Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 54 (2018) 87–94

Journal of Nutritional Biochemistry

# Antiobesity effect of *Lactobacillus reuteri* 263 associated with energy metabolism remodeling of white adipose tissue in high-energy-diet-fed rats☆,☆☆

Li-Han Chen[a], Yi-Hsing Chen[b], Kuan-Chen Cheng[c], Ting-Yi Chien[d], Ching-Hung Chan[d], Shu-Ping Tsao[d], Hui-Yu Huang[d,*]

[a]YongLin Biomedical Engineering Center, National Taiwan University, Taipei City 10617, Taiwan
[b]Research and Development Department, GenMont Biotech Incorporation, Tainan 741, Taiwan
[c]Institute of Food Science, National Taiwan University, Taipei City 10617, Taiwan
[d]Department of Food Science, Nutrition, and Nutraceutical Biotechnology, Shih Chien University, Taipei City 10462, Taiwan

Received 17 July 2017; received in revised form 26 September 2017; accepted 11 November 2017

**Abstract**

Obesity is a serious and costly issue to the medical welfare worldwide. Probiotics have been suggested as one of the candidates to resolve the obesity-associated problems, but how they combat obesity is not fully understood. Herein, we investigated the effects of *Lactobacillus reuteri* 263 (*L. reuteri* 263) on antiobesity using four groups of Sprague–Dawley rats ($n$=10/group), namely, C (normal diet with vehicle treatment), HE [high-energy diet (HED) with vehicle treatment], 1X (HED with $2.1\times10^9$ CFU/kg/day of *L. reuteri* 263) and 5X (HED with $1.05\times10^{10}$ CFU/kg/day of *L. reuteri* 263), for 8 weeks. *L. reuteri* 263 improved the phenomenon of obesity, serum levels of proinflammatory factors and antioxidant enzymes. More importantly, *L. reuteri* 263 increased oxygen consumption in white adipose tissue (WAT). The mRNA expressions of thermogenesis genes *uncoupling protein-1*, *uncoupling protein-3*, *carnitine palmitoyltransferase-1 and cell death-inducing DFFA-like effector-a* were up-regulated in WAT of the 5X group. Moreover, *L. reuteri* 263 might induce browning of WAT due to the higher mRNA levels of browning-related genes *peroxisome proliferator-activated receptor-γ*, *PR domain containing-16*, *Pparγ coactivator-1α*, *bone morphogenetic protein-7* and *fibroblast growth factor-21* in the 1X and 5X groups compared to the HE group. Finally, *L. reuteri* 263 altered the expressions of genes involved in glucose and lipid metabolisms in WAT, including increasing the levels of *glucose transporter type 4* and *carbohydrate-responsive element-binding protein* and decreasing the expression of *Acetyl-CoA carboxylase-1*. The results suggest that *L. reuteri* 263 may treat obesity through energy metabolism remodeling of WAT in the high-energy-diet-induced obese rats.


*Keywords:* Antiobesity; Browning of white adipose tissue; Energy metabolism remodeling; Lactobacillus reuteri 263; Probiotics

## 1. Introduction

Obesity is defined as abnormal accumulation of fat in the body. It is a medical condition that is linked to, and may increase the likelihood of, multiple other health disorders. According to World Health Organization, the prevalence of obesity increases more than twofold from 1980 to 2014 [1]. Since obesity-linked diseases, such as diabetes, hypertension, atherosclerosis and cancer [2,3], place an enormous personal and economic burden on those affected, it is critical to find ways to prevent and treat obesity.

Because obesity is caused by an imbalance of energy intake and expenditure, the best way to treat obesity is to cause a decrease in the former and an increase in the latter. Previous studies have shown that decreasing energy intake also leads to decreased energy expenditure [4]. Thus, current obesity treatments have focused on increasing energy expenditure. WAT, which was previously believed to be an energy storage tissue, has been found to be also involved in energy expenditure. WAT can up-regulate an *uncoupling protein* (*Ucp*) 1 and thus remodel its energy metabolism through a process called browning [5]. Browning transforms an original WAT to a beige adipose tissue, which has a higher respiratory rate, thermogenesis rate and energy consumption rate, similar to BAT [6,7]. After browning, the WAT displays a metabolism that expended glucose and lipid energy instead of storing it [8]. Thus, browning of WAT can be one of the ways to increase energy expenditure and prevent obesity.

Browning of WAT is regulated by several genes, including *peroxisome proliferator-activated receptor γ* (*Pparγ*), *PR domain containing 16* (*Prdm16*), *Pparγ coactivator-1α* (*Pgc1α*), *bone morphogenetic protein 7* (*Bmp7*) and *fibroblast growth factor 21* (*Fgf21*). Pparγ is the master transcription factor for fat differentiation and survival of adipocytes [9]. Pparγ stabilizes and recruits Prdm16 to activate *Pgc1α*, which results in browning of WAT [10,11]. Bmp7 and Fgf21 have been reported to induce *Pgc1α* and *Ucp1*, which increase energy expenditure [10,12,13]. Browning also induces mitochondrial biogenesis genes such as *carnitine palmitoyltransferase 1* (*Cpt1*) and *cell death-*

☆ Financial support: Funds raised by ourselves.
☆☆ Conflict of Interest: All authors declare no conflict of interest.
* Corresponding author. Tel.: +886 6600 2178.
*E-mail address:* maggieh323@hotmail.com (H.-Y. Huang).

https://doi.org/10.1016/j.jnutbio.2017.11.004
0955-2863/

*inducing DFFA-like effector a* (*Cidea*). Taken together, these genes are the biomarkers of beige adipose tissue.

Daily oral probiotic supplements are one of the potential candidates to tackle the issues of obesity because several different species of probiotics were demonstrated for their antiobesity effect. For example, *Lactobacillus rhamnosus* PL60 has been found to reduce weight and body fat in DIO mice despite no decrease of food intake [14]. *Lactobacillus paracasei* CNCM I-4270, *Lactobacillus rhamnosus* I-3690 and *Bifidobacterium animalis subsp. lactis* I-2494 also attenuated weight gain in mice without reducing food intake [15]. *Bifidobacterium spp*. showed similar results when they were added to an HFD in rats [16,17]. Probiotics were believed to modulate obesity by improving energy metabolism in brown adipose tissue (BAT) and skeletal muscle [18], but the effects of probiotics on energy regulation in white adipose tissue (WAT) were still unclear.

*Lactobacillus reuteri* 263 (*L. reuteri* 263) is a probiotic that has beneficial effects on renal fibrosis and insulin resistance, and ameliorates hepatic steatosis and hyperlipidemia [19–21]. It was first reported in 2010 and became a patented strain for improving the syndrome of diabetes (US 20110300117 A1) and renal fibrosis in diabetes (US 20120183504 A1) [19].

Although the previous studies did not focus on antiobesity effect of *L. reuteri* 263, lower body weight gain was observed following treatment of *L. reuteri* 263 [21–24]. Moreover, Hsieh et al. (2013 and 2016) and Liao et al. (2016) also revealed that treating rats with *L. reuteri* 263 at ~$2\times10^9$ CFU/rat/day attenuated obesity symptoms and enhanced expression of *Pparγ* in WAT [22–24]. Since WAT remodeling is a mechanism of antiobesity and is regulated by *Pparγ*, we hypothesized that *L. reuteri* 263 can attenuate HED-induced obesity *via* energy metabolism remodeling of WAT. To test our hypothesis, SD rats were fed with HED and were given *L. reuteri* 263 at either $2.1\times10^9$ CFU/rat/day or $1.05\times10^{10}$ CFU/rat/day for 8 weeks. At the end of the eighth week, we analyzed the energy expenditure of WAT, the genes related to browning, and metabolisms of glucose and lipid in WAT. These results confirmed that *L. reuteri* 263 induced energy metabolism remodeling of WAT.

## 2. Materials and methods

### 2.1. Animals and diets

Male Sprague–Dawley (SD) rats (8 weeks old, ~300 g) were purchased from local supplier (LASCO, Taipei, Taiwan) and singly housed under standard laboratory conditions (12/12-h light/dark cycle, 22°C–24°C, 40%–60% humidity) with free access to food and water. All rats were given 7 days to acclimate to their new environment and fed with normal diet in the acclimation period. Control group (C) consisted of 10 rats which were given normal diet (see Table 1 for ingredient composition) and placebo (distilled water). The other 30 rats were fed with high-energy diet (HED, see Table 1 for ingredient composition) and were randomly selected and assigned to three groups including the HE group, the 1X group (given HED and $2.1\times10^9$ CFU/kg/day of *L. reuteri* 263) and the 5X group (given HED and $1.05\times10^{10}$ CFU/kg/day of *L. reuteri* 263). The recommended dose of *L. reuteri* 263 for humans is $2\times10^{10}$ CFU/day. The dosage given to rats was converted from a human equivalent dosage using the following formula from the US Food and Drug Administration: assuming a human weight of 60 kg, the human equivalent dose for $2\times10^{10}$ CFU/60 kg/day×6.2=$2.1\times10^9$ CFU/kg/day; a conversion coefficient of 6.2 was used to account for differences between rats and humans.

Table 1
The effects of *L. reuteri* 263 on total food intake, BW and food consumption efficiency in HED-fed rats

| | C | HE | 1X | 5X |
|---|---|---|---|---|
| Food intake (g/day)/rat | 21.4± 2.1[a] | 19.5±1.6[a] | 19.4±1.3[a] | 19.3±1.2[a] |
| Food energy (kcal/ day)/rat | 41.5±4.5[a] | 88.5±6.7[b] | 88.1±6.2[b] | 87.6±6.0[b] |
| BW gain (g)/rat | 217.9±17.3[a] | 366.2±19.2[b] | 314.4±16.7[c] | 308.9±12.1[c] |
| FCE (%) | 16±0.9[a] | 30±1.2[b] | 26±1.1[c] | 25±3.1[c] |

Control, vehicle control; HE, high-energy diet control; 1X, HED with $2.1\times10^9$ CFU/kg/d of *L. reuteri* 263; 5X, HED with $1.05\times10^{10}$ CFU/kg/d of *L. reuteri* 263. All values are means ±S.EM. ($n$=10 rats/group). Different superscript letters (a, b, c) differ significantly at $P<.05$ by one-way ANOVA with Duncan posttest.

Moreover, ~$2\times10^9$ CFU/rat/day of *L. reuteri* 263 showed to attenuate obesity and enhance *Pparγ* in the WAT in previous studies. Thus, $2.1\times10^9$ CFU/kg/day and 5 times the dose were used in this study [22–24]. The *L. reuteri* 263 used was live *L. reuteri* 263 powdered by lyophization and purchased from GenMont (GenMont, Tainan, Taiwan). The CFU of powdered *L. reuteri* 263 was counted just before the beginning of this study. The powdered *L. reuteri* 263 was dissolved in 1 ml of distilled water and orally intubated by gavage to the 1X and the 5X groups at 9 a.m. every day for 8 weeks, while 1 ml of distilled water was given to the C and HE groups instead. Food and water intakes were recorded every day, and body weights were measured weekly. The rats were sacrificed at the eighth week. All animal experiments were performed in accordance with the protocol (IACUC-10302) approved by the Institutional Animal Care and Use Committee of Shih Chien University.

### 2.2. Adipose tissues sampling

WATs from mesenteric, epididymal and perirenal sites and interscapular BAT were separated and rinsed twice with a physiological saline solution, and then weighed immediately. All collected samples were stored at −80°C for further analysis.

### 2.3. Serum biochemical profiles

After the rats fasted for 8 h, blood was collected to determine their blood sugar (Roche, Indianapolis, IN, USA) and centrifuged at 3500×*g* for 10 min at 4°C to obtain serum. Serum triacylglycerol (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine transaminase (ALT) and aspartate transaminase (AST) levels were measured by using SYNCHRON Systems (Beckman Coulter Inc., Fullerton, CA, USA). Free fatty acid (FFA) levels were determined by enzymatic colorimetric assay according to the manual of commercial kit (Free fatty acids, Half-micro test, Boehringer Mannheim, Stuttgart, Germany).

### 2.4. Determination of adipocyte size of WATs

Epididymal fat pads (EFPs) were fixed in 4% paraformaldehyde (pH 7.2) at 4°C for 16 h. The samples were subsequently dehydrated in absolute ethanol, cleared in xylene and then embedded in paraffin. The paraffin blocks were cut into 4-μm sections, which were stained with Harris hematoxylin, counterstained with eosin and photographed under light microscopy (Nikon, Tokyo, Japan). The area, approximate diameter, perimeter and shape factor of the samples were recorded in 100 adipocyte units (5 slides for each rat, 6 rats per group) by using Sigma ScanPro4 program (Sigma, St. Louis, MO, USA) to analyze the digital images.

### 2.5. Measurement of oxygen consumption rates (OCRs)

OCRs were determined by using the XF24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA, USA) following the manufacturers' protocols. Briefly, 0.3 g of EFPs was separated from each rat and homogenized using a Polytron homogenizer in ice-cold MAS buffer (115 mM KCl, 10 mM $KH_2PO_4$, 2 mM $MgCl_2$, 3 mM HEPES and 1 mM EGTA) containing 0.2% fatty-acid-free BSA. The samples were then centrifuged at 700×*g* for 10 min to remove nuclei and cell debris, and then centrifuged at 8000×*g* for 10 min to collect mitochondria. After washing with ice-cold MAS buffer, the mitochondria pellet was resuspended in MAS buffer. The samples' protein concentrations were measured by using a BCA assay. Subsequently, the isolated mitochondria were seeded at 50 μl (10 μg of protein) per well in XF24 V7 microplates (Seahorse Bioscience, North Billerica, MA, USA). The plates were centrifuged at 2200×*g* for 20 min at 4°C; 625 μl of MAS buffer (containing 5 mM glutamate, 5 mM malate and 5 mM succinate) was then added into each well at 37°C. The XF24 plate was transferred to XF24 Extracellular Flux analyzer at 37°C and equilibrated for 10 min. Four assay cycles (30-s mix and 3-min determining period) were used to measure basal respiration. Following addition of oligomycin (4 μM; inhibiting ATP synthase) by automatic pneumatic injection (three assay cycles), FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone) (0.5 μM) was injected to completely uncouple the mitochondria. Finally, a cocktail of ofrotenone (4 μM) and antimycin A (2 μM) was injected to correct for nonmitochondrial respiratory rate. OCR measurements were recorded at set interval time points. All materials in the assay were obtained from Sigma-Aldrich. Bioenergetic Health Index (BHI) was calculated following the description of the study [25] using the BHI = log [(reserve capacity×ATP-linked)/(nonmitochondrial×proton leak)].

### 2.6. RNA extraction and quantitative RT-PCR

RNA was isolated from tissues by using RNeasy Mini kit (Qiagen, Hilden, Germany), and 500 ng of RNA from each sample was reverse-transcribed using iScript cDNA Synthesis Kit (Bio-Rad, USA), according to the instructions of the manufacturer. Quantitative PCR was performed in a MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) The sequences of the gene-specific primers (Purigo, Taipei, Taiwan) are shown in Table 2. β-Actin was used as an internal control for normalizing the mRNA levels of tested genes.