Latilactobacillus sakei WIKIM31 Decelerates Weight Gain in High-Fat Diet-Induced Obese Mice by Modulating Lipid Metabolism and Suppressing Inflammation

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Introduction

Obesity is a serious disease worldwide and is associated with many metabolic diseases, such as diabetes, fatty liver, and cardiovascular diseases [1]. Recent studies have shown that the gut microbiota, which is affected by changes in bacterial compositions and function, is closely related to obesity [2]. Changes in specific intestinal microbiota, particularly, the ratio of the phyla Firmicutes and Bacteroidetes, which are representative dominant bacteria, is the main cause of obesity [3]. These changes in dominant bacteria affect the energy metabolism of the host, which can cause metabolic problems [4]. In addition, emerging research indicates that chronic inflammation is highly associated with the development of obesity [5]. Thus, obese individuals have increased circulating levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein-1 (MCP-1) [6]. Obesity induced inflammation is related with leakage of microbial, gut-derived molecules, such as lipopolysaccharide (LPS) [7]. Alterations in gut microbiota induced by a high-fat diet cause intestinal inflammation, which leads to the leakage of gut microbes and contributes to inflammation associated with obesity [8]. Therefore, modulation of the gut microbiota is very important as a potential therapeutic probiotic for obesity.

Keywords: Latilactobacillus sakei WIKIM31, probiotics, obesity, inflammation

Obesity and related metabolic diseases are major problems worldwide. Some probiotics are currently considered potential therapeutic strategies for obesity. We aimed to investigate the anti-obesity efficacy of Latilactobacillus sakei WIKIM31 in obese mice induced by a high fat diet. The administration of a high-fat diet with L. sakei WIKIM31 reduced body weight gain, epididymal fat mass, triglyceride and total cholesterol levels in the blood, and remarkably decreased the expression of lipogenesis-related genes in the epididymal adipose tissue and liver. Interestingly, intake of L. sakei WIKIM31 improved gut barrier function by increasing the gene expression of tight junction proteins and suppressing the inflammatory responses. Additionally, L. sakei WIKIM31 enhanced the production of short-chain fatty acids, such as butyrate and propionate, in the intestinal tract. These results showed that L. sakei WIKIM31 can be used as a potential therapeutic probiotic for obesity.

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Materials and Methods

Isolation and Preparation of *L. sakei* WIKIM31

*L. sakei* WIKIM31 was isolated from homemade kimchi (Gangwon province, Republic of Korea). The kimchi was homogenized and filtered by stomacher filter bag. The homogenate was spread onto Man-Rogosa-Sharpe (MRS; BD Difco, USA) agar. The plate were incubated to at 30°C for 48 h. Isolated strains were obtained from subculture. After pre-screening for anti-obesity effects in vitro, selected strain was identified by 16S rRNA gene sequence. WIKIM31 was deposited at Korean Federation of Culture Collection as KFCC 11654P. To prepare the strain for use in animal experiments, WIKIM31 was cultured at 30°C for 18 h in MRS broth. The strain was harvested by centrifugation and washed twice with PBS. Next, the strain was resuspended in PBS (1 × 10⁹ CFU) for oral administration to mice.

Cell Culture and Oil Red O Staining

3T3-L1 (ATCC-CL-173) preadipocyte cells were maintained in Dulbecco Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% newborn calf serum (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin. For differentiation to adipocytes, preadipocytes were cultured to full confluency, and the medium was changed with differentiation media (DMEM supplemented with 10% fetal bovine serum [FBS]) with 1 μg/ml insulin, 0.1 μM dexamethasone, and 0.5 mM isobutyl methylxanthine. After 2 days, the cells were cultured in DMEM containing 10% FBS for additional 4 days. For Oil Red O staining, cells were washed with PBS, fixed with 10% formalin for 1 h, and rinsed with 60 % isopropyl alcohol. Cells were stained with Oil Red O solution for 10 min, and washed with distilled water.

Animals and Experimental Design

The care and study of the laboratory mice followed the protocols of the Institute Animal Care and Use Committee for the World Institute of Kimchi (WIKIM IACUC 201608). 6-week-old male C57BL/6 mice were purchased from OrientBio (Korea) and acclimated to the environment for 1 week. All mice were cared in individually ventilated cages where temperature and humidity were controlled on a 12 h light/dark cycle. Food and water were supplied ad libitum. Each group of mice were fed a normal diet (ND mice, 10% kcal as fat, D10001, Research Diet Inc., USA), high-fat diet (HFD mice, 45% kcal fat, D12451, Research Diet), or HFD with WIKIM31 (HFD-WIKIM31 mice) for 12 weeks. WIKIM31 was orally administered at a daily (10 am) concentration of 1 × 10⁹ CFU per 200 μl. The well-being of the animals, body weight, and food intake were measured weekly. The body composition of the mice was determined by MRI relaxometry (EchoMRI-500, Echo Medical System, USA). After 12 weeks, the mice were sacrificed using CO₂ asphyxiation. Serum separated from blood was stored at -80°C until analysis.

Histological Analysis

Histological analysis was performed as described previously [18]. Histological images of the liver and adipose tissue were examined under a microscope (Olympus DP73, Japan).

Measurements of Triglyceride, Cholesterol, Glucose and Adipokines Levels

Serum triglyceride, cholesterol, and glucose levels were determined by a FUGI DRI-CHEM 7000 (Fujifilm, Japan) according to the manufacturer’s protocol. The concentrations of leptin, adiponectin, and resistin were measured using a Bio-Plex Pro Mouse Diabetes-Plex Assay (Bio-Rad, USA).

Isolation of Stromal Vascular Fraction and Measurements of Cytokine Levels

Isolation of stromal vascular fraction (SVF) was performed as previously described [18]. Briefly, epididymal fat (EF) tissues were digested using 2 mg/ml collagenase (Sigma-Aldrich, USA) at 37°C for 45 min, filtered using 100 μm cell strainers (BD Falcon), and centrifugated at 300 × g for 10 min at room temperature. The adipocyte layer and the supernatant were separated, and SVF pellets were collected. For the measurement of pro-inflammatory cytokine secretion, the isolated SVFs (5 × 10⁵ cells/well) were seeded in a 96-well plate, added to LPS (100 ng/ml), and cultured for 24 h at 37°C. Levels of IL-6, TNF-α, and MCP-1 in the cultured supernatant were measured using a cytometric bead array kit (BD Biosciences, USA).

RNA Isolation and Real-Time PCR Analysis

Total RNA was isolated from frozen tissues and cDNA was synthesized using a TOPscript cDNA synthesis kit (Enzynomics, Korea). Real-time PCR was performed using a CFX9600 (Bio-Rad). PCR experiments were performed under the same cycling conditions: 95°C for 5 min, followed by 39 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 15 s. The PCR primer sequences are listed in Table 1. β-Actin RNA levels were used to normalize each gene.

Statistical Analysis

All data are presented as the mean ± standard error (S.E.). Student’s t-test was used for the statistical analysis of data. Analysis of variance (ANOVA) was used to determine the level of significance.
Results
Selection and Identification of a Candidate Strain with Anti-Obesity Efficacy
To screen lactic acid bacteria (LAB) with high anti-obesity efficacy in vitro, we measured the level of lipid accumulation by LAB treatment in 3T3-L1 adipocytes. Among 20 isolates, we selected a LAB10 strain for further study as it showed the highest inhibition of lipid accumulation in 3T3-L1 adipocytes (Fig. 1A). Even in a repeated assay, LAB10 reduced by about 44% compared to the control (Fig. 1B). In addition, we monitored the expression of metabolic genes in the liver and EF was determined by real-time RT-PCR. The expression levels of hepatic or EF of mice fed a HFD (Fig. 2A, p = 0.0098; B, p = 0.0022), although food intake was not different (Fig. 2C). WIKIM31 led to a significant reduction in fat mass gain (29%), without suppressing lean mass gain, compared to HFD mice (p = 0.0043) (Fig. 2D). In addition, the weights of epididymal (p = 0.01) and abdominal adipose tissue (p = 0.0113) in HFD-WIKIM31 mice were significantly lower than those in HFD mice (Fig. 2E). Consistently, adipocyte size in HFD-WIKIM31 mice were significantly lower than those in HFD mice (Fig. 2F). Furthermore, the serum levels of triglycerides, cholesterol, and glucose in HFD-WIKIM31 mice were reduced by 35% (p = 0.0006), 13% (p = 0.0155), and 16% (p = 0.0195), respectively (Fig. 2G). Alterations in serum adipocytokine levels are closely associated with obesity. For example, serum leptin and resistin levels increased, whereas adiponectin levels decreased in HFD mice. Therefore, we investigated whether WIKIM31 administration to mice during HFD feeding affected the serum adipocytokine levels. Leptin (p = 0.0072) and resistin (p = 0.0098) serum levels decreased, and adiponectin (p = 0.0177) levels in HFD-WIKIM31 mice increased (Fig. 2H). Together, these results indicate that WIKIM31 administration suppresses HFD-induced obesity.

L. sakei WIKIM31 Administration Suppresses Weight Gain in HFD-Induced Obese Mice
To determine whether WIKIM31 can improve HFD-induced obesity, WIKIM31 was orally administered to 7 week old male mice for 12 weeks. Mice fed both HFD and WIKIM31 showed reduced weight gain compared to HFD mice (p = 0.0019; EF, p = 0.0103; B, p = 0.0088) serum levels of triglycerides, cholesterol, and glucose in HFD-WIKIM31 mice were reduced by 35% (p = 0.0006), 13% (p = 0.0155), and 16% (p = 0.0195), respectively (Fig. 2G). Alterations in serum adipocytokine levels are closely associated with obesity. For example, serum leptin and resistin levels increased, whereas adiponectin levels decreased in HFD mice. Therefore, we investigated whether WIKIM31 administration to mice during HFD feeding affected the serum adipocytokine levels. Leptin (p = 0.0072) and resistin (p = 0.0098) serum levels decreased, and adiponectin (p = 0.0177) levels in HFD-WIKIM31 mice increased (Fig. 2H). Together, these results indicate that WIKIM31 administration suppresses HFD-induced obesity.

L. sakei WIKIM31 Administration Alters Metabolic Gene Expression in HFD-Induced Obese Mice
To identify the mechanism underlying the phenotype of HFD-WIKIM31 mice, the expression of lipid metabolic genes in the liver and EF was determined by real-time RT-PCR. The expression levels of hepatic or EF of lipogenesis-related genes, such as PPARγ (liver, p = 0.0233; liver, p = 0.00043), C/EBPα (liver, p = 0.0088; liver, p = 0.0034), FAS (liver, p = 0.024; liver, p = 0.0009), SREBP-1c (liver, p = 0.0042; liver, p = 0.0044), SCD1 (EF, p = 0.0045), and CD36 (liver, p = 0.0013; EF, p = 0.0117) were substantially lower in HFD-WIKIM31 mice than in HFD mice (Figs. 3A and 3B). However, the expression of UCP2 (liver, p = 0.0204; liver, p = 0.0005) and CPT-1α (liver, p = 0.0019; EF, p = 0.0001) genes involved in β-oxidation, was remarkably increased in HFD-WIKIM31 mice (Figs. 3C and 3D). These results indicate that WIKIM31 administration improves lipid and energy metabolism in the liver and adipose tissue by modulating the expression of lipogenesis- and β-oxidation-related genes.

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It is known that the secretion of pro-inflammatory cytokines and gene expression increases in HFD-induced obesity [5]. Therefore, we examined the expression levels of pro-inflammatory cytokine genes to determine whether WIKIM31 administration could reduce the inflammatory response. The expression of TNF-α, IL-6, and MCP-1, pro-inflammatory gene, were remarkably increased in the liver and ileum of HFD mice compared to those in ND mice. However, WIKIM31 administration strongly reduced the expression of these genes (TNF-α; liver: \( p = 0.0048 \), ileum: \( p = 0.0003 \), IL-6; liver: \( p = 0.0042 \), ileum: \( p = 0.0058 \), and MCP-1; liver: \( p = 0.0001 \), ileum: \( p = 0.0052 \)) (Figs. 4A and 4B). Obesity is associated with chronic inflammation characterized by macrophages and monocytes infiltration, and pro-inflammatory cytokines production [5]. To address whether oral administration of WIKIM31 regulates the suppression of inflammatory response in the adipose tissue, SVF isolated from EF were re-stimulated with LPS for 24 h, and the levels of pro-inflammatory cytokines in the supernatant were measured. The increased levels of IL-6 (\( p = 0.047 \)), TNF-α (\( p = 0.041 \)), and MCP-1 (\( p = 0.0489 \)) production in the HFD mice were remarkably decreased in the WIKIM31 mice (Fig. 4C). Intestinal inflammation is associated with intestinal barrier dysfunction [19, 20]. The effect of oral administration of WIKIM31 on the expression of tight junction proteins in the ileum of mice was further explored. Compared to HFD mice, WIKIM31 administration upregulated the expression levels of tight junction protein genes, such as claudin-2 (\( p = 0.0019 \)), claudin-5 (\( p = 0.0017 \)), occluding (\( p = 0.001 \)), and zonula occludens-1 (ZO-1) (\( p = 0.0018 \)) (Fig. 4D).

Fig. 1. The inhibitory effect of *L. sakei* WIKIM31 on lipid accumulation. (A, B) 3T3-L1 cells were treated with 10% bacterial extracts for 10 days, and then intracellular lipid were stained with Oil Red O (Pre, no differentiated cells; Con, fully differentiated cells) (C) Gene expression of lipogenesis were determined by real-time RT-PCR. LAB10 was renamed as WIKIM31. Data are presented as the mean ± SD of triplicate tests. *\( p < 0.05 \), **\( p < 0.001 \) vs. Con.
indicate that WIKIM31 treatment suppresses the immune responses related to obesity and further improves intestinal barrier function.

**L. sakei WIKIM31 Administration Increases SCFA Content in HFD-Induced Obese Mice**

SCFAs are metabolites of the intestinal microbiota and are beneficial to the host body [21]. The contents of propionate, butyrate, and valerate in HFD mice were distinctly lower than those in ND mice (Fig. 5A). After WIKIM31 administration, these SCFAs increased in comparison with HD mice (propionate, \( p = 0.0014 \); butyrate, \( p = 0.0006 \); valerate, \( p = 0.0035 \)). However, acetate content was not significantly different. Consistent with these results, the expression of G protein-coupled receptor (GPR) 40 (\( p = 0.0102 \)) and GPR43 (\( p = 0.038 \)), known as receptors for SCFAs, was remarkably increased (Fig. 5B).

**Discussion**

The pharmacological approach to the treatment of obesity can cause serious side effects, and probiotic administration has been considered an alternative [22]. Several clinical trials and studies have demonstrated the usefulness of probiotics in the treatment of metabolic diseases [23]. Probiotics improve obesity status by regulating the host’s energy metabolism and gut microbiota [24, 25].
In this study, to obtain LAB with anti-obesity from kimchi, we isolated 20 LAB strains from kimchi and examined their ability to inhibit lipid accumulation in vitro. *L. sakei* WIKIM31 was selected as it showed the highest inhibitory effect. Our in vivo results showed that the oral administration of *L. sakei* WIKIM31 in HFD-induced obese mice remarkably reduced body weight and fat mass gain without reducing food intake (Fig. 2). The liver and white adipose tissues are central organs with metabolic and lipogenic functions in the body [1, 26]. Therefore, dysregulation of energy metabolism in these organs is one of potential factors for metabolic disorders, such as obesity and diabetes [27]. It has been previously reported that *L. sakei* strains OK67 and ADM14 isolated from kimchi induce anti-obesity effects by modulating lipid metabolism in adipose tissue [16, 17]. Similarly, *L. sakei* WIKIM31 may exhibit anti-obesity effects by modulating lipid metabolism in adipose tissue.
sakei WIKIM31 administration effectively reduced serum triglyceride contents by inhibiting the expression of lipogenesis-related genes (PPARγ, C/EBPα, FAS, SREBP-1c, SCD1, and CD36) and promotes energy expenditure by increasing the expression of β-oxidation-related genes, UCP2 and CPT-1α in liver and white adipose tissues.

In obese individuals, as tight junction protein levels decrease, intestinal permeability increases, resulting in the infiltration of pro-inflammatory molecules that lead to inflammatory responses [28, 29]. Tight junction proteins such as occludin and claudins, play important roles in the intestinal barrier [30]. Previous studies have reported that ingestion of probiotic strains increases the expression of tight junction proteins in the intestine [16-18]. In the present study, L. sakei WIKIM31 not only increased the expression of tight junction protein genes to the level of the normal group, but also reduced the expression of pro-inflammatory cytokines in the ileum. GPRs, receptors for SCFAs, have been known to modulate the expression of tight junction proteins upon activation, as well as to improve intestinal inflammation and host energy metabolism [21, 31, 32]. However, it is unclear whether L. sakei can regulate the expression of GPRs in the intestine. In this study, we clearly demonstrated that L. sakei WIKIM31 administration increases the level of propionate and butyrate, resulting in the enhanced expression of their receptors, GPR41 and GPR43.

In conclusion, oral administration of L. sakei WIKIM31 ameliorated obesity by suppressing lipogenesis and inflammatory responses via improvement of gut barrier functions and the production of SCFAs. These results suggest that L. sakei WIKIM31 can be used as a therapeutic agent for obesity.

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Conflict of Interest
The authors have no financial conflicts of interest to declare.

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