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NOTE

Effects of *Lactobacillus gasseri* BNR17 on Body Weight and Adipose Tissue Mass in Diet-Induced Overweight Rats

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We investigated the weight-gain suppressive effect of *Lactobacillus gasseri* BNR17 isolated from human breast milk. Rats were fed a high-carbohydrate diet and administered BNR17 (BNR17 group) twice daily for twelve weeks. Changes were observed in body weight and white adipose tissue mass. The percent increase in body weight ($P=0.0331$) and fat pad mass ($P<0.01$) was significantly lower in the BNR17 group, and the FER was moderately lower ($P=0.0769$). These data suggest that BNR17 can prevent diet-induced overweight and may become an alternative method for treating weight problems and obesity.

***Keywords*:** probiotics, body weight gain, breast milk, white adipose tissue

Probiotics are defined as live microorganisms that produce a health benefit when administered to animals, including humans. Several studies have reported the health-promoting effects of probiotics, including the maintenance of intestinal mucosal resistance to pathogenic microorganisms (Mennigen and Bruewer, 2009), the prevention of diarrhea (Guandalini, 2008), immunomodulation (Perdigón *et al.*, 2001), reduced serum cholesterol levels (Nguyen *et al*., 2007), and the prevention of allergic diseases and cancer (Isolauri and Salminen, 2008; Kumar *et al*., 2010).

Recently, additional beneficial effects of probiotics or probiotic-containing food products on body weight control and metabolic disorders such as diabetes, hypertension, and hypercholesterolemia have been reported (Lee *et al*., 2007; Bhathena *et al*., 2009; Lye *et al*., 2009). Meanwhile, many reports suggesting that the human gut flora may be a contributing factor to body weight differences among individuals have been published (Ley *et al*., 2005, 2006; Turnbaugh *et al*., 2006). These findings suggest that the manipulation of gut microbial communities could be an alternative treatment for obesity. From this perspective, it is worth investigating whether *Lactobacillus* affects obesity, since *Lactobacillus* is the most well-known probiotics and a critical constituent of the gut flora. Indeed, *Lactobacillus* has been shown to help maintain body weight; however, its anti-obesity effects have not been thoroughly examined.

Lactobacillus gasseri, in the genus of *Lactobacillus acidophilus*, is the most well-known probiotics and one of the most critical constituents of gut flora. *Lactobacillus gasseri* BNR17 was isolated from breast milk collected from healthy lactating females within two weeks of parturition in our laboratory. We previously evaluated the following probiotics properties of this strain: acid and bile resistance, binding to human colonic cell (Caco-2 cell), antibacterial activity against food pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Bacillus cereus*), and the production of antibacterial substances, bacteriocin (data not shown).

To the best of our knowledge, the effect of human breast milk-derived probiotics on body weight and fat pad weight has not been reported. In this study, we investigated the effect of BNR17 on weight gain, food efficiency ratio (FER), and white adipose tissue in diet-induced overweight rats.

BNR17 suspensions (final concentration of 10^9 CFU/0.5 ml) were prepared daily in sterilized phosphate-buffered saline (PBS, pH 7.4) by the centrifugation of an MRS broth culture (5,500×g for 10 min at 4°C), which was washed twice with PBS prior to resuspension in the same buffer.

Inbred-specific, six-week-old male Sprague-Dawley (SD) rats (n=20, average weight: 135 g) were obtained from Koatech Animal Inc. (Pyeongtaek, South Korea). All animals were housed in standard plastic cages (two rats per cage), and maintained under a 12-h light-dark cycle; the temperature and humidity were held at 23±1°C and 55±5%, respectively. All animals involved in this study were treated according to the standards of the Animal Ethics Committee at our institution. A high-carbohydrate diet (AIN-76A; 200.00 g/kg casein, 3.00 g/kg DL-methionine, 150.00 g/kg corn starch, 500.00 g/kg sucrose, 50.00 g/kg cellulose, and 50.00 g/kg corn oil; Central Laboratory Animal Inc., South Korea) and water were administered *ad libitum*. The rats were randomly assigned to the control or BNR17 group. The BNR17 group was administered the BNR17 preparation orally twice a day, while the control group was administered PBS.

Body weight and food intake were measured once a week

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Table 1. Effect of *Lactobacillus gasseri* BNR17 administration on morphometric and metabolic parameters in diet-induced overweight SD rats

	Control group	BNR17 group	P-value
	Mean±SD	Mean±SD	
Initial weight (g)	170.08±6.81	167.89±0.60	0.5018
Final weight (g)	436.64±26.01	408.91±20.59	**0.0331***
Weight gain (g/day)	3.17±0.29	2.87±0.21	**0.0282***
Food intake (g/day)	17.72±0.87	17.12±0.31	0.1011
Food efficiency ratio (%)	0.17±0.01	0.16±0.01	0.0769
Total cholesterol (mg/dl)	99.66±11.66	96.32±6.27	0.2959
Triglycerides (mg/dl)	115.90±11.00	122.59±5.51	0.1371
HDL-cholesterol (mg/dl)	38.51±4.63	40.76±1.81	0.2256
LDL-cholesterol (mg/dl)	22.72±1.96	21.53±3.44	0.1697
Plasma glucose (mg/dl)	88.90±12.09	79.20±4.09	0.1919
Total protein (g/dl)	8.59±0.26	9.04±0.26	0.1120
Liver weight (g)	15.95±1.34	14.19±0.83	**0.0069****
Kidney weight (g)	2.59±0.31	2.55±0.22	0.7444
Spleen weight (g)	0.92±0.10	0.88±0.10	0.3916
MFP weight[a] (g)	5.95±0.95	4.64±0.74	**0.0083****
PFP weight[b] (g)	7.83±1.10	5.45±1.48	**0.0026****
EFP weight[c] (g)	7.59±0.58	5.65±1.07	**0.0005*****

Food efficiency ratio (FER): Weight gain (g/day)/Food intake (g/day)
[a] MFPs, mesenteric fat pads; [b] PFPs, perirenal fat pads; [c] EFPs, epididymal fat pads
* P<0.05, ** P<0.01, *** P<0.001

during the experiment. At the end of the twelve-week treatment period, the rats were starved for 15 h before being anesthetized with Zoletil (30 mg/kg body weight). Blood samples were collected by heart puncture into heparinized tubes and centrifuged at 2,000×g for 20 min at 4°C. Plasma glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, total protein, and triglyceride levels were analyzed using an automatic blood chemical analyzer 7020 (Hitachi, Japan). The liver, kidneys, and spleen of the sacrificed animals were aseptically excised and weighed. The white adipose tissue (mesenteric fat pads [MFPs], perirenal fat pads [PFPs], and epididymal fat pads [EFPs]) weight was also determined.

All experimental data are presented as mean values with the standard deviation. A paired-sample *t*-test was applied to compare the groups using SPSSWIN 12.0 software (SPSS Inc., USA) with the significance level set at P<0.05.

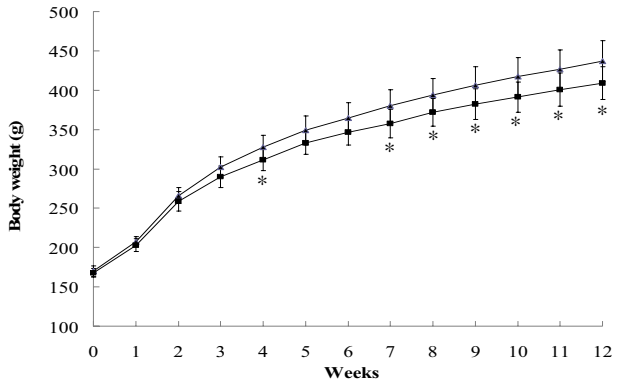

Fig. 1. Change in body weight of SD rats administered *Lb. gasseri* BNR17 for 12 weeks.
* P<0.05, compared with the value of control. (▲) Control, (■) BNR17

No significant difference in daily food intake was observed between the control and BNR17 groups; however, the BNR17-treated group showed less weight gain for 12 weeks (Fig. 1). At the end of the experiment (Table 1), the control group animals had gained 436.64 g in body weight, while those administered BNR17 gained 408.91 g (13.1% decrease; P=0.0331). The average daily weight gain of the BNR17-treated animals was significantly lower than that of the control animals (P=0.0282), and the FER was moderately lower (P=0.0769). Weights of the MFPs (P=0.0083), PFPs (P=0.0026), and EFPs (P=0.0005) extracted from the BNR17-treated animals were dramatically reduced compared to those of the control animals.

A recent review focused on the link between metabolic disturbances in adipose tissue during obesity and the development of insulin resistance and type 2 diabetes mellitus (T2DM) (Cusi, 2010). That report demonstrated that an excess nutrient supply caused adipocyte hypertrophy, macrophage infiltration, and adipocyte insulin resistance, leading to T2DM via pancreatic β cell dysfunction. We previously showed that BNR17 effectively reduced blood glucose levels and improved diabetic symptoms in T2DM mice (Yun *et al*., 2009). Although we could not clarify the mechanism involved, we speculated that BNR17 exerted anti-obesity and -diabetes effects by preventing metabolic disturbances in adipose tissue.

The levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, total protein, and triglyceride were not different between the two groups. Although statistical significance was not attained, the blood glucose levels in the BNR17 group were lower than those in the control group.

No significant differences in kidney and spleen weights were observed between the control and BNR17 animals. However, we observed an abnormal increase in liver weight in the control group but not in the BNR17 group. It is known that obesity induces hepatomegaly by sinusoidal dilatation, microvesicular steatosis, or fibrosis (Altunkaynak and Ozbek, 2009). Our data suggests that BNR17 prevents morphological alteration of the liver resulting from excess weight or obesity.

Numerous papers have addressed the role of probiotics in the development of obesity. Raoult (2009) stated that dietary supplements containing probiotics of the *Firmicutes* group, including *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus* spp., are frequently used to maintain the health and weight of livestock. That paper also described weight gain in children given *Lactobacillus* spp. as a treatment for diarrhea, suggesting that probiotics may be related to obesity. In opposition to this view, Ehrlich (2009) and Delzenne and Reid (2009) asserted that humankind has been consuming probiotics throughout most of its history without inducing obesity in healthy adults. They insisted that probiotics are unrelated to obesity because *Firmicutes* contains not only *Lactobacillus* but also deadly pathogens such as *Bacillus anthracis.* In the midst of these discussions, our work collectively shows that *Lactobacillus* treatment can control body weight gain in diet-induced overweight rats.

The BNR17 used in this study was derived from breast milk. Because the bacterial composition of breast milk affects the normal flora of infants, there is a strong possibility that the presence of probiotic bacteria in breast milk contributes to the gastrointestinal disease resistance of breast-fed infants (Olivares *et al*., 2005). Owen *et al*. (2006, 2008) showed that breast-feeding in infancy was associated with a reduced mean body mass index and risk of type 2 diabetes. Although it is not entirely clear which components of breast milk are responsible for these effects, our results suggest that the probiotics included in breast milk may be a factor.

In conclusion, we found that the oral administration of *L. gasseri* BNR17 derived from human breast milk to diet-induced overweight rats prevented increases in body weight and adipose tissue. In future, we plan to investigate the effects BNR17 in a clinical trial of obese and overweight adults to determine whether this effect can be reproduced in human subjects.

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