**Note**

*Lactobacillus plantarum* OLL2712 Regulates Glucose Metabolism in C57BL/6 Mice Fed a High-Fat Diet

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**Summary** The aim of the present study was to determine the effect of oral administration of *Lactobacillus plantarum* OLL2712 (*L. plantarum* OLL2712) on glucose and lipid metabolism in mice with high-fat diet-induced obesity. Mice that had been administered 10^9 cfu heat-killed *L. plantarum* OLL2712 for 12 wk showed significant reduction of blood glucose levels in response to insulin. Furthermore, mRNA expression of interleukin-1β in adipose tissue and serum levels of nonesterified fatty acids in mice administered *L. plantarum* OLL2712 were significantly lower than those in control mice. These results indicate that *L. plantarum* OLL2712 regulates glucose metabolism.

**Key Words** *Lactobacillus plantarum*, obesity, inflammation, adipose tissue

The incidence of obesity is increasing worldwide and this is problematic since obesity is a high-risk factor for the development of metabolic syndrome—a melange of pathological conditions that include insulin resistance, glucose intolerance and toxicity, hepatic steatosis and dyslipidemia, as well as the risk of developing type 2 diabetes (1). Type 2 diabetes and obesity, which both involve genetic and environmental factors (2), are major global causes of morbidity and mortality, as current nonsurgical therapies are inadequate (3). With accumulating evidence of some bacteria being beneficial to human health and metabolism, interest in foods containing live bacteria is increasing and food manufacturers are now adding beneficial bacteria to a wide variety of foods and beverages. Probiotics are defined as live microbial food components that are beneficial for humans (4). We have been evaluating the effects of probiotic bacterial strains on lipid metabolism using an in vitro 3T3-L1 cell culture system and we have found that some bacterial strains have regulatory properties for lipid metabolism. One of the candidate strains is *Lactobacillus plantarum* OLL2712 (*L. plantarum* OLL2712). The aim of the present study was to determine the effect of oral administration of *L. plantarum* OLL2712 on glucose and lipid metabolism in mice with high-fat diet-induced obesity.

*Lactobacillus plantarum* OLL2712 was grown in de Man, Rogosa and Sharpe broth (Becton Dickinson, CA, USA) at 37°C for 18 h. After fermentation, the cells were harvested in a refrigerated centrifuge (10,000 g, 15 min) and washed twice with saline solution followed by one wash with water. The cells were resuspended in distilled water, heat-killed at 70°C for 30 min, and lyophilized. The lyophilized cells were resuspended in distilled water at a concentration of 5×10^8 cfu/mL. Five-week-old specific pathogen-free C57BL/6J male mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Mice were given a high-fat diet. HFD-60 (Oriental Yeast Co., Ltd., Tokyo, Japan), from 6 wk of age. Daily intragastric administration of 1×10^9 cfu heat-killed *L. plantarum* OLL2712 was started from 6 wk of age and continued until 18 wk of age. Control mice were given distilled water intragastrically. Food intake and body weight (BW) were measured throughout the experimental period. All experimental procedures were approved by the Animal Research Committee of the University of Tokushima.

To evaluate sensitivity to insulin, mice were administered 0.75 U of insulin per kg of BW. Blood samples were collected from the tip of the tail vein at 0, 30, 60, 90 and 120 min. Blood glucose levels were measured by the EAD-glucose dehydrogenase method with a GLUCO-CARD GT-1820 device (Arklay, Tokyo, Japan).

Serum levels of total cholesterol, triglycerides and nonesterified fatty acid (NEFA) were analyzed by enzymatic kits (Cholesterol E test Wako, Triglyceride E test Wako and NEF A C test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum levels of total cholesterol, triglycerides and nonesterified fatty acid (NEFA) were analyzed by enzymatic kits (Cholesterol E test Wako, Triglyceride E test Wako and NEF A C test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Total RNA was isolated from epididymal fat and the liver using an RNasey Micro kit (Qiagen Science, MD, USA). First-strand cDNA was reverse-transcribed at 42°C for 60 min and at 95°C for 5 min from 2 μg of the extracted total RNA with reverse transcriptase (Invitrogen, CA, USA) and a random primer. We performed real-time PCR using specific primers and SYBR green dye (Takara Bio, Japan) in a Light Cycler real-time PCR system (Roche Diagnostics, Germany) according to the manu-
Lactobacillus plantarum OLL2712 Regulates Glucose Metabolism in Mice

Fig. 1. Effects of L. plantarum OLL2712 on weight gain and insulin tolerance in C57BL/6 mice fed a high-fat diet. (A) Body weights in control and L. plantarum OLL2712-treated mice were measured weekly. Weight gain was expressed as percentage of weight gain from the start of the experiment. (B) Control and L. plantarum OLL2712-treated mice were injected with insulin and their blood glucose levels were measured at 0, 30, 60, 90 and 120 min after treatment. Filled circles and open squares indicate control and L. plantarum OLL2712-treated mice, respectively. Data are shown as means±SE for 9 to 10 mice. Differences between the control and experimental groups is shown at *p<0.05 and **p<0.01.

Table 1. Inflammatory cytokine expression levels in adipose tissue in mice treated with L. plantarum OLL2712.

<table>
<thead>
<tr>
<th>Relative mRNA expression/36B4 mRNA expression</th>
<th>Control</th>
<th>L. plantarum OLL2712</th>
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</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>24.9±14.7a</td>
<td>21.3±9.6a</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.53±0.7a</td>
<td>1.63±0.28b</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.47±0.43b</td>
<td>0.56±0.12a</td>
</tr>
<tr>
<td>IL-6</td>
<td>8.76±2.51a</td>
<td>7.40±1.44a</td>
</tr>
</tbody>
</table>

1 Values are expressed as means with SE (n=9 or 10). Values in each group with the same letters are not significantly different (p<0.05).

Feeding of the high-fat diet increased BW up to 30% greater than that of mice fed normal chow at 18 wk of age under our facility conditions. During the experimental period, we did not observe significant differences in BW gain (Fig. 1A), final BW (control vs L. plantarum OLL2712: 33.2±1.0 g vs 33.8±0.7 g) or food intake (control vs L. plantarum OLL2712: 2.3±0.1 g/d vs 2.3±0.1 g/d) between the control and L. plantarum OLL2712 groups. Furthermore, no difference was observed in liver weight (control vs L. plantarum OLL2712: 1.25±0.08 g vs 1.17±0.03 g), epidymal fat weight (control vs L. plantarum OLL2712: 0.99±0.13 g vs 1.34±0.27 g) or fasting glucose level (control vs L. plantarum OLL2712: 84.3±5.4 mg/dL vs 76.2±2.7 mg/dL). First, we compared magnitudes of reduction in serum glucose concentration in response to insulin in the control and L. plantarum OLL2712 groups. Serum glucose concentrations were lower in the L. plantarum OLL2712 group than in the control group at 30, 60, 90 and 120 min after treatment with insulin (Fig. 1B). The magnitude of reduction was also evaluated by area under the concentration-time curve (AUC). AUC data also showed that administration of L. plantarum OLL2712 improves insulin resistance (control vs L. plantarum OLL2712: 9.53±2.126 vs 13.146±2.494).

We also investigated mRNA expression levels of inflammatory cytokines (IL-1β, IL-6) and found that IL-1β mRNA expression was significantly reduced in L. plantarum OLL2712-treated mice (Table 1). It has been shown that several proinflammatory cytokines (5), including IL-1β, are involved in disruption of insulin signaling (6). Randomized clinical trials...
have demonstrated that blockade of IL-1β signaling by
Analinra, a recombinant human IL-1 receptor antago-
nist, leads to sustained reduction in systemic inflamma-
tion and improvement of type-2 diabetes (7, 8). In an
animal study, mRNA expression of IL-β in visceral adi-
pose tissue was shown to correlate with BW and adi-
posity (9). The exact mechanism by which L. plantarum
OLL2712 regulates the expression of IL-1β mRNA in
adipose tissue was not determined in this study. In addi-
tion to glucose metabolism, we determined serum total
cholesterol, triglyceride and NEFA levels. Although sig-
nificant differences in serum total cholesterol and tri-
glyceride levels were not found, a significant reduction
of NEFA levels in L. plantarum OLL2712-treated mice
was observed (Table 2). Expression levels of the lipid
metabolism-related genes peroxisome proliferator-acti-
ated receptor α, carnitine palmitoyltransferase 1β, carni-
tine palmitoyltransferase 2, adipocyte triglyceride
lipase and hormone sensitive lipase were determined
by real-time PCR. We did not find a causal relationship
between these mRNA expressions and lipid metabolism
in L. plantarum OLL2712 mice (data not shown).

To our knowledge, this is the first study to show that
probiotic bacteria alter glucose metabolism without
change in BW. Although some studies have shown alter-
ation of glucose metabolism and lipid metabolism, these
alterations were accompanied by BW reduction and/or
fat weight reduction (10–13). The regulatory mecha-
nism of glucose and lipid metabolism by L. plantarum
OLL2712 is not known, though there are some possi-
bilities for the regulatory mechanism. One is the effect
of L. plantarum OLL2712 on microflora because it has
been shown that microflora play an important role in
body metabolism. Recent studies have shown the impor-
tance of gut microbiota in the development of obesity
and metabolic syndrome (14, 15). However, this possi-
bility is not likely because there has been no report that
showed heat-killed bacteria alter microflora. In our pre-
liminary experiment, intestinal microflora in the con-
trol and L. plantarum OLL2712 groups were compared by
the terminal restriction fragment length polymorph-
ism assay. No significant difference was found in the
pattern of peak areas of amplified 16S ribosome gene
products digested with HhaI or MspI (data not shown).
Another possibility is that L. plantarum OLL2712 regu-
lates immune cell function and then changes mechani-
isms. Recent studies have shown that immune cells,
especially T cells, contribute to the development of met-
abolic syndrome (16–18). It has been shown that T cells
in adipose tissue crucially contribute to differentiation
of inflammatory macrophages and also metabolic syn-
drome. In fact, modulation of regulatory T cell subsets
by anti-CD3 mAb or depletion of CD8+ T cells by anti-
CD8 mAb improved glucose metabolism in animals with
high-fat diet-induced obesity (16, 18). Probiotic bacte-
ria are known to affect T cell function and to have anti-
allergic action (19, 20). Further study is needed to show
the contribution of T cell function in adipose tissue to
the alteration of glucose metabolism in L. plantarum
OLL2712-treated mice.

One recent focus in studies on probiotic bacteria is
anti-obesity action. Some investigators have found use-
ful bacterial strains that prevent obesity and/or improve
metabolism (10–13). Bifidobacterium breve strain B-3
and Lactobacillus rhamnosus PL60 have been shown to
suppress weight gain in mice fed a high-fat diet (11, 12).
Increases in cell numbers and proportion of bifidobac-
teria in the microbiota and increase in production of
conjugated linoleic acid were speculated as mechanisms
for weight reduction in the case of Bifidobacterium breve
strain B-3 and in the case of Lactobacillus rhamnosus
PL60, respectively. Although they did not affect BW,
Lactobacillus gasseri SBT2055 and Lactobacillus plantar-
rum No. 14 reduced adipocyte size and/or adipose tis-
ue weight (10, 13). All of these bacterial strains reduce
body and/or fat weight. It thought that altered glucose
and lipid metabolism might be due to the reduction of
BW and fat weight. Lactobacillus plantarum OLL2712
can regulate glucose metabolism without reduction in
BW or visceral adipose tissue weight. Lactobacillus
plantarum OLL2712 provides a new insight into the
mechanism by which probiotic bacteria mediate glucose
metabolism regulation.

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IKK-β links inflammation to obesity-induced insulin

Table 2. Serum levels of total cholesterol, triglyceride
and nonesterified fatty acid in mice treated with L. plan-
tarum OLL2712.

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>L. plantarum OLL2712</th>
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</table>
| Total choles-
terol (mg/dL) | 156.4±13.5a | 173.5±9.6b          |
| Triglyceride
(mg/dL)       | 53.2±17.2a | 50.4±7.7a           |
| Nonesterified
fatty acid (mEq/L) | 1.37±0.03a | 1.14±0.03b         |

1 Values are expressed as means with SE (n=9 or 10). Val-
eses in each group with the same letters are not signifi-
cantly different (p<0.05).


