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Supplementation with two probiotic strains, *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032, reduces fasting triglycerides and enhances apolipoprotein A-V levels in non-diabetic subjects with hypertriglyceridemia

Hyeon Yeong Ahn [a], Minjoo Kim [b], Jey Sook Chae [b], Young-Tae Ahn [c], Jae-Hun Sim [c], Il-Dong Choi [c], Sang-Hyun Lee [d], Jong Ho Lee [b, e, f, g, *]

[a] *Interdisciplinary Course of Science for Aging, Yonsei University, Seoul, Republic of Korea*
[b] *Research Center for Silver Science, Institute of Symbiotic Life-TECH, Yonsei University, Seoul, Republic of Korea*
[c] *Korea Yakult Co., Ltd., Yongin, Gyeonggi, Republic of Korea*
[d] *Department of Family Practice, National Health Insurance Corporation Ilsan Hospital, Goyang, Republic of Korea*
[e] *National Leading Research Laboratory of Clinical Nutrigenetics/Nutrigenomics, Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul, Republic of Korea*
[f] *Department of Food and Nutrition, Brain Korea 21 PLUS Project, College of Human Ecology, Yonsei University, Seoul, Republic of Korea*
[g] *Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea*

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ABSTRACT

Objective: Previous studies have indicated that supplementation with probiotics might improve lipid metabolism. The objective of the study was to evaluate the effect of supplementation with probiotic strains *Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032 on triglyceride (TG) and apolipoprotein A-V (apo A-V) levels.
Methods: A randomized, double-blinded, placebo-controlled study was conducted with 128 non-diabetic subjects with hypertriglyceridemia. Over a 12-week test period, the probiotic group consumed 2 g/day of a powdered supplement containing *L. curvatus* HY7601 and *L. plantarum* KY1032, whereas the placebo group consumed a powder lacking probiotics.
Results: After the treatment, the probiotic group showed an 18.3% ($P < 0.001$) reduction in TGs and increases of 21.1% ($P = 0.001$) and 15.6% ($P < 0.001$) in the apo A-V and LDL particle size, respectively. The probiotic group had a significant reduction in TGs ($P = 0.040$) and increases in the plasma apo A-V ($P = 0.003$) and LDL particle size ($P < 0.001$) compared with the placebo group. In the probiotic group, the reduction in the TG levels was negatively correlated with changes in the apo A-V and baseline TGs, regardless of the *APOA5* -1131T > C genotype.
Conclusion: The consumption of two probiotic strains for 12 weeks reduced TGs and increased the apo A-V and LDL particle size in hypertriglyceridemic subjects. This effect was more pronounced in subjects with higher levels of fasting TGs regardless of their *APOA5* -1131T > C genotype.

1. Introduction

Accumulating evidence suggests that high triglyceride (TG) levels might be a significant, independent risk factor for coronary artery disease [1]. Thus, understanding and controlling TG metabolism is important to treat hypertriglyceridemia effectively. Moderate hypertriglyceridemia in some individuals could be managed by changes in diet or exercise; however, some patients do not respond positively to these lifestyle changes [2–4]. The substantial interindividual differences in response to dietary treatment could be partly driven by genetic factors.

Over the past ten years, probiotics have rapidly emerged as natural therapeutics with the potential to improve lipid

* Corresponding author. Department of Food & Nutrition, College of Human Ecology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea.
E-mail address: jhleeb@yonsei.ac.kr (J.H. Lee).

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metabolism [5–10]. A recent study showed that probiotic treatment with *Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032 at 10^{10} colony-forming units per day (CFU/d) reduced average plasma TG levels by 40% compared with a placebo treatment in rats fed a high-fructose diet [10]. The aim of this study was to investigate the effect of dual supplementation with probiotic strains *L. curvatus* HY7601 at 0.5×10^{10} CFU/d and *L. plantarum* KY1032 at 0.5×10^{10} CFU/d on TG-lowering efficacy in non-diabetic and borderline to moderate hypertriglyceridemic (TG, 150–499 mg/dL) subjects. Additionally, we analyzed the plasma concentration of apolipoprotein A-V (apo A-V), a significant modulator of serum TG [11], and genotyped the *APOA5* -1131T > C polymorphism in this patient cohort.

2. Materials and methods

2.1. Study subjects

The study subjects were recruited from the Health Service Center (HSC) during routine check-ups at National Health Insurance Corporation Ilsan Hospital, Goyang, Korea (June 2012–March 2014). Based on the data screened from HSC, subjects who were non-diabetic with borderline to moderate hypertriglyceridemia were referred to the Family Medicine or Internal Medicine departments. The patients were rechecked with their health and lipid profiles, and those who satisfied the study criteria were recommended for participation in the 12-week interventional study. Those patients who consented to the program in writing were included in this study. Hypertriglyceridemia was defined according to the National Cholesterol Education Program (NCEP) – Adult Treatment Panel (ATP) III [12]. A total of 128 subjects with borderline to moderate hypertriglyceridemia were enrolled in this study, and the protocol was approved by the Institutional Review Board (IRB) of Yonsei University and National Health Insurance Corporation Ilsan Hospital. Subjects were excluded by the following criteria: treatment with lipid-lowering medications; treatment with medications or supplements known to affect lipid metabolism or any probiotic products in the past month; a diagnosis of dyslipidemia, diabetes mellitus, hypertension, liver disease, renal disease, cardiovascular disease, cerebrovascular disease, pancreatitis, or cancer; medication or alcohol abuse; or pregnancy or breast feeding. The study subjects were randomly assigned to the probiotic group or the placebo group. The groups were created by computer-generated block randomization (probiotic:placebo = 1:1). Seven subjects dropped out of the study, six for personal reasons and one by loss of contact. Ultimately, 121 subjects [probiotic group (n = 58), placebo group (n = 63)] completed the 12-week interventional study. Of the 121 participants, 64 had TG levels between 150 and 199 mg/dL, 54 had TG levels between 200 and 399 mg/dL, and 3 had TG levels between 400 and 499 mg/dL.

2.2. Study design and intervention

A 12-week, double-blinded, placebo-controlled, randomized study was conducted with 128 non-diabetic and hypertriglyceridemic subjects divided into two groups: the probiotic group and the placebo group. The individuals in the probiotic group (n = 64) consumed 2 g daily of a powder containing the following probiotic strains: *L. curvatus* HY7601 and *L. plantarum* KY1032, each at 0.5×10^{10} CFU/d. The individuals in the placebo group (n = 64) consumed an identical powder that did not contain probiotics (NCT02215694; http://www.clinicaltrials.gov). The products were provided by Korea Yakult Co., Ltd. (Seocho-gu, Seoul, Korea). The study was divided into the following periods: pre-ingestion, in which the non-diabetic subjects with hypertriglyceridemia did not ingest the probiotic or placebo for 14 days, and the ingestion period, the 12 weeks in which the probiotic or placebo product was administered.

2.3. Anthropometric parameters, blood pressure, and blood collection

The weight and height were measured without clothes or shoes, and the BMI was calculated by kilograms per square meter (kg/m^2). The lipid accumulation product (LAP) was defined as [waist circumference (WC) (cm) – 65] x [TG concentration (mmol/L)] for men and [WC (cm) – 58] x [TG concentration (mmol/L)] for women [13]. The waist and hip circumferences were measured using a plastic measuring tape to calculate the waist to hip ratio (WHR). After a 20-min rest, the blood pressure (BP) was measured 2 times in the left arm of seated subjects with an automatic BP monitor (FT-200S, Jawon Medical, Gyeongsan, Korea). After a 12-h fasting period, venous blood specimens were collected in EDTA-treated whole blood tubes and serum tubes. The blood samples were centrifuged to obtain plasma and serum. The collected samples were stored at −70 °C.

2.4. Serum lipid profile and free fatty acids

The fasting TG and total cholesterol were measured using a Hitachi 7600 Autoanalyzer (Hitachi, Ltd., Tokyo, Japan). The HDL cholesterol remaining in the supernatant fraction was measured with an enzymatic method after the precipitation of other lipoproteins. The LDL cholesterol was indirectly calculated using the Friedwald formula, as follows: LDL cholesterol = total cholesterol – [HDL cholesterol + (TG/5)] for the subjects with a serum TG level <400 mg/dL. The LDL cholesterol was measured directly using a Hitachi 7600 Autoanalyzer for the subjects with a serum TG level ≥400 mg/dL. The free fatty acids (FFA) were measured by an enzymatic assay with the acylCoA synthetase-acylCoA oxidase (ACS-ACOD) method using a Hitachi 7600 Autoanalyzer.

2.5. Fasting glucose and related biomarkers

The fasting serum glucose was measured by the hexokinase method with a Hitachi 7600 Autoanalyzer. The serum insulin was measured by an immunoradiometric assay kit from DIAsource ImmunoAssays S.A. (Louvain, Belgium). The serum C-peptide was measured using an immunoradiometric assay with a C-peptide IRMA kit (Immunotech, Czech).

2.6. Serum high-sensitivity C-reactive protein and LDL particle size

The serum high-sensitivity C-reactive protein (hs-CRP) was measured using an ADVIA® 2400 Clinical Chemistry System (Siemens, Ltd., Tarrytown, NY) and high-sensitivity CRP-Latex (II) X2 kit (Denka-Seiken Co., Ltd., Tokyo, Japan). The LDL particles were isolated using sequential flotation ultracentrifugation and particle size distribution (1.019–1.063 g/mL) and examined on non-denaturing polyacrylamide gels containing a linear gradient of 2–16% acrylamide (CBS Scientific, CA, USA) using a pore gradient lipoprotein system (CBS Scientific Company, Inc., San Diego, CA). The relative migration rates of each band were estimated using latex beads (30 nm), thyroglobulin (17 nm), ferritin (12.2 nm), and catalase (10.4 nm). The gels were scanned with a GS-800 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Inc., Hercules, CA).

2.7. Plasma apo A-V and genotyping of APOA5 -1131T > C

The plasma apo A-V was measured using an enzyme immunoassay (Human Apolipoprotein A ELISA kit, Millipore, MO, USA), and the color reaction was read at 450 nm using a Victor2 spectrometer (Perkin Elmer Life Sciences, Turka, Finland). The genomic DNA was extracted from 5 mL of whole blood with a commercially available DNA isolation kit (WIZARD Genomic DNA purification kit, Promega Co., Madison, WI, USA). Genotyping of *APOA5* -1131T > C was performed using single primer extension technology (SNPstream 25K™ system, Orchid BioSciences, NJ, USA) with SNP-IT™ assays. The colorimetric reaction was read using an enzyme-linked immunosorbent assay (ELISA) reader, and the genotype was determined by QCReview™ software.

2.8. Statistical analysis

The statistical analysis was performed with SPSS version 21.0 (IBM/SPSS Corp., Chicago, IL, USA). Logarithmic transformation was performed on the skewed variables. An independent *t*-test was used to compare the parameters between the placebo and probiotics groups. The general linear model test was applied to adjust for potential confounding factors. Pearson's correlation coefficient was used to examine relationships between the variables. A heat map was generated to visualize correlations among the variables. The results were expressed as the mean ± SEM. A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. The characteristics at baseline and the distribution of the APOA5 -1131T > C polymorphism

Table 1 shows the general and biochemical characteristics of the baseline values in the placebo and probiotic groups. There were no significant differences between the two groups in age, gender distribution, BMI, smoking, drinking, BP, serum lipid profiles, hs-CRP, apo A-V, glucose, insulin, or C-peptide. The estimated total caloric intake, physical activity, and percentages of protein, fat, and carbohydrate intake were not significantly different between the two groups (data not shown). The genotype distribution of the *APOA5* -1131T > C was within the limits of the Hardy–Weinberg equilibrium for the entire cohort, as well as for the placebo and probiotic groups individually (Table 1). In the entire set of non-diabetic hypertriglyceridemic subjects, the frequency of the −1131T > C minor allele was 0.38, which was consistent with previous observations in a hypertriglyceridemic Korean population [2,14]. To increase the statistical power for this test, the carriers of the less common C allele (T/C + C/C) were pooled.

3.2. Effects of a 12-week consumption of probiotics on the BMI, LAP, TG, apo A-V, FFA, and LDL particle size

There were no significant mean changes in any of the tested clinical and biochemical characteristics between the placebo and probiotic groups (data not shown), except for the serum TG, plasma apo A-V levels, and LDL particle size. The mean changes in the BMI and LAP tended to differ between the two groups ($P = 0.068$ and $P = 0.056$, respectively). The data in Fig. 1 show that after 12 weeks of treatment, the individuals in the probiotic group demonstrated a significant reduction of 18.3% in their serum TG ($P < 0.001$) levels. Additionally, the probiotic group showed significant increases of 21.1% ($P = 0.001$) and 15.6% ($P < 0.001$) in their plasma apo A-V levels and LDL particle size, respectively. The placebo group did not exhibit significant changes over the same time period. When the TG, apo A-V levels, and the LDL particle size changes (the differences from the baseline) in the placebo and probiotic groups were compared, the probiotic group had a greater reduction in the serum TG ($P = 0.040$) levels and greater increases in the plasma apo A-V ($P = 0.003$) levels and the LDL particle size ($P < 0.001$).

The circulating TG and apo A-V levels were tested before and after the treatment as a function of the *APOA5* -1131T > C genotype. There was no significant association between these values and the *APOA5* -1131T > C genotype, which was consistent with previous observations [2]. The circulating TG, apo A-V levels, and the LDL particle size changes were compared according to the *APOA5*

Table 1
Clinical and biochemical characteristics of placebo and probiotic groups at baseline.

	Total (n = 121)		*P*
	Placebo group (n = 63)	Probiotics group (n = 58)	
Age (year)	52.2 ± 1.17	53.6 ± 1.14	0.412
Male/female n, (%)	15 (23.8)/48 (76.2)	18 (31.0)/40 (69.0)	0.373
Body mass index (kg/m^2)	24.8 ± 0.33	24.9 ± 0.42	0.923
Waist hip ratio	0.91 ± 0.01	0.90 ± 0.01	0.826
Cigarette smoker n, (%)	7 (11.1)	7 (12.1)	0.869
Alcohol drinker n, (%)	33 (52.4)	33 (56.9)	0.618
Systolic BP (mmHg)	120.3 ± 1.63	121.2 ± 1.88	0.709
Diastolic BP (mmHg)	79.3 ± 1.11	79.1 ± 1.33	0.911
Triglyceride (mg/dL)$^{\phi}$	204.4 ± 5.95	211.0 ± 9.01	0.535
Total-cholesterol (mg/dL)$^{\phi}$	211.8 ± 4.30	209.5 ± 4.59	0.716
HDL-cholesterol (mg/dL)$^{\phi}$	47.3 ± 0.98	47.8 ± 1.36	0.759
LDL-cholesterol (mg/dL)$^{\phi}$	123.7 ± 4.15	121.4 ± 4.38	0.701
LDL particle size (nm)$^{\phi}$	23.1 ± 0.10	22.7 ± 0.45	0.408
Free fatty acid (μEq/L)$^{\phi}$	524.3 ± 19.4	524.0 ± 21.0	0.992
hs-CRP (mg/dL)$^{\phi}$	1.22 ± 0.12	1.20 ± 0.14	0.912
Apolipoprotein A-V (ng/mL)$^{\phi}$	236.4 ± 26.6	219.5 ± 23.6	0.637
Glucose (mg/dL)$^{\phi}$	89.1 ± 1.61	89.5 ± 1.53	0.863
Insulin (μIU/dL)$^{\phi}$	10.5 ± 0.59	10.4 ± 0.52	0.899
C-peptide (μEq/L)$^{\phi}$	2.20 ± 0.09	2.17 ± 0.11	0.844
ApoA5 -1131 T > C n, (%)			0.344
T/T	22 (34.9)	21 (36.2)	
T/C	30 (47.6)	32 (55.2)	
C/C	11 (17.5)	5 (8.6)	

Mean ± SEM. $^{\phi}$Tested by logarithmic transformation, *P*-values derived from independent *t*-test.

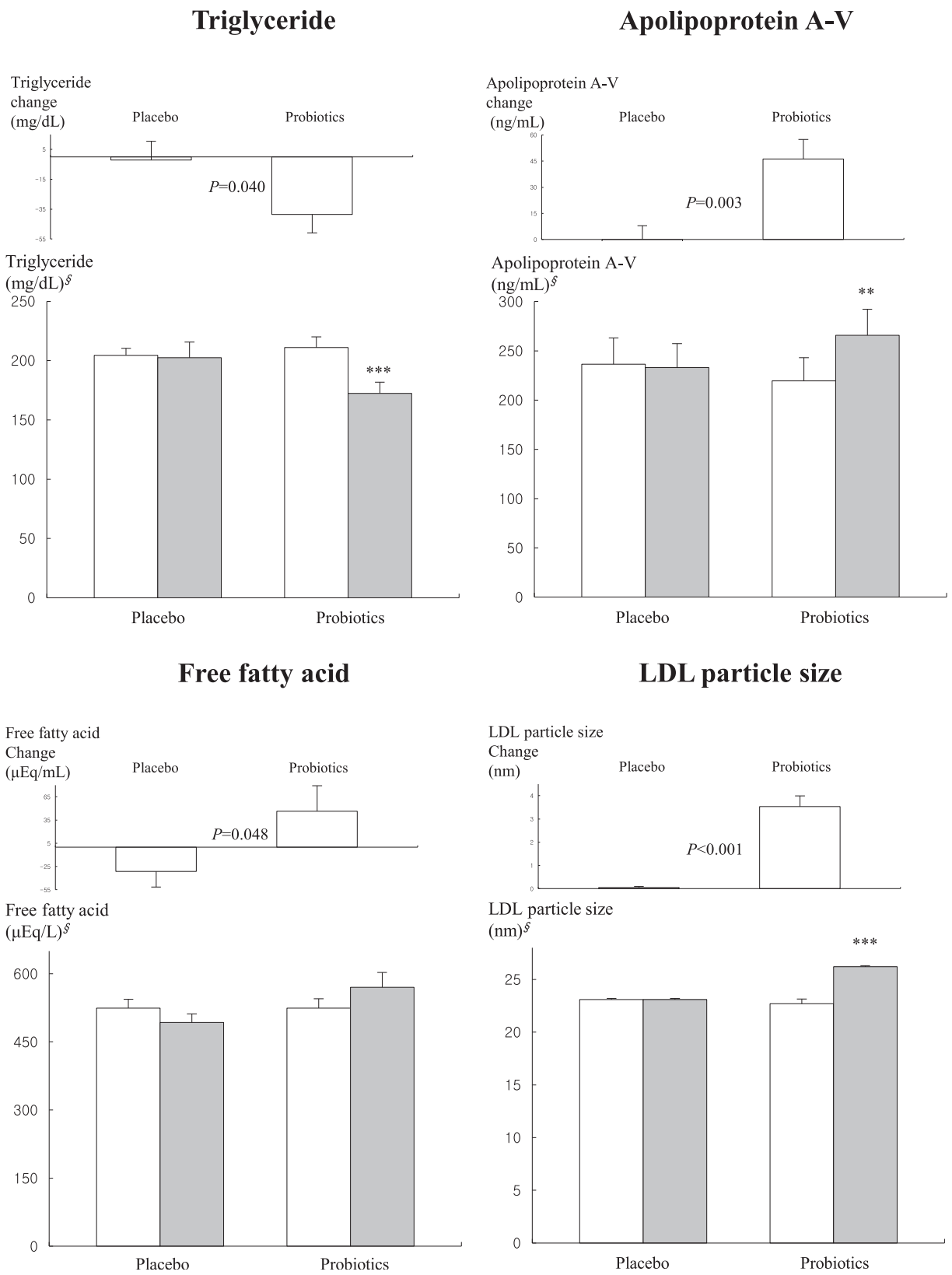

Fig. 1. The triglyceride, apolipoprotein A-V, fasting free fatty acid, and LDL particle size levels at the initial visit (□) and at the 12-week follow-up (■) the mean changes according to the treatment. Means ± SEM. The data included 63 (placebo) and 58 (probiotics) participants. §Tested by logarithmic transformation. *P*-values determined using an independent t-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with baseline values in each group as determined by the paired *t*-test.

-1131T > C between the placebo and the probiotic groups. The results show that the C allele carriers in the probiotic group had a greater reduction in serum TG (placebo: 5.95 ± 16.3 mg/dL; probiotics: -43.1 ± 16.9, $P = 0.040$) and greater increases in plasma apo A-V (placebo: -3.49 ± 16.2 ng/dL; probiotics: 48.6 ± 13.6, $P = 0.017$) and the LDL particle size (placebo: 0.01 ± 0.05 nm; probiotics: 3.85 ± 0.71, $P < 0.001$) than those in the placebo group *APOA5* -1131T > C genotypes.

3.3. Relationship between the changes in TG, apo A-V, and FFA

The correlations between the changes in the levels of TG, apo A-V, and FFA were determined after adjusting for the age, sex, and BMI differences between the placebo and probiotic groups (Fig. 2). In the probiotic group, the TG change (Δ) negatively correlated with the Δ in the apo A-V and ΔFFA, whereas ΔFFA positively correlated with Δapo A-V. Additionally, ΔTG negatively correlated with ΔHDL cholesterol in the placebo group ($r = -0.295$, $P = 0.019$). The probiotic group exhibited a ΔTG that negatively correlated with the baseline TG ($r = -0.611$, $P < 0.001$) and ΔHDL cholesterol ($r = -0.317$, $P = 0.015$), and the ΔFFA negatively correlated with the baseline FFA ($r = -0.351$, $P = 0.007$). Then, the cohort was subdivided according to the *APOA5* -1131T > C genotype. In the placebo group, ΔTG correlated positively with Δapo A-V in the individuals with the T/T genotype (Fig. 3). In the probiotic group, ΔTG negatively correlated with Δapo A-V and ΔFFA, and Δapo A-V positively correlated with ΔFFA, regardless of the genotype.

Because the regulation of circulating TG is complex, a multiple linear regression analysis was performed to determine the independent effects of the following variables on the changes in the TG levels in the placebo and probiotic groups: *APOA5* -1131T > C genotypes; age; BMI; baseline values of TG, HDL cholesterol, insulin, FFA, and apo A-V; and changes in HDL cholesterol, insulin, FFA, and apo A-V. Changes in the TG levels of the placebo group were affected by the BMI, baseline insulin, and ΔHDL ($r^2 = 0.344$, $P = 0.027$). The probiotic-mediated changes in the TG levels were affected by the age, baseline TG, baseline FFA, ΔHDL, and Δinsulin ($r^2 = 0.582$, $P < 0.001$).

4. Discussion

The lipid improvement effect of probiotics has raised interest in recent years. In a randomized, single blind, placebo-controlled and parallel study, a low-fat yogurt containing *Bifidobacterium longum* BL1 decreased the serum total-cholesterol, LDL-cholesterol and TGs in 42 subjects after 4 weeks [15]. A previous clinical study reported that a probiotic strain, *Enterococcus faecium* M−74, reduced the serum cholesterol level by 12% in 43 volunteers after 56 weeks [16]. Another clinical study demonstrated the hypocholesterolemic effect of a probiotic diet in humans, in which the administration of a milk containing *Lactobacillus acidophilus* was associated with the reduction of total cholesterol [17]. In the meta-analysis of 13 randomized controlled trials, the consumption of probiotics has beneficial effects on the total-cholesterol and LDL-cholesterol levels [18]. Most clinical trials regarding probiotics have focused on individuals suffering from hypercholesterolemia or hyperlipidemia. There have been few clinical studies on the efficacy of probiotics for TG control. A recent study showed that dual probiotic supplementation (*L. curvatus* HY7601 and *L. plantarum* KY1032) reduced the average plasma TG levels by 40% compared with placebo treatment in rats [10]. We hypothesized that this effect might be extrapolated to humans and that a clinical study of the effects of dual probiotic strains on hypertriglyceridemia would inform the understanding of the lipid-regulating mechanism of probiotics. To justify the TG-lowering effect of probiotics, we investigated the supplementation effect of dual probiotic strains containing *L. curvatus* HY7601 and *L. plantarum* KY1032 on TG-lowering efficacy in borderline and moderate hypertriglyceridemic subjects. Daily ingestion of 2 g of powder containing these two probiotic strains led to a significant reduction of 18% in the serum TG and significant increases of 21% and 15.6% in the plasma apo A-V levels and LDL particle size, respectively, in non-diabetic subjects with mild to moderate hypertriglyceridemia. When we compared the TG, apo A-V, and LDL particle size changes in the placebo and probiotic groups, the probiotic group had a greater reduction in serum TG and greater increases in the plasma apo A-V and LDL particle size.

Although many studies have demonstrated the beneficial effect of probiotics on the modulation of serum lipid levels [5–9], some studies have not observed any improvement in the blood lipid profiles [19,20]. The mechanism underlying the modulation of serum lipid profiles by probiotics remains largely obscure. According to Park et al. [10], 10^{10} CFU/d of probiotic treatment given for 3 weeks to rats with high fructose-induced metabolic syndrome led to transcriptional changes in peroxisome proliferator-activated receptor-α (PPAR-α), carnitine palmitoyltransferase 2 (CPT 2), sterol regulatory element-binding protein-1 (SREBP-1), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) expression, which resulted in a significant reduction in the plasma TG levels. Probiotics were found to down-regulate the mRNA and protein expression of PPAR-γ2, CCAAT/enhancer binding protein-α (C/EBPα), fatty acid synthase, and adipocyte-fatty acid binding protein in a cell culture system [21]. The significant increase we observed in the plasma apo A-V levels in the probiotic group might result from up-regulation of apo A-V or a close association between circulating TG and apo A-V, which is secreted primarily from the liver with HDL and is associated with VLDL [22,23]. The importance of apo A-V in the lipolysis regulation of plasma TG levels through its direct interaction with lipoprotein lipase has been found [11,24,25]. This previous finding could explain the negative correlation between the changes in serum TG with the changes in FFA and apo A-V and the positive correlation between the changes in apo A-V and FFA in the probiotic group of this study.

Some interindividual differences in the reduction of serum cholesterol as a result of probiotic ingestion has been attributed to the effect of genetic variation in apolipoprotein E [6]. Similarly, the variability of the *APOA5* -1131T > C genotypes have been associated with TG levels. A previous study showed that carriers of the C allele (TC or CC) of the *APOA5* -1131T > C had higher TG levels than the TT allele carriers [14]. The substantial interindividual differences in response to dietary treatment could be partly driven by genetic factors. Some earlier studies have shown that *APOA5* -1131T > C polymorphism is associated with dietary factors (particularly dietary fat) in the determination of the TG levels [26,27]. The *APOA5* -1131T > C gene variation has been shown to affect individual responses to dietary therapy in patients with impaired fasting glucose or type 2 diabetes [28,29]. However, a multiple linear regression analysis in this study showed that the probiotic-mediated changes in the TG levels were affected by the baseline levels of TG and FFA and not by the *APOA5* -1131T > C genotypes. This lack of relationship between the change in the TG level and the *APOA5* -1131T > C genotypes could be because of the different subjects (type 2 diabetes vs. non-diabetic hypertriglyceridemia), different intervention (dietary therapy vs. probiotic supplementation), or small sample size (probiotic group: n = 58) in this study. However, the strongly negative correlation between the change in the TG and baseline TG levels suggests that non-diabetic subjects with higher levels of TG might benefit from higher TG reductions regardless of the *APOA5* -1131T > C genotypes after treatment with dual probiotic strains containing *L. curvatus* HY7601 and

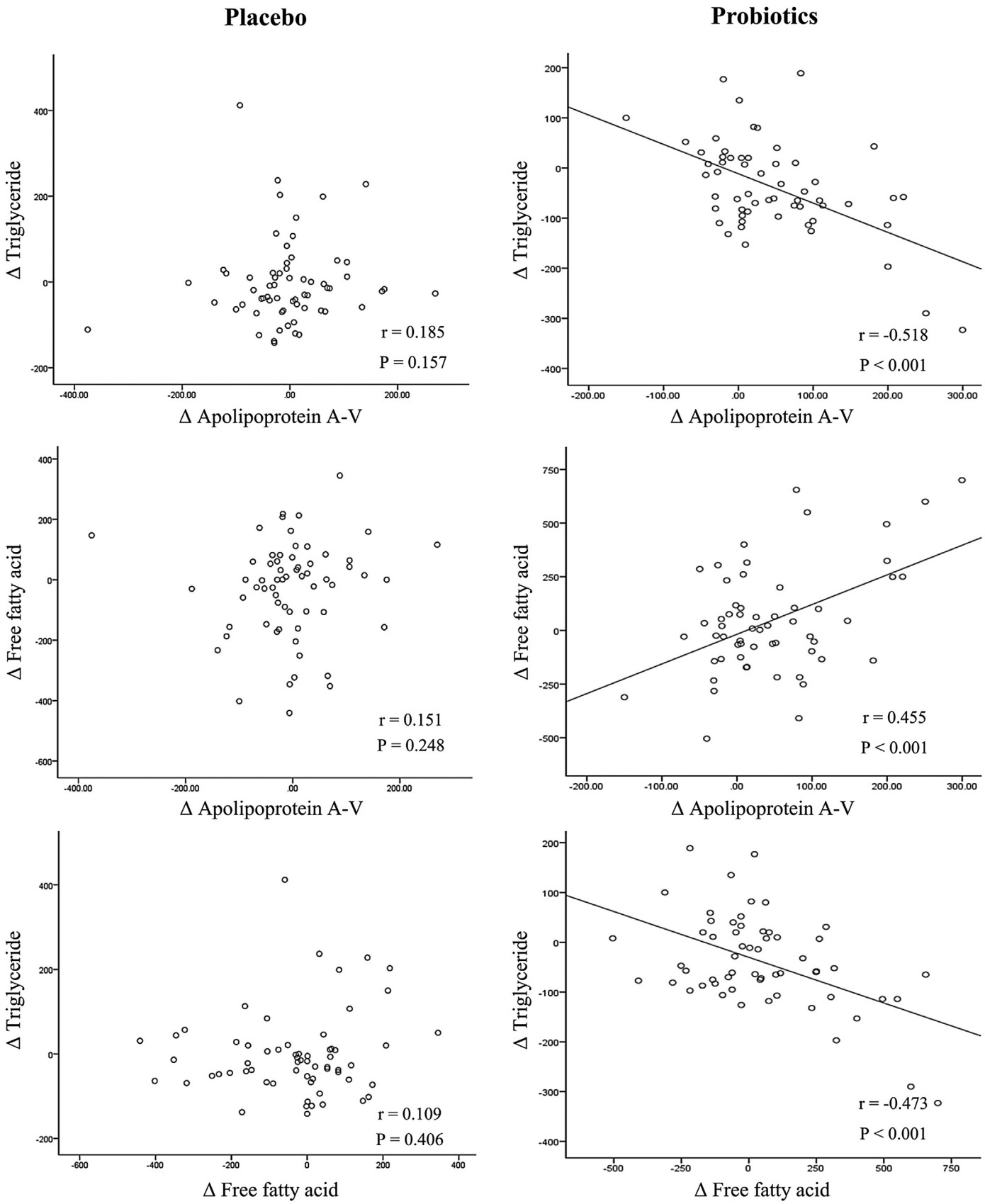

Fig. 2. The correlation between the changes (the difference from baseline) in the triglyceride, apolipoprotein A-V l, and fasting free fatty acids levels. *r*: Pearson's correlation coefficients; adjusting for age, sex, and BMI change.

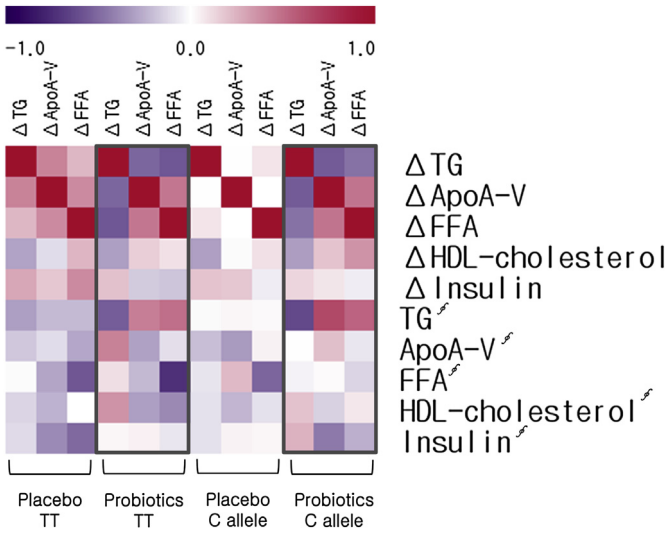

Fig. 3. The correlation matrix among the changes in the atherogenic risk factors in each group according to the *APOA5* −1131T/C genotype. $Tested by logarithmic transformation. The correlations were obtained by deriving a Spearman's correlation coefficient. *Red* and purple are positive and negative correlations, respectively.

L. plantarum KY1032.

Plasma levels of TG and HDL cholesterol are inversely related. A greater blood TG level is associated with a lower blood HDL-cholesterol level. An apo A-V deficiency might delay TG hydrolysis and reduce the availability of the surface components of TG-rich lipoproteins (which contribute to HDL-cholesterol formation), thereby leading to a decreased formation of HDL cholesterol [30]. Additionally, apo A-V-deficient mice have shown decreased lipoprotein lipase activity and the accumulation of larger VLDL particles [31], which are precursors of small, dense LDL, an independent predictor of coronary artery disease [32]. In this study, the probiotic group showed a significant increase in the apo A-V, a significant decrease in the TG, and changes in the HDL cholesterol and LDL particle size.

The LAP, which is calculated from the WC and fasting TG, has been proposed as an alternative measure of excessive lipid accumulation and as a better marker than BMI for identifying a risk for cardiovascular disease [13] and diabetes [33,34]. The LAP was created to describe the extent to which a subject had experienced an increase in waist and TG. In this study, the LAP decreased after the 12-week follow-up, and the change in the LAP tended to differ in the two groups ($P = 0.068$). This finding was consistent result with a change in the BMI ($P = 0.056$), which did not change significantly after probiotic supplementation. This study suggests that two probiotic strains, *L. curvatus* HY7601 and *L. plantarum* KY1032, reduced the TG level and increased the apo A-V level and LDL particle size in hypertriglyceridemic subjects, whereas the BMI and LAP did not have a conspicuous effect.

There are several limitations in our study design. First, the dietary intake was based on reports by the subjects and thus was not monitored for errors. However, other studies have shown that measurement errors from self-reported dietary intake and lifestyle variables are relatively small [35]. Second, because of the small sample size, the results of the genetic analyses should be interpreted with caution. Finally, we focused specifically on Korean non-diabetic subjects with mild to moderate hypertriglyceridemia subjects. Therefore, our data could not be generalized to other ethnic groups or patients with severe hypertriglyceridemia.

Despite these limitations, the data show that supplementation with two probiotic strains, *L. curvatus* HY7601 and *L. plantarum* KY1032, for 12 weeks in non-diabetic, mild to moderate hypertriglyceridemic subjects significantly reduced the serum TG levels, with the most robust effect observed in the subjects with the highest levels of fasting TG. Probiotic supplementation showed greater increases in the plasma apo A-V levels and LDL particle size than in the placebo group.

Conflict of interest

No potential conflicts of interest were disclosed.

Acknowledgments

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