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Triglyceride-Lowering Effects of Two Probiotics, *Lactobacillus plantarum* KY1032 and *Lactobacillus curvatus* HY7601, in a Rat Model of High-Fat Diet-Induced Hypertriglyceridemia

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The triglyceride-lowering effect of probiotics *Lactobacillus plantarum* KY1032 and *Lactobacillus curvatus* HY7601 were investigated. Male SD Wistar rats were randomly divided into three groups and fed high-fat diet (HFD), HFD and probiotics (5×10^9 CFU/day of *L. plantarum* KY1032 and 5×10^9 CFU/day of *L. curvatus* HY7601), or normal diet for 6 weeks. Probiotic treatment significantly lowered the elevated plasma triglyceride and increased plasma free fatty acid, glycerol, and plasma apolipoprotein A-V (ApoA-V) levels. The probiotic-treated group showed elevated hepatic mRNA expression of PPARα, bile acid receptor (FXR), and ApoA-V. These results demonstrate that *L. plantarum* KY1032 and *L. curvatus* HY7601 lower triglycerides in hypertriglyceridemic rats by upregulating ApoA-V, PPARα, and FXR.

Keywords: Triglyceride, probiotics, *Lactobacillus plantarum*, *Lactobacillus curvatus*, apolipoprotein A-V

Triglyceride (TG), one of the most important lipids, is composed of free fatty acids (FFAs) ester-linked to a glycerol backbone [11]. TGs are synthesized in liver and intestinal cells, and then packaged into very low-density lipoprotein (VLDL) and TG-rich lipoprotein chylomicron, respectively. Elevated plasma TG levels and prolonged circulation of lipoprotein remnants are independent risk factors for coronary artery disease, the most common cardiovascular disease [6, 22]. The primary causes of hypertriglyceridemia are various genetic defects leading to disordered TG metabolism. Secondary hypertriglyceridemia is the result of acquired causes such as high-fat diet (HFD), obesity, diabetes, hypothyroidism, and certain medications [16].

Probiotics are defined by the World Health Organization as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." Most bacteria with probiotic properties belong to the genera *Lactobacillus* and *Bifidobacterium*, which are common but non-dominant members of the indigenous microbiota of the human gastrointestinal tract [19, 25]. In the last decade, probiotics have rapidly emerged as natural therapeutics with the potential to improve lipid metabolism [3, 4, 9, 12, 13, 14].

A recent study showed that treatment with the probiotic *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 at 10^{10} CFU/day significantly reduced average plasma TG levels by 46% compared with placebo treatment in high-fructose-fed rats [14].

A more recent clinical study using *L. curvatus* HY7601 and *L. plantarum* KY1032 reported that probiotics treatment reduced serum TG levels by 18.3% and increased plasma apolipoprotein A-V (ApoA-V) levels by 21% in non-diabetic subjects with hypertriglyceridemia [1].

The aim of this study was to determine the TG-lowering effect of naturally derived probiotic strains *L. curvatus*

Table 1. Composition of experimental diets (g/kg).

Ingredient	Normal diet	High-fat diet
Casein	200	200
DL-Methionine	3	3
Corn starch	150	150
Sucrose	500	150
Cellulose	50	50
Corn oil	50	0
Salt mix	35	35
Vitamin mix	10	10
Choline bitartrate	2	2
Beef tallow	0	400
Total	1,000	1,000

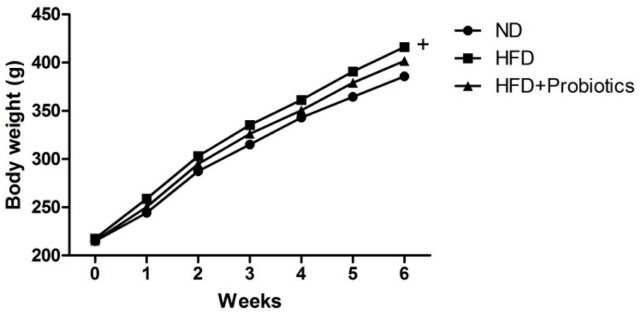

Fig. 1. Effect of probiotics on body weight during the experimental period.

Significant differences between the high-fat-diet-fed control group (HFD, $n = 10$) and the normal control group (ND, $n = 10$) are indicated as $^{+}p < 0.05$.

HY7601 and *L. plantarum* KY1032 in an HFD (containing 40% beef tallow)-induced hypertriglyceridemia model. Furthermore the relationship between the TG level and the expression of ApoA-V, an important determinant of plasma TG levels, was elucidated.

Male SD rats ($n = 30$) aged 5 weeks were purchased from Raon Bio (Korea). All rats were individually housed at a constant temperature (22°C ± 2°C) and humidity (55% ± 10%) with a 12 h light/dark cycle. After 1 week adaptation, rats were randomly assigned to two groups and fed an HFD ($n = 20$) to induce hypertriglyceridemia or a normal diet (ND group, $n = 10$) for 6 weeks. Of the HFD-fed rats, some were randomly assigned to receive probiotics (HFD + probiotic group, $n = 10$) or PBS (HFD group, n = 10) for the experimental period. The experimental design was approved by the Ethics Committee at Korea Yakult Company Limited R&D Center (KYIACUC-2014-00023-Y-2). The probiotics were suspended in sterilized PBS and mixed with the diet immediately before feeding. Once the rats consumed the initial amount of food, an additional amount was added to the feed jar so that all the rats could consume all of the available food/probiotic mix every day. The composition of the diets was based on the AIN-76 semi-synthetic diet (Table 1).

Body weight and food intake were measured every week for 6 weeks. During the experimental period, the body weights of all groups increased every week. At the end of the experiment, the body weight gains of the HFD and HFD + probiotics groups were higher than that of the ND group. The body weight of the HFD group was significantly increased compared with the ND group but the differences between the HFD and HFD + probiotics groups were not significant (Fig. 1)

Hypertriglyceridemia was induced by using HFD. In a previous study [2], HFD promoted a significant increase in serum TG concentration over the 7-week experimental period. Therefore, in this study, at the end of the experiment, the plasma levels of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglyceride (TG) in each group were measured (Fig. 2). Plasma TG, TC, and HDL-C were enzymatically determined using a commercial kit (Asan Co.). Plasma LDL-C was calculated using the formula by Friedewald *et al*. [7]. The TC, LDL-C, and TG levels of the HFD group were higher than those of the ND group and HFD + probiotic group. In particular, TG levels in the HFD + probiotics group were 22% ($p < 0.05$) lower than that in the HFD group. The HDL-C levels did not differ significantly among groups.

Next, the concentrations of plasma free fatty acid (FFA), glycerol, and ApoA-V were measured (Fig. 3) The plasma FFA and glycerol levels in the HFD group were lower than those in the ND group. In contrast, the FFA and glycerol levels in the HFD + probiotics group were 13% ($p < 0.05$) and 18% ($p < 0.05$) higher than that in the HFD group ($p < 0.01$). These results suggest that *L. curvatus* and *L. plantarum* exert their TG-lowering effect in hypertriglyceridemic rats by degrading TG. To investigate the relationship between apolipoprotein and TG levels, the concentrations of ApoA-V, ApoC-II, and ApoC-III were determined at the protein level using an ELISA kit. The levels of ApoC-II and ApoC-III were not significantly different among the three groups. However, after administration of probiotics, the plasma ApoA-V level in the HFD + probiotic group was dramatically increased by 37% ($p < 0.05$). These results indicate that ApoA-V, not ApoC-II and ApoC-III, participates in TG

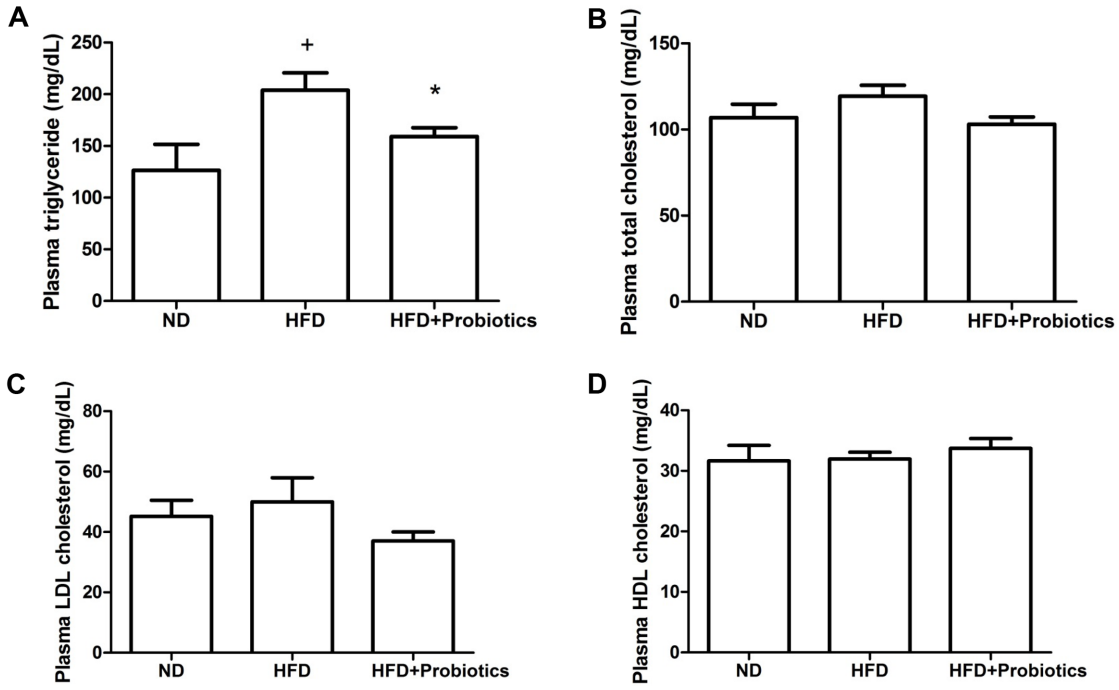

Fig. 2. Effect of probiotics treatment on plasma triglyceride (**A**), total cholesterol (**B**), LDL cholesterol (**C**), and HDL cholesterol (**D**) in the high-fat-diet-fed rats.

The results are expressed as the mean ± SE. Significant differences between the high-fat-diet-fed control group (HFD, $n = 10$) and the normal control group (ND, $n = 10$) are indicated as $^{+}p < 0.05$. Significant differences between the probiotics treatment group (HFD + Probiotics, $n = 10$) and the high-fat-diet-fed control group are indicated as $^{*}p < 0.05$.

metabolism.

Next, the expressions of TG-related mRNAs in the liver of rats were examined (Fig 4). Total RNA was isolated from whole livers using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed to cDNA using the QuantiTect reverse transcription kit (Qiagen). RNA expression was quantified by real-time quantitative PCR using the Quanti-Tect SYBR Green RT-qPCR kit (Qiagen). GAPDH was used as an internal control, and relative gene expression was calculated using the 2-DDCt method. As shown in Fig. 4, HFD intake significantly decreased the expression of ApoA-V, PPARα, and FXR genes. However, after administration of the probiotics, the mRNA levels of ApoA-V, PPARα, and FXR increased significantly ($p < 0.05$). We also examined the expression of genes involved in fatty acid metabolism: sterol regulatory element binding protein-1 (SREBP-1), fatty acid synthase (FAS), stearoyl-coenzyme A desaturase-1 (SCD-1), and carnitine palmitoyltransferase-1 (CPT-1). None of the genes showed a significant difference in expression among the groups. These results suggest that *L. curvatus* and *L. plantarum* exert their TG-lowering effect via upregulation of ApoA-V, and the increased ApoA-V level is associated with and, most likely, due to increased hepatic PPARα and FXR expression.

TGs are the initiators of the metabolic changes leading to atherogenic dyslipidemia, a major inducer of atherosclerosis resulting from quantitative and qualitative changes in lipoprotein subclass distributions [10]. The direct relationship between TG and atherosclerosis is still controversial, because atherosclerotic plaques possess primarily cholesterol and not triacylglycerol [20]. However, genome-wide association studies suggest that triglyceride plays an indirect role in disease progression through its association with other genetically regulated components [21].

ApoA-V is strongly associated with plasma TG levels and modulates the occurrence of both moderate and severe hypertriglyceridemia. ApoA-V is 366 amino acids long and is expressed in the liver; it binds to very-low-density lipoprotein, high-density lipoprotein, and chylomicrons in plasma [5]. Pennacchio *et al*. [17] reported that mice expressing a human ApoA-V transgene had one-third the plasma TG concentration in control mice. However, ApoA-V knockout mice had four times as much plasma TGs as controls. Another group generated mice with adenoviral

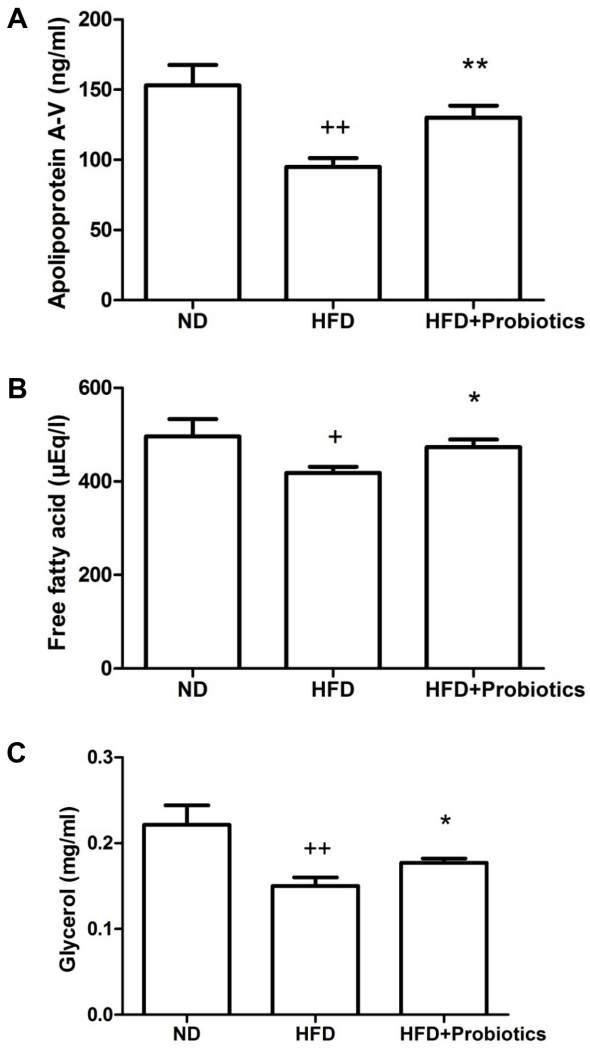

Fig. 3. Effect of probiotics treatment on serum apolipoprotein A-V (**A**), free fatty acid (**B**), and glycerol (**C**) in high-fat-diet-fed rats.

The results are expressed as the mean ± SE. Significant differences between the high-fat-diet-fed control group (HFD, n = 10) and the normal control group (ND, n = 10) are indicated as $^{+}p < 0.05$ and $^{++}p < 0.01$. Significant differences between the probiotics treatment group (HFD + Probiotics, n = 10) and the high-fat-diet-fed control group are indicated as $*p < 0.05$ and $**p < 0.01$.

overexpression of ApoA-V and analyzed plasma lipid profiles and observed a reduction of VLDL TG, and cholesterol levels in ApoA-V-overexpressing mice, suggesting that ApoA-V participates in the regulation of plasma TG and cholesterol levels [23].

PPARα agonists are known to have hypotriglyceridemic effect, and two studies in 2003 have shown that the ApoA-V gene is highly upregulated by PPARα and FXR [18, 24].

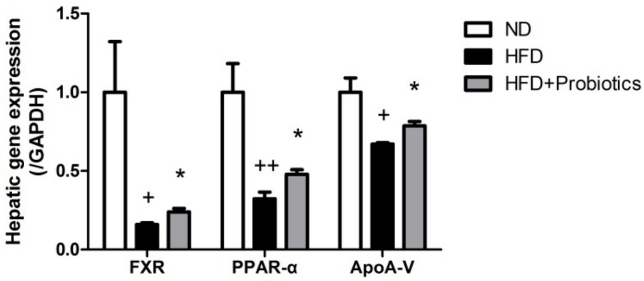

Fig. 4. Effect of probiotics treatment on triglyceride-secretion-related gene expression in the hepatic tissue of high-fat-diet-fed rats.

The results are expressed as the mean ± SE. Significant differences between the high-fat-diet-fed control group (HFD, n = 10) and the normal control group (ND, n = 10) are indicated as $^{+}p < 0.05$ and $^{++}p < 0.01$. Significant differences between the probiotics treatment group (HFD + Probiotics, n = 10) and the high-fat-diet-fed control group are indicated as $*p < 0.05$.

However, despite these strong correlations between ApoA-V and TGs, the mechanism linking these two parameters remains undefined [8].

L. curvatus HY7601 and *L. plantarum* KY1032 were isolated from Korean traditional fermented cabbage. *L. curvatus* HY7601 and *L. plantarum* KY1032 have been reported to have beneficial effects in diet-induced animal models. These probiotics have been shown to reduce diet-induced obesity and to modulate genes associated with metabolism and inflammation in the liver and adipose tissue [15, 25]. A combination of these probiotics also suppressed the clinical characteristics of high-fructose-induced metabolic syndrome [14].

In this study, we investigated the TG-lowering effect of two probiotic strains, *L. plantarum* KY1032 and *L. curvatus* HY7601, in HFD-induced hypertriglyceridemic rats. Our findings showed that these probiotics exert their TG-lowering effects by upregulating the expression of ApoA-V, PPARα, and FXR. These probiotics may provide a natural alternative for the treatment of diet-induced hypertriglyceridemia.

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