Obesity is characterized by an imbalance between energy intake and expenditure, resulting in fat accumulation, and is associated with an increased risk of developing insulin resistance and type 2 diabetes [8]. Recent studies have shown that the gut microbiota plays a major role in health and disease in humans and is regarded as a dynamic organ whose cellular composition is affected not only by diet and immune status but also by host physiology such as obesity [5]. Since the microbiota is involved in energy harvest and storage as well as in a variety of metabolic functions, both obesity and type 2 diabetes are associated with the altered composition and/or activity of gut microbiota, known as dysbiosis [3, 5]. High-fat (HF) diet feeding has recently been found to induce strong and rapid microbial shifts in the gut and favors the development of obesity and associated metabolic disorders [12].

Recently, *Lactobacillus rhamnosus* GG (LGG) was shown to exert insulin-sensitizing and adiposity-reducing effects in high-fat (HF) diet-fed mice. In the present study, we observed that the effects were correlated with the extent of dysbiosis induced by HF diet feeding before LGG administration. LGG-treated mice were protected from HF diet-induced adiposity and/or insulin resistance when LGG was treated after, not along with, HF diet feeding. Results indicate that, under HF dietary condition, supplemented LGG reverses insulin resistance, but does not block its onset.

**Keywords:** *Lactobacillus rhamnosus* GG, insulin resistance, adiposity, adiponectin, dysbiosis

and/or treat metabolic diseases through promoting specific alterations in gut microbiota in a state of dysbiosis. *Lactobacillus rhamnosus* GG (LGG), which is one of the most extensively studied probiotic bacteria, has been reported to exert a hypoglycemic effect in diabetic animal models [7, 9, 16]. However, the detailed mechanisms underlying the reported effect of LGG on metabolic homeostasis are still unclear and remain to be addressed. To better understand the mechanisms involved in the enhanced glucose tolerance and reduced adiposity by LGG treatment, studies should focus on the metabolic impact of LGG, particularly in an animal model with HF diet-induced dysbiosis. In spite of its evident anti-diabetic effect, it is required to examine whether LGG blocks insulin resistance by interrupting the onset of dysbiosis or by recovering insulin sensitivity in a state of dysbiosis. In this study, the hypothesis to be tested was that LGG reverses host metabolic alterations caused by dysbiosis, a consequence of HF-diet ingestion, and that this occurs only when LGG is treated after, not along with, dysbiosis induction. To test this, mice were maintained on a HF diet with varying periods of LGG treatment, and body weight and glucose tolerance were measured. The HF diet contained 60 kcal% fat, 20 kcal% protein, and 20 kcal% carbohydrate, providing 5.24 kcal/g of energy (D12492; Research Diets, New Brunswick, NJ, USA). LGG-treated
and control mice were orally administered a daily dose of LGG harvested at late-log phase (1 × 10^8 CFU per mouse) and PBS, respectively. As shown in Fig. 1, we set three experimental sets with varying period of dysbiosis induction before LGG administration; that is, starting LGG treatment and HF diet feeding at the same time (Experiment 1), and starting LGG treatment after 1 week (Experiment 2) and 9 weeks (Experiment 3) of HF diet feeding. Experiment 1 was set for testing whether LGG could prevent the occurrence of dysbiosis itself, and Experiments 2 and 3 were for testing the protective effect of LGG against low-grade and high-grade dysbiosis, respectively. We preliminarily checked the effect of a HF diet on gut microbiota composition to confirm the induction of dysbiosis. By measuring total 16S rRNA gene copies in fecal samples of mice by qPCR, the abundance of two quantitatively dominant phyla, Firmicutes and Bacteriodetes, was determined. Results showed that there was a significant decrease in Firmicutes (by 32%) and a significant increase of Bacteriodetes (by 290%) in HF diet-fed compared with normal diet-fed mice, indicating that a dramatic change in intestinal microbial composition, that is, dysbiosis, was induced by HF diet feeding (data not shown).

In Experiment 1, during 15 weeks on a HF diet, there was no significant difference in body weight between HF-diet LGG-co-treated (HFL1) and HF-diet PBS-treated control (HFP) mice (Fig. 2A). However, in Experiment 2, mice on a 12-week HF diet with LGG treatment for the latter 11 weeks (HFL2) had reduced body weight compared with HFP controls showing a significant reduction at two measured points (Fig. 2B). In Experiment 3, mice on a 16-week HF diet with LGG treatment for the latter 7 weeks (HFL3) had a significantly lower body weight compared with HFP controls (Fig. 2C). These results indicated that LGG could protect mice against adiposity only when treated after dysbiosis induction by HF feeding, but this was not the case when LGG was co-treated from the beginning of HF feeding. It was also observed that the weight of epidyymal adipose tissue was significantly lower in mice of the HFL3 group of Experiment 3, confirming the significant reduction of adiposity in HFL3 mice (Fig. 2D). Interestingly, the weight of liver tissue was reduced significantly in the HFL2 group mice of Experiment 2, but not in HFL3 mice of Experiment 3 (Fig. 2D).

We also observed that glucose tolerance was enhanced in LGG-treated mice of Experiment 2, but not of Experiment 1, which indicated that the adiposity reduction caused by LGG treatment was associated with increased insulin sensitivity (Figs. 3A and 3B). Although LGG treated from the beginning of HF diet feeding could not prevent the occurrence of HF diet-induced insulin resistance, the 1 week delayed treatment of LGG reversed the insulin resistance that was already induced by preemptive HF diet intake. However, in Experiment 3, we unexpectedly found that, despite their lower adiposity, LGG-treated (HFL3) mice did not show enhanced glucose tolerance compared with HFP control mice (Fig. 3C). These results indicated that the point of starting LGG treatment during the process of HF diet-induced dysbiosis was a crucial factor for the insulin-sensitizing and adiposity-reducing effects exerted by LGG. This finding demonstrates that the effect of LGG in reversing host metabolic alterations associated with dysbiosis in response to a HF-diet is not universally applicable, but depends on the host metabolic state, which could be an important issue in application of probiotics as a therapeutic strategy to prevent the development and progression of metabolic disorders.

Adiponectin, an adipokine produced almost exclusively in adipose tissues and secreted abundantly, powerfully affects the glucose and fatty acid metabolism in peripheral tissues through enhancing insulin sensitivity and inhibiting inflammatory responses [4]. We previously reported that LGG treatment enhanced insulin sensitivity and elevated adiponectin production with improving metabolic parameters in HF-diet-induced obese mice [9]. To confirm this effect of LGG on adiponectin production and compare it with those obtained in each experimental set of the present study, we
measured the protein level of adiponectin in the serum. As shown in Fig. 3D, a significant enhancement of serum adiponectin level was observed in LGG-treated mice of Experiments 2 and 3 (5.3-fold and 2.5-fold increase in blot intensity, respectively), whereas there was no significant difference between LGG-treated mice and their controls in Experiment 1. In addition, we found that the significance level of difference in serum adiponectin between LGG-treated and control mice was higher in Experiment 2 (\(p < 0.01\)) compared with Experiment 3 (\(p < 0.05\)). As expected from the very significantly higher production of adiponectin in HFL2 mice, the enhancing effect of LGG on glucose tolerance in Experiment 2 was correspondently significant (Fig. 3B). However, in Experiment 3, despite a significant increase in serum adiponectin in HFL3 mice relative to HFP controls, there was no significant improvement in glucose tolerance of HFL3 mice, which indicated that insulin resistance induced by a 9-week prefeeding of HF in Experiment 3 was too severe to be reversed by LGG treatment.

On the other hand, consistent with the changes in adiponectin production, the liver weight of HFL2 mice was significantly lower, and that of HFL3 mice was apparently lower without significance, compared with their HFP controls, whereas there was no reduction in the liver weight between HFL1 and HFP control mice, as shown in Fig. 2D. A higher production of adiponectin may trigger higher activation of AMPK in the liver, leading to a consequent inhibition of fatty acid synthesis and reduction in fat accumulation, and as a result, a lower weight of the liver was observed.

Taken together, it is conclusively proved that the LGG effect on the host is more effective in the early dynamic phase of weight gain and insulin resistance than in the late static phase, which was reached after a prolonged HF diet. Although we did not measure any direct effect of LGG on dysbiosis in this study, we found that the insulin-sensitizing effect of LGG exerted under the HF diet-induced dysbiosis condition depends on the period of dysbiosis induction, not...
the level of host adiposity. In addition, based on the fact that low-grade and chronic inflammation is one of the major contributors in obesity-induced insulin resistance and type 2 diabetes [14], an elevated serum adiponectin level caused by LGG treatment appears to be a strong protector from the low-grade inflammation and insulin resistance. Our findings provide a detailed insight regarding the LGG effect on the host metabolism under HF-dietary and dysbiosis condition, which is important to determine the strategies to apply probiotics to the host. It is also suggested that probiotics intake could play a crucial role in reversing metabolic disturbance during the progression of adiposity and glucose homeostasis impairment, but not in preventing the onset of insulin resistance. Further studies are required to investigate the mechanism of how the colonization of supplemented LGG affects gut ecology and host metabolism through restoring healthy microbiota.

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References


