins, fecal samples of 6 mothers were selected and isolated. All twin pairs and mothers are from the Korea Twin Family Cohort (32, 33).

One gram fecal samples were placed in 10 ml PBS and suspended by vortexing for 1min. The fecal materials were serially diluted 10-fold from 10^{-5} to 10^{-8}, and 0.1 ml was spread onto selective media. The culture media used in this study for the isolation and identification of bacterial strains are shown in Table 2. Four types of genus were targeted, *Lactobacillus spp., Bifidobacterium spp., Bacteroides spp. Fusobacterium spp*. The condition of whole method was maintained in anaerobic condition at 37℃. Colonies were harvested from each plate and picked by loop & needle 1 μL (SPL, USA). Each colony was cultured in each media

for 24 hours at 37℃ in anaerobic chamber (Coy Lab, USA). Stocks were stored duplicate in -80℃ freezer (2).

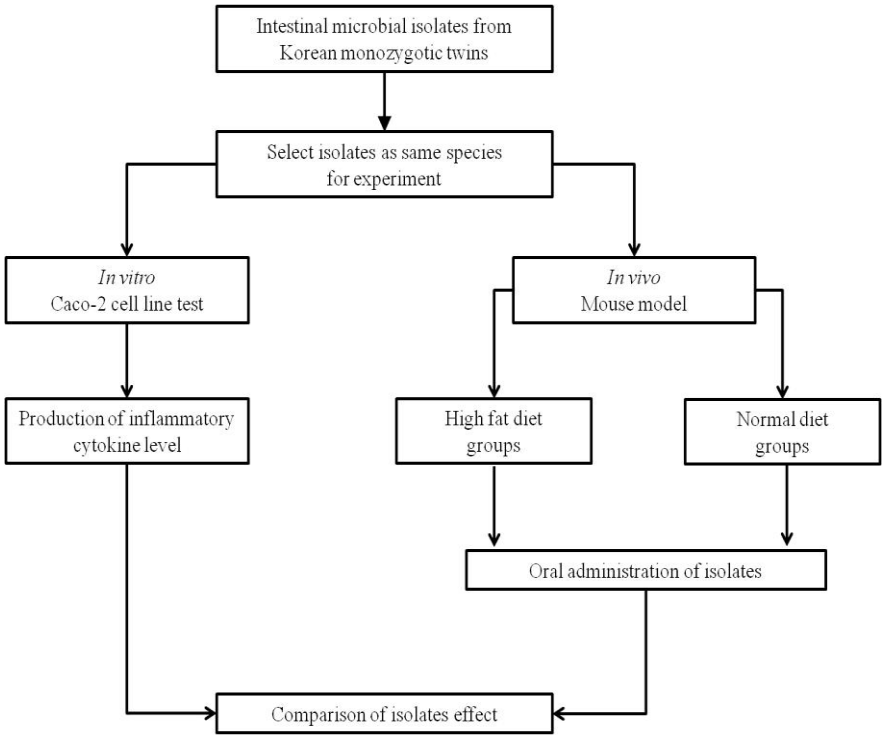

Fig. 1. The flow of the present study design.

Table 1. Discordant twins samples for isolation

Twin ID	Individual[a)]	BMI (kg/m^2)	Discordance rate[b)] (%)	Age	Gender
1	OB1	28.51	16.62575	36	Male
	NO1	23.77			
2	OB2	26.09	16.94136	37	Male
	NO2	21.67			
3	OB3	27.45	15.62842	38	Male
	NO3	23.16			
4	OB4	27.38	13.36742	39	Male
	NO4	23.72			
5	OB5	30.49	18.49787	40	Male
	NO5	24.85			
6	OB6	31.27	17.6527	41	Male
	NO6	25.75			
7	OB7	25.9	13.35907	42	Male
	NO7	22.44			
8	OB8	22.58	15.36758	43	Male
	NO8	19.11			
9	OB9	24.74	12.85368	44	Male
	NO9	21.56			
10	OB10	28.05	10.23173	45	Male
	NO10	25.18			

Abbreviations: a) OB, obesity twin; NO, normal twin; b) Discordance rate of BMI (%) = (OB-NO) / 0.5 (OB+NO) * 100.

Table 2. Culture media and methods for isolation

Media[a]	Targeted microorganisms	Incubation time	Incubation temperature
MRS medium[b]	*Lactobacillus spp.*	48 hours	37℃
GAM medium[c]	*Bifidobacterium spp.*		
BPRM medium[d]	*Bacteroides spp.*		
RCM medium[e]	*Fusobacterium spp.*		

[a]Culture media were prepared on a clean bench. Petri dishes were stored in a vinyl anaerobic chamber (Coy Lab, USA) containing 90% N_2, 5% CO_2, 5% H_2.

[b]MRS, Lactobacilli deMan, Sogosa and Sharpe, (Difco, USA)

[c]GAM, General anaerobic medium (Nissui, Japan)

[d]BPRM, Bacteroides Phage Recovery Medium (Conda, Spain)

[e]RCM, Reinforced Clostridial Media (Difco, USA)

2. DNA extraction and 16S rRNA sequencing

DNA was extracted from isolates using G-spin Genomic DNA Extraction Kit (iNtRON Biotech., Korea) according to the manufacturer`s instructions. DNA concentration and quality was determined a NanoDrop ND-1000 spectrophometer (NanoDrop Technologies, USA).

To identify each isolates, 16S rRNA genes were amplified using universal primer set, forward primer (27F, 5'-AGA GTT TGA TCM TGG CTC AG-3') and reverse primer (1492R, 5'-GGY TAC CTT CTT ACG ACT T-3'). Reactions were incubated for 2 min at 95℃, followed by 40 cycles of 30 sec at 94℃, 30 sec at 45℃, 90 sec at 72℃, and a final extension at 72℃ for 5 min.

After the PCR reaction, the quality of the amplified PCR products was confirmed by electrophoresis with 1 μL of the PCR reaction mixture in 1.5% agarose gel (0.5X TAE buffer) and purified using QIAquick PCR Purification kit (Qiagen, Germany). PCR products concentration and quality was confirmed by NanoDrop. The identification of colony sequence was used by BLAST in NCBI (National Center for Biotechnology Information).

3. Experiments in Caco-2 cell line

The human colon cancer cell line Caco-2 from was cultured in 24-wells culture plates in Dulbecco`s Modified Eagle Medium (DMEM) supplemented with 20% heat inactivated fetal bovine serum (FBS), 10 mM HEPES, 10 mM sodium bicarbonate, gentamicin 50 g/L, and 10 mM Earle`s minimal essential-nonessential amino acid (all from Gibco, USA) at 37℃ in 5% CO_2/air atmosphere. Media was changed every day. Experiments were initiated on days 14 after seeding (31). Twenty-four hours before bacterial challenge, the culture media was changed for a medium without FBS.

Twin ID 1 and 5 were selected for this test. All bacteria from microbial isolates between obese and normal twins were cultured at 37℃ for 21 hours and were harvested by centrifugation 3000g for 15 min after 24 hours of cultivation at the stationary growth phase and were washed three times with phosphate buffered saline (PBS). Bacteria were added at a multiplicity of infection (MOI) of 100 in 0.5 ml DMEM. After 3, 6, and 9 hours incubation periods, cell supernatants were collected and frozen at -20℃ until assay. (8)

TNF-α, IL-6, IL-10, IL-12, IL-1β and IFN-γ cytokines were measured in Caco-2 cells. All cytokine measurements were performed using Human Cytokine/Chemokine Magnetic Panel (Millipore, USA) and high sensitivity xMAP Technology in a Luminex 100 System (Millipore, USA) (27).

4. Preparing of probiotics for oral administration

Bifidobacterium longum, Bifidobacterium pseudocatenulatum were used in this study and isolated from Korean discordant twins as described. Two species of *Bifidobaterium* were isolated from Twin ID 1 and 5 same as Caco-2 cell line test.

Administered cultures were prepared once a day, two species of *Bifidobacterium* were maintained in anaerobic condition and stored at 37℃ for 21 hours. Cells were harvested, washed twice in PBS and obtain 1×10^{8}~1×10^{9} CFU/ml. Prepared cultures were administered 0.2 ml to mice daily and non feeding probiotics groups were administered 0.2 ml PBS to mice.

5. Animals and treatment

A total of 48 male C57BL/6J mice aged 5 weeks were obtained from ORIENT BIO (KOREA) and fed on a standard diet (Zelgler Bros. Inc., USA) for 2 weeks to stabilize all metabolic conditions. Food and water were supplied *ad libitum* and each cage contained two mice. The mice were exposed to a 12-h light/dark cycle. All the animal study and protocol were approved by Institutional Animal Care and Use Committee of Seoul National University. The mice were randomly selected and assigned to six groups (8 mice per group) and these are shown in Table 3; a standard diet group (SD), a standard diet group with probiotics from obese twin (SD-OB), a standard diet group with probiotics from normal twin (SD-NO), a high fat diet group (HFD), a high fat diet group with probiotics from obese twin (HFD-OB), a high fat diet group with probiotics from normal twin (HFD-NO).

After 1 week of stabilization, oral administration began for all groups. Four groups were administered with daily probiotics from obese and normal twins and two groups were orally administered 0.2 ml of PBS once a day until end of study. After 2 weeks of feeding probiotics, HFD groups were provided with 45% high-fat diet (TD.06414, harlan, USA) for six weeks (Table 4).

Their body weight and food intake were measured weekly and fasting blood glucose measurements were made by Accucheck Performa glucometer device (Roche, Switzeland). This study was maintained for 10 weeks and Figure 2 shows all experimental schedules.

At 0, 2 and 7 week of the experiment, blood samples were collected via the ophthalmic venous plexus after fasting 9 hours. After collection of the whole blood, centrifuge at 3000 g for 10 min in order to collect serum (11). All serum samples were frozen and stored at -70℃ before assays (28).

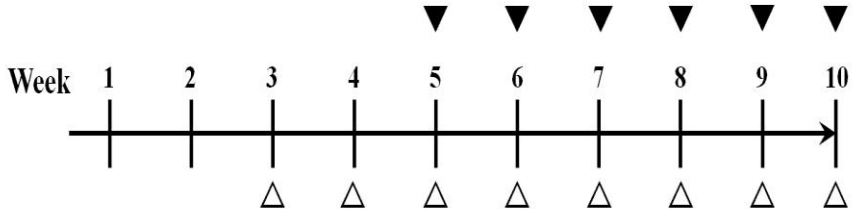

Fig. 2. Experimental schedule of a mouse model for obesity and probiotics. △, oral administration of *Bifidobacterium* or saline; ▼, feeding of high-fat diet or standard diet.

Table 3. Group and feeding schedules in this study.

Groups	Mice(n)	Food	Treatment
SD	8	Standard diet	0.2 ml of PBS
SD-OB		Standard diet	0.2 ml of probiotics from obese twin
SD-NO		Standard diet	0.2 ml of probiotics from normal twin
HFD		45% High fat diet	0.2 ml of PBS
HFD-OB		45% High fat diet	0.2 ml of probiotics from obese twin
HFD-NO		45% High fat diet	0.2 ml of probiotics from normal twin

Abbreviations: SD, a standard diet group; SD-OB, a standard diet group with probiotics from obese twin, SD-NO, a standard diet group with probiotics from normal twin; HFD, a high fat diet group; HFD-OB, a high fat diet group with probiotics from obese twin; HFD-NO, a high fat diet group with probiotics from normal twin

Table 4. Nutrient information of the experimental diets (% by weight)

	Standard Diet[a]	**High Fat Diet**[b]
Protein	18.0	21.7
Carbohydrate	60.0	41.4
Fat	4.0	22.8

[a]Standard diet, 413110 (Zeigler, USA)

[b]High fat diet, TD06415 (harlan, USA)

6. Oral glucose tolerance test

The evaluation of glucose intolerance was performed using oral glucose tolerance test (OGTT). After 8 hours of fasting, glucose was administrated to mice at a dose of 2 g kg^{-1} body weight by oral gavage. Blood glucose level was measured by Accucheck Performa glucometer device (Roche, Switzeland).

7. Statistical analysis

The results were presented as mean ± standard error of mean (SEM). The statistical significance of differences was analyzed by one-way ANOVA and student`s t test. A value of $p \leq 0.05$ is considered to be statistically significant. Microsoft Excel 2010 (Microsoft Corporation, USA) and Sigmaplot for windows ver. 10.0 (Systat software Inc, USA) were used for the statistical analysis.

Ⅲ. Results

1. Comparison of microbial diversity between obese and normal twins.

Fecal sample of total 10 pairs of discordant twins were isolated in each four different media. Figure 3 shows the difference of Korean discordant twin gut microbiota at genus level. The intestinal microbial isolates were only cultured bacteria. Obesity twin gut microbiota was a higher proportion of *Enterococcocus* and *Bifidobacterium* than normal twin. However, a higher proportion of *Bacteriodes* and *Streptococcus* was found in normal twin gut microbiota.

All distances for sequence of *Bifidobacterium* between twins were shown the phylogenetic tree (Fig. 4). The trend of this figure showed mostly grouping with same ID and species. Consequentially, we used 4 type of Bifidobacterium (OB1 *Bifidobacterium longum*, NO1 *Bifidobacterium longum*, OB5 *Bifidobacterium pseudocatenulatum* and NO5 *Bifidobacterium psedocatenulatum*) for in vitro and in vivo test.

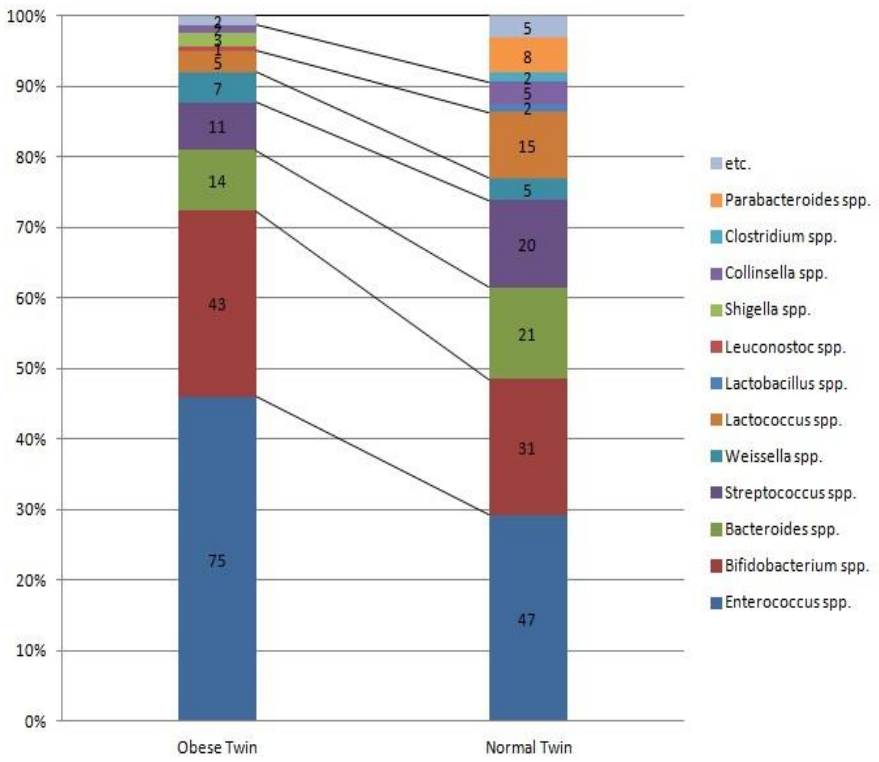

Fig. 3. Composition of intestinal microbial isolates from obese and normal twins. Abundance of bacteria isolates sequence of 16S rRNA genes from 10 pairs of Korean discordant twins classified at genus level with database from Basic Local Alignment Search Tool (BLAST).

Fig 4. The phylogenetic tree of sequence of *Bifidobacterium* in bacterial isolates from discordant twin. A phylogenetic tree was inferred by the UPGMA method. ○, obesity twin; ●, normal twin; OB, obesity twin; NO, normal twin.

2. Cytokine production in Caco-2 cells and intestinal microbial isolates

To analyze cytokine production by Caco-2 cells and bacteria, TNF-α, IL-6, IL-10, IL-12, IL-1β and IFN-γ were measured. Challenging bacteria was *Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Enterococcus faecium.* Table 5 shows the production of each cytokine level for 9 hours and data presented a mean ± standard error of mean. Specifically, *Bifidobacterium longum* and *Bifidobacterium pseudocatenulatum* from obese twin samples were significantly high level of IL-6 and TNF- α secretion compare to normal twin samples. Furthermore, *B. longum* (OB), *B. longum* (NO), *B. pseudocatenulatum* (OB), *B. pseudocatenulatum* (NO) and *E. faecium* (OB) in TNF-α and IL-6 levels were higher secretion than control ($P < 0.05$). The effect of bacteria on IL-10, IFN-γ and IL-12 was not significantly found (Table 5).

Table 5. Secretion of cytokine levels from Caco-2 cells in response to intact intestinal microbial isolates

Microbial isolates	IL-6	IL-1β	IL-10	IL-12	TNF-α	IFN-γ
B.longum (OB)	1.54±0.05*	0.22±0.08	1.71±0.08	2.63±0.04	0.92±0.02*	0.28
B.longum (NO)	1.09±0.02*	0.18±0.06	1.71±0.08	2.63±0.04	1.06±0.03*	0.28
p-value†	0.0478	0.6796	-	-	0.004	-
B.pseudocatenulatum (OB)	1.06±0.09*	0.27±0.09	1.72±0.02	2.63±0.08	1.02±0.04*	0.28
B.pseudocatenulatum (NO)	0.40±0.05*	0.17±0.01	1.69±0.07	2.68±0.07	0.77±0.07*	0.28
p-value†	0.0379	0.2001	0.5776	0.4918	0.0112	-
E.faecium (OB)	0.04	0.1±0.01	1.68±0.04	2.65±0.04	0.63±0.05*	0.28
E.faecium (NO)	0.04	0.15±0.04	1.58±0.05	2.57±0.04	0.59±0.01	0.28
p-value†	-	0.1497	0.0974	0.1012	0.0058	-
Control	0.04	0.07±0.01	1.46	2.46±0.02	0.58	0.28

Data are presented a mean ± SEM (pg/ml) values of triple cultures. Each bacterial species was added into Caco-2 cell for 9 hours. After culturing, the amount of IL-6, IL-1β, IL-10, IL-12, TNF-α and IFN-γ in the supernatants were determined by specific ELISA. *, statistical different ($P < 0.05$ *vs.* control; ANOVA test); †, p-value between obesity and normal twin isolates.

3. Anti-obesity effect of *Bifidobacterium* from discordant twins

To demonstrate the anti obesity effect of *Bifidobacterium*, we observed mice body weight every week. The body weight of all groups was increased especially that of the HFD groups compared to the SD groups during 10 weeks. After 2 weeks of adjustment period, the oral administration of Bifidobateria was started. At five week of experiment, the diet change began and significant differences in body weight gains were observed. At 10 week, the mean body weight was 27.1 ± 1.6g (P < 0.05, *vs*. HFD), 26.2 ± 1.7g (P < 0.05, *vs*. HFD), 27.6 ± 1.6g (P < 0.05, *vs.* HFD), 30.5 ± 1.7g (P < 0.05, *vs.* HFD), 31.2 ± 3.4g and 32.5 ± 1.4g for SD-OB, SD-NO, SD, HFD-OB, HFD-NO and HFD, respectively. Body weight of *Bifidobacterium* fed groups was lower than both standard diet and high-fat diet fed group (Fig. 5).

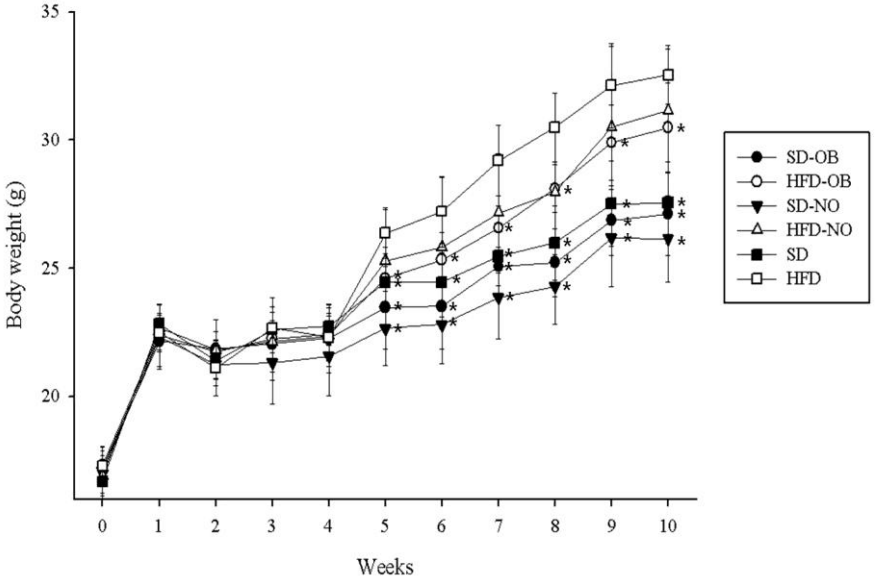

Fig. 5. Body weight change in mice fed with *Bifidobacterium*. ●, the group fed a standard diet and *Bifidobacterium* from obese twin; ○, the group fed a high fat diet and *Bifidobacterium* from obese twin; ▼, the group fed a standard diet and *Bifidobacterium* from normal twin; △, the group fed a high fat diet and *Bifidobacterium* from normal twin; ■, the group fed a standard diet; □, the group fed a high diet; *, statistical different ($P < 0.05$ *vs.* HFD; ANOVA test).

4. Effect of the experimental diets and oral administration on fasting blood glucose, oral glucose tolerance test.

Table 6 and 7 show the effects on fasting blood glucose levels and OGTT (oral glucose tolerance test) at 8 week. Blood glucose levels after 8 hours fasting were measured at 10 week after daily treatment. Compared with HFD groups, SD groups had significantly lower fasting blood glucose concentration after 5 week (Table 6). At 5, 7 and 8 week, feeding *Bifidobacterium* groups in HFD were lower fasting blood glucose concentration than non-feeding *Bifidobacterium* groups in HFD. A similar trend was also observed in the OGTT, with blood glucose levels after glucose loading being significantly lower at 30, 60, 90 and 120 min in HFD groups (Table 7). However, there is no statically difference in same diet groups and the effect of *Bifidobacterium* on glucose resistance was not demonstrated in the mouse model except glucose loading after 60 min.

Table 6. Effect of *Bifidobacterium* and high fat diet on fasting blood glucose concentrations.

Experiment week	SD-OB	SD-NO	SD	HFD-OB	HFD-NO	HFD
1	129.75±9.92	133±30.54	136.63±29.73	134.125±27.25	135±32.41	124.38±15.48
2	132.25±20.12	129.75±26.38	122.63±32.60	102±22	111±25.58	108.25±17.52
3	124.38±23.11	114.75±20.12	135.38±35.27	119.25±17.35	108.25±13.83	85.25±23.58
4	90.17±7.81	92.71±17.67	107.25±19.78	90.875±13.10	90.38±13.35	107.43±11.83
5	116.13±21.56*	100.29±11.80*	131.25±15.64*	136.38±27.51	127.5±22.02*	157.14±20.48
6	112.38±16.83*	107.71±10.59*	109.5±24.87	133±31.98	119.5±28.76	135.29±21.29
7	118.86±15.50*	101.43±23.96*	112.13±27.08*	127.75±35.76	116.63±18.25*	128.71±30.94
8	88.86±13.29*	93.71±19.64	158.74±17.44	94.75±17.89*	101.5±28.59	117±18.95

Fasting blood glucose level (mg/dl) was measured by Accucheck Performa glucometer device (Roche, Switzeland). Diet change started at 5 week. Data is presented as the mean ± SEM. Statistical analysis of fasting blood glucose concentrations was performed using the ANOVA. *, statistical different ($P < 0.05$ *vs.* HFD). SD, a standard diet group; SD-OB, a standard diet group with probiotics from obese twin, SD-NO, a standard diet group with probiotics from normal twin; HFD, a high fat diet group; HFD-OB, a high fat diet group with probiotics from obese twin; HFD-NO, a high fat diet group with probiotics from normal twin.

Table 7. Effect of *Bifidobacterium* and high fat diet on oral glucose tolerance test.

Time after glucose loading (min)	SD-OB	SD-NO	SD	HFD-OB	HFD-NO	HFD
0	143.71±22.52	159.33±25.88	128.88±13.82*	168.75±23.04	151.75±42.82	172.71±27.79
30	189.29±22.73*	182.33±26.41*	180.63±17.86*	200.25±35.18	221.63±43.21	220.14±18.95
60	174.14±23.12*	159.17±7.75*	164±25.22*	181±16.64*	201.88±24.78	208.29±26.70
90	187.43±16.05*	183.17±19.27*	158.63±22.72*	213.88±26.10	233.88±47.28	208.71±15.70
120	174.29±6.92*	167.67±17.36*	153.13±23.57*	193.5±21.52	202.38±45.32	196.43±20.67

Oral glucose tolerance was measured by Accucheck Performa glucometer device (Roche, Switzeland). Time points after glucose loading were 0, 30, 60, 90 and 120 min. Data is presented as the mean ± SEM. Statistical analysis of blood glucose concentrations (mg/dl) was performed using the ANOVA. *, statistical different ($P < 0.05$ *vs.* HFD). SD, a standard diet group; SD-OB, a standard diet group with probiotics from obese twin, SD-NO, a standard diet group with probiotics from normal twin; HFD, a high fat diet group; HFD-OB, a high fat diet group with probiotics from obese twin; HFD-NO, a high fat diet group with probiotics from normal twin.

Ⅳ. Discussion

Obesity and dietary fat intake is known to the most important factors which chronic diseases such as hypertension, diabetes and heperlipidemia. Many studies have reported anti-obesity effect of some bacterial strains such as *Bifidobacterium.* However, the previous studies used breast milk or food in *Bifidobacterium* study. Our study is the first using isolates from Korean monozygotic twins. In the present study, we isolated specific bacteria between Korean obese and normal monozygotic twins. We selected 2 species of *Bifidobacterium* and observed difference of effect in two bacterial species for cell line experiments and mouse model.

Significant differences have been demonstrated in the diversity of the microbiotas of rodents and humans ingesting high fat and normal diets (3, 10, 36). Furthermore, a number of studies have revealed the anti-obesity effect of orally administrating *Bifidobacterium* in diet-obese induced mouse model (14, 16). However, recent studies have not demonstrated differences between bacteria from host especially BMI. In the present study, we succeed isolate several specific bacteria such as *Bifidobacterium*, *Bacteroides*, *Streptococcus*, and *Lactobacillus*. Specifically, the proportion of *Bacteroides* and *Lactobacillus* in obese twins was lower than normal twins. Recent study showed that reduced levels of *Bacteroides* and *Lactobacillus* in obese participants (29, 34). Furthermore, intestinal

microbial isolates we have might analysis their effect and influence of obese in human body and metabolite in the future study.

To demonstrate differences of inflammatory responses between intestinal microbial isolates from obesity and normal twins, we observed production of inflammatory cykokine levels in enterocyte cell line Caco-2 and isolated bacteria. Our results confirm slightly difference between bacteria from obese and normal twin in Caco-2 cell line by inflammatory cytokine levels, especially IL-6 and TNF-α (Table 5). IL-6 and TNF-α are expressed by subcutaneous adipose tissue (24). Furthermore, omental adipose tissue released 2-3 times more IL-6 than did sc adipose tissue (7). In the present study, the levels of IL-6 and TNF-α were differently measured in *Bifidobacterium longum* and *Bifidobacterium pseudocatenulatum*. Two species of *Bifidobacterium*, intestinal isolate, have the potential pro-inflammatory response in Caco-2 cells.

Probiotics are traditionally defined live microorganisms and confer a health benefit. Many studies have reported anti-obesity effect of *Bifidobacterium* in rodent models (19, 38). In the present study, we observed that feeding of 45% high fat diet for 6 weeks produced significant increases in body weight, and oral administration of two types of *Bifidobacterium* reduced body weight and glucose intolerance. Significant differences have been demonstrated in and the diversity of microbiotas of rodents and obese with high fat and standard diets (3, 10). However, these studies do use 60% high fat diet due to extremely induce

obese. Although the results reduced the obesity effect, we did use 45% high fat diet for more similar recent human diet pattern than normal diet induced obese murine models. Most of studies showed the results of weight gain with high fat diet are more than 20 grams (15), but the results of this study are less than 15 grams. Following these reason, the anti-obesity effect of oral administration of *Bifidobacterium* was not higher than 60% high fat diet feeding studies. However, feeding 45% high fat diet was also showed the difference of body weight between groups even lower. Probiotics effects were appeared as soon as beginning changing diet (Fig. 5), but there is no difference in standard diet groups. Similar effects have been observed in other studies using mouse (14, 25). Figure 5 demonstrated anti obesity effect of *Bifidobacterium* isolates from Korean gut twins. However, the difference of BMI between hosts was not showed in the present study. In the present study, the reduction of inflammatory, bile acid and cholesterol was not experiment. Recently, western diet associated with cholesterol and bile acid metabolisms (6). Growing evidence indicates that increased intestinal permeability and inflammatory disorders, including inflammatory bowel disease (30). Probiotics have been shown to prevent the development of inflammatory diseases in mouse models (21, 22). It remains to be resolved whether *Bifidobacterium* isolates from Korean twins can improve inflammatory diseases.

Obesity increase the problem of developing type 2 diabetes and insulin resistance considered risk of metabolic syndrome. In the present study, we

observed fasting glucose blood levels every week and result showed Table 6. The data was not hard to demonstrate probiotics effect of insulin resistance. Compare to recent studies, the experiment period is not enough to induce diabetes and feeding 45% high fat diet is possible to reason. Furthermore, a glucose tolerance test is a medical test for diabetes, insulin resistance. Our result showed the difference of between HFD and SD groups but there was hard to find *Bifidobacterium* effect of glucose tolerance (Table 7). In addition, insulin tolerance test (ITT) was not hard to compare each groups and data was not shown.

In summary, the present study firstly showed isolate to Korean discordant monozygotic twin as BMI. We can do extensional test using obesity associated bacteria such as *Bifidobacterium, Lactobacillus, Bacteroides, Enterococcus*. Second, we observed production of inflammatory between Caco-2 cell and isolated bacteria. The result of IL-6 and TNF-α showed the difference between host and BMI especially *Bifidobacterium*. Third, diet induced mouse model showed the effectiveness of probiotics strain, *Bifidobacterium longum, Bifidobacterium pseudocatenulatum,* on preventing obesity and glucose tolerance. Additional studies are necessary to observe inflammatory marker change, cholesterol and leptin in mouse model, and detailed mechanisms of microbiotas as the difference of host and BMI.

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국문초록

정상인과 비만인 쌍둥이에서 분리한 장내 미생물 균주의 비교 연구

서울대학교 보건대학원

환경보건학과 환경보건학 전공

홍 성 호

지도교수 고 광표

본 연구의 목적은 한국인 일란성 쌍둥이에서의 차이를 규명하는데 있다. 먼저, 우리는 일란성 쌍둥이 중 비만인과 정상인에게서 장내 세균을 순수분리 하였다. 비만인과 정상인 쌍둥이에서 분리한 균의 차이를 알아보기 위하여 장 상피 세포주인 Caco-2 세포주를 통하여 염증반응의 차이를 살펴보았으며 IL-6 와 TNF-α 에서 비만에게서 온 분리균의 염증반응이 유의적으로 일반인에 비해 높게 나타났다. 또한 48마리의 마우스(mouse)를 통하여 고지방식이 섭취와 일반식이군, 프로바이오틱스 섭취군으로 나뉘어 집단간의 항 비만효과와 대사작용 변화 및 장내 미생물 군집 변화(microbiome)를 살펴보았다. 프로바이오틱스인 비피도균 (*Bifidobacterium*) 섭취 군에서 체중이 주요하게 적게 나타났으며 공복혈당과 구강 혈당 부하 검사(Oral glucose tolerance test)에서도 유의적으로 낮은 결과를 보여주었다. 결론적으로 한국인 일란성 쌍둥이에서 분리한 비피도균은 비만 유도 마우스 모델 실험에서 항비만효과를 나타내고 당불내성 억제효과를 나타냈으나, 숙주의 비만여부에 따른 같은 종의 균의 차이는 비만인의 분리균의 염증 반응 외에는 나타나지 않았다.

주요 단어 : 마이크로비옴, 비만, 쌍둥이, 프로바이오틱스

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