Supplementation with two probiotic strains, *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032, reduced body adiposity and Lp-PLA₂ activity in overweight subjects

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ABSTRACT

The effect of consumption of two probiotic strains, *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032, on weight loss, body adiposity and lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activities in overweight subjects was examined. After 12-week probiotic treatment, the probiotic group presented reductions in body weight (−0.65 ± 0.23 kg), body fat percentage (−0.57 ± 0.19%) and body fat mass (−616 ± 161 g) measured using DEXA, and L₁ subcutaneous fat area (−2.68 ± 1.31 cm²) measured using CT, compared to baseline. The probiotic group exhibited greater reductions in oxidized low density lipoprotein (oxid-LDL) (P < 0.001) and Lp-PLA₂ (P < 0.001) and a greater increase in low density lipoprotein (LDL) particle size (P = 0.001) than the placebo. The change (△, difference from baseline) in total fat mass correlated with △Lp-PLA₂ (r = 0.480, P < 0.001), which correlated with △oxid-LDL (r = 0.537, P < 0.001). Probiotic-induced weight loss was associated with reductions in fat mass, which correlated with the changes in Lp-PLA₂ activities (ClinicalTrials.gov: NCT02492698).

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1. Introduction

It is well established that obesity is associated with low-grade inflammation, which is detected via increased levels of several inflammatory markers that might be reduced by losing weight. Probiotic supplementation with or without weight loss may protect against inflammation and adiposity (Zarrati et al., 2014). Especially many studies reported that several *Lactobacillus* species showed antiobesity effects in diet-induced
2. Materials and methods

2.1. Study subjects

Study subjects were recruited through an advertisement in Seoul. Based on data screened by the Clinical Nutrition Lab, Yonsei University, subjects who were nondiabetic (fasting blood glucose < 126 mg/dL and 2-hour blood glucose < 200 mg/dL) and overweight (25 kg/m² ≤ BMI < 30 kg/m²) were referred to the Department of Family Medicine, Yonsei University Severance Hospital. Subjects were rechecked for general health, and blood tests, including serum glucose, were performed. Subjects who satisfied the study criteria were recommended to participate in the study. Inclusion criteria were age between 20 and 65 years, nondiabetic, and overweight. Exclusion criteria were constant consumption of any probiotics product within the month before screening; unstable body weight (body-weight change > 1 kg within 3 months before screening); hypertension; type 2 diabetes; cardiovascular disease; cerebrovascular disease; thyroid disease; dietary supplementation within 6 months before screening; pregnancy or breast-feeding; and consumption of medication that affects body weight, energy expenditure, glucose control or antibiotic treatment within 3 months before screening. Further exclusion criteria were acute or chronic infections, liver disease, kidney disease, gastrointestinal disease, or any other acute or chronic disease requiring treatment. Finally, those who consented to the program were included in this study. Written informed consent was obtained from all participants, and the protocol was approved by the institutional review board (IRB) of Yonsei University and Yonsei University Severance Hospital according to the Helsinki Declaration (1040917-201405-BR-167-04).

2.2. Study design and intervention

A 12-week, double-blind, placebo-controlled, randomized study was conducted on 120 nondiabetic and overweight subjects divided into two groups: a probiotic group [individuals (n = 60) who consumed 2 g of powder of two probiotic strains, L. curvatus HY7601 and L. plantarum KY1032, each at 2.5 × 10⁹ cfu, twice a day (immediately after breakfast and dinner)] and a placebo group [individuals (n = 60) who consumed the same amount of powder that did not contain any probiotics]. The 2 g of probiotic powder contained 0.1 g of L. curvatus HY7601, 0.1 g of L. plantarum KY1032, 1.24 g of crystalline cellulose, 0.5 g of lactose, and 0.06 g of blueberry-flavouring agent. The 2 g of placebo powder contained 1.34 g of crystalline cellulose, 0.6 g of lactose, and 0.06 g of blueberry-flavouring agent. The products were provided by Korea Yakult Co., Ltd. (Yongin, Gyeonggi, Korea). The randomization was performed via computer-generated block randomization (placebo: test = 1:1, 60 individuals respectively). The study was divided into two periods: pre-ingestion, during which nondiabetic and overweight subjects did not ingest probiotic or placebo products for 2 weeks, and the ingestion period, during which subjects ingested the probiotic or placebo product for 12 weeks.

2.3. Daily energy intake and physical activity measurements

The subjects were instructed to maintain their eating habits and physical activity during the study period (pre-ingestion – 2 weeks; ingestion period – 12 weeks) to ensure that any body weight fluctuations were not due to diet or physical activity. A standardized 3-day dietary record (2 weekdays and 1 weekend day) was obtained from each participant. This record was completed at home after the participants received detailed explanations from a dietician. A computerized version of the Korean Nutrition File (Can-Pro 3.0; The Korean Nutrition Society,
Seoul, Korea) was used to determine the macronutrient content of foods and total daily energy intake. This measurement was repeated at weeks 6 and 12. A standardized 3-day physical activity record was also completed at home on the same days that the dietary record was completed. In addition, the participants completed a 24-item dietary recall with the assistance of the dietician at weeks 0, 6 and 12.

2.4. Anthropometric parameters and blood pressure

Body weight (in light clothes and without shoes) (UM0703581; Tanita, Tokyo, Japan) and height (GL-150; G-tech International, Uijeongbu, Korea) were measured in the morning, and BMI was calculated in units of kilograms per square meter (kg/m²). Waist circumference (directly on the skin) was measured at the umbilical level after normal expiration with the subject in an upright standing posture using a plastic measuring tape with measurements to the nearest 0.1 cm. Anthropometric parameters were assessed at screening, baseline and week 12. During each testing session, systolic and diastolic blood pressures (BPs) were assessed in the supine position after a resting period (20 min). The BPs were measured twice on the left arm with an automatic BP monitor (FT-200S; Jawon Medical, Gyeongsan, Korea), and we used the average of the two measurements.

2.5. Abdominal fat area and body composition measurements

Body fat distribution and muscle areas were measured via computed tomography (CT) scanning using a General Electric (GE) Medical System HiSpeed Advantage® (Milwaukee, WI, USA). Two cross-sectional images were made for each subject: the abdomen at the levels of 1st lumbar (L1) and 4th lumbar (L4) vertebrae. The scanning parameters were a slice thickness of 1 mm, 200 mAs, 120 kVp, and 48-cm field of view. The body composition of the study participants was measured via dual-energy X-ray absorptiometry (DEXA) (Discovery A; Hologic, Bedford, MA, USA) at baseline and week 12 to determine fat percentage, fat mass and lean body mass. The resulting data were analyzed with volume integration software (APEX 4.0.2 (13.4.1); Hologic, Bedford, MA, USA).

2.6. Blood collection and serum lipid profile

Blood samples were collected at approximately 8 a.m. following an overnight fast of at least 12 hours. Venous blood specimens were collected in EDTA-treated whole-blood tubes and serum tubes (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The blood samples were centrifuged to obtain plasma and serum. The collected samples were stored at −70 °C. Fasting triacylglycerol and total cholesterol were measured via enzymatic assay using Pureateau S TG-N and Pureateau S CHO-N kits (Daichii, Tokyo, Japan), respectively. High density lipoprotein (HDL) cholesterol was measured via selective inhibition using Cholestat N-HDL kits (Daichii). The resulting colour reaction was monitored with an ADVIA 2400 (Siemens, Tarrytown, NY, USA). LDL cholesterol was indirectly calculated using the Friedewald formula: LDL cholesterol = total cholesterol – [HDL cholesterol + (triaclyglycerol/5)].

2.7. Serum fasting glucose, insulin and hs-CRP

Fasting serum glucose was measured via the hexokinase method with glucose kits (Siemens). Serum insulin was measured via an immunoradiometric assay using kits (Diasource ImmunoAssays S.A., Louvain-la-Neuve, Belgium). Serum high-sensitivity C-reactive protein (hs-CRP) was measured using CRP-Latex(II) X2 kits (Denka-Seiken, Tokyo, Japan). The resulting colour reaction was monitored with an ADVIA 2400 (Siemens) for fasting glucose and hs-CRP and SR-300 (Stratec, Birkenfeld, Germany) for insulin.

2.8. Plasma Lp-PLA₂, activity, ox-LDL and LDL particle size

The activity of Lp-PLA₂ was measured via a high-throughput radiometric activity assay using MicroBeta (PerkinElmer, Shelton, CT, USA). Plasma oxidized low density lipoprotein (ox-LDL) was measured using an enzyme immunoassay (Mercodia AB, Uppsala, Sweden). The resulting colour reaction was monitored at 450 nm with a Wallac 1420 Victor² multilabel counter (PerkinElmer Life Sciences, Boston, MA, USA). To measure the LDL particle size, LDL particles were isolated using sequential flotation ultracentrifugation, and the particle size distribution (1.019–1.063 g/mL) was examined on non-denaturing polyacrylamide gels containing a linear gradient of 2–16% acrylamide (CBS Scientific, San Diego, CA, USA) using a pore gradient lipoprotein system (CBS Scientific). 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, Hercules, CA, USA).

2.9. Statistical analysis

Statistical analysis was performed with the SPSS version 21.0 software package (IBM/SPSS, Chicago, IL, USA). Logarithmic transformation was performed on skewed variables. Independent t-tests were used to compare parameters between the placebo and probiotics groups. Paired t-tests were used to compare parameters between the baseline and 12 week follow-up. A general linear model test was applied to adjust for potential confounding factors. Pearson’s correlation coefficient was used to examine the relationships between variables. The results are expressed as the mean ± standard error (SE). A P-value <0.05 was considered statistically significant.

3. Results

3.1. Effects of 12-week consumption of probiotics on anthropometric parameters

After 12 weeks, 25 of 120 subjects (14 placebo and 11 probiotic) dropped out (one participant no longer satisfied the screening criteria (placebo); two participants developed severe colds that required antibiotics (placebo); and eight participants wanted to drop out voluntarily (4 placebo and 4 probiotic); seven participants denied CT, DEXA or anthropometric measurements
(4 placebo and 3 probiotic); and seven participants failed to follow their habitual eating or physical activity patterns (3 placebo and 4 probiotic). No adverse events were reported as a dropout reason. Table 1 presents anthropometric parameters and abdominal fat areas at baseline and 12 weeks for the placebo and probiotic groups. At baseline, there were no significant differences between the two groups in terms of age, gender distribution, smoking and drinking, body weight, BMI, or waist circumference. The estimated total calorie intake, physical activity, protein intake percentage, fat intake percentage, and carbohydrate intake percentage were not significantly different between the two groups at baseline, week 6 and week 12 (data not shown). After 12 weeks of treatment, individuals in the probiotic group exhibited reductions in body weight ($P = 0.003$) and total fat ($P = 0.003$) at the L1 level were observed in the placebo group; in contrast, a significant reduction in subcutaneous fat was observed at the L1 level in the probiotic group ($P = 0.047$). There were significant differences in the changes in the L1 subcutaneous fat ($P = 0.009$), L1 total fat ($P = 0.033$), L4 subcutaneous fat ($P = 0.029$), and L4 total fat ($P = 0.008$) areas between the two groups, after adjusting for the baseline. At 12 weeks, the L4 total fat area in the probiotic group showed a trend to be lower than in the control group ($P = 0.060$) (Table 1).

### 3.3 Changes in body composition measured using DEXA in response to probiotics

At baseline, there were no significant differences between the two groups in terms of fat percentage, fat mass and lean body mass measured using DEXA (Fig. 1). The total fat mass decreased by 616 g from baseline in the probiotic group ($P < 0.001$), and the fat percentage decreased by 0.57% from baseline ($P = 0.005$). The reductions in total fat mass (225 ± 160 vs. −616 ± 161 g; $P < 0.001$) and fat percentage (0.09 ± 0.17 vs. −0.57 ± 0.19%; $P = 0.012$) were greater in the probiotic group than in the control. At 12 weeks, the total fat mass was lower ($P = 0.046$) in the probiotic group compared with the control. Although reductions in total fat mass and fat percentage were observed in the probiotic group, the total lean body mass did

### Table 1 – Effects of 12-week consumption of probiotics on anthropometric parameters and abdominal fat areas.

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 95)</th>
<th>Placebo group (n = 46)</th>
<th>Probiotic group (n = 49)</th>
<th>$P^a$</th>
<th>$P^b$</th>
<th>$P^c$</th>
<th>$P^d$</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td></td>
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<tr>
<td>Age (year)</td>
<td>37.8 ± 1.63</td>
<td>40.1 ± 1.48</td>
<td>0.294</td>
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<tr>
<td>Male/Female, n, (%)</td>
<td>16 (34.8)/30 (65.2)</td>
<td>18 (36.7)/31 (63.3)</td>
<td>0.843</td>
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<tr>
<td>Cigarette smoker, n, %</td>
<td>5 (10.9)</td>
<td>5 (10.2)</td>
<td>0.916</td>
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<tr>
<td>Alcohol drinker, n, %</td>
<td>33 (71.7)</td>
<td>30 (61.2)</td>
<td>0.279</td>
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<tr>
<td>Weight (kg)</td>
<td>73.5 ± 1.42</td>
<td>73.5 ± 1.28</td>
<td>0.845 ± 0.01</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>Change</td>
<td>0.42 ± 0.21</td>
<td>−0.65 ± 0.23</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 0.22</td>
<td>27.0 ± 0.21</td>
<td>0.845 ± 0.01</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>Change</td>
<td>0.14 ± 0.07</td>
<td>−0.24 ± 0.09</td>
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<tr>
<td>Waist (cm)</td>
<td>91.1 ± 0.86</td>
<td>90.1 ± 0.77</td>
<td>0.349 ± 0.19</td>
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<tr>
<td>CT evaluation (L1)</td>
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<tr>
<td>Visceral fat area (cm²)</td>
<td>102.4 ± 6.40</td>
<td>113.9 ± 4.75</td>
<td>0.149 ± 0.36</td>
<td>0.187</td>
<td>0.334</td>
<td></td>
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<tr>
<td>Change</td>
<td>7.26 ± 2.30</td>
<td>2.67 ± 2.55</td>
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<td></td>
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<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>137.4 ± 5.86</td>
<td>132.4 ± 4.90</td>
<td>0.507 ± 0.14</td>
<td>0.009</td>
<td>0.005</td>
<td></td>
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<tr>
<td>Change</td>
<td>3.29 ± 1.82</td>
<td>−2.68 ± 1.31</td>
<td></td>
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<td></td>
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<tr>
<td>Total fat area (cm²)</td>
<td>239.9 ± 8.58</td>
<td>246.3 ± 6.15</td>
<td>0.540 ± 0.67</td>
<td>0.026</td>
<td>0.033</td>
<td></td>
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<tr>
<td>Change</td>
<td>10.6 ± 3.33</td>
<td>−0.01 ± 3.27</td>
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<tr>
<td>CT evaluation (L4)</td>
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<tr>
<td>Visceral fat area (cm²)</td>
<td>88.1 ± 4.69</td>
<td>87.3 ± 4.12</td>
<td>0.902 ± 0.49</td>
<td>0.316</td>
<td>0.255</td>
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<tr>
<td>Change</td>
<td>1.53 ± 1.84</td>
<td>−1.64 ± 2.51</td>
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<td></td>
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<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>217.9 ± 7.31</td>
<td>213.6 ± 6.09</td>
<td>0.650 ± 0.17</td>
<td>0.053</td>
<td>0.029</td>
<td></td>
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<tr>
<td>Change</td>
<td>4.45 ± 3.50</td>
<td>−3.66 ± 2.30</td>
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<tr>
<td>Total fat area (cm²)</td>
<td>306.0 ± 7.11</td>
<td>295.6 ± 5.69</td>
<td>0.579 ± 0.06</td>
<td>0.023</td>
<td>0.008</td>
<td></td>
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<tr>
<td>Change</td>
<td>5.98 ± 3.68</td>
<td>−5.31 ± 3.23</td>
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</tbody>
</table>

The data points correspond to the mean ± SE. 
$P^a$ values derived from independent t-tests in baseline. 
$P^b$ values derived from independent t-tests in follow-up. 
$P^c$ values derived from independent t-tests in changed value. 
$P^d$ values are adjusted for baseline. 
* $P < 0.05$, **$P < 0.01$ derived from paired t-tests.
not significantly differ in response to probiotic treatment. No statistically significant differences in the total fat mass, body fat percentage and lean body mass were observed for the placebo treatment (Fig. 1).

### 3.4. Responses of the serum lipid profiles, Lp-PLA₂ activity, ox-LDL and LDL particle size to probiotics

Table 2 presents the BP, serum lipid profiles, glucose, insulin and hs-CRP at baseline and 12 weeks for the placebo and probiotic groups. There were no significant differences at baseline and no significant mean changes in the systolic and diastolic BPs; triacylglycerol; total, HDL and LDL cholesterol; glucose; insulin; and hs-CRP in the placebo and probiotic groups after 12 weeks of treatment (Table 2). No significant differences in Lp-PLA₂ activity, ox-LDL, and LDL particle size between the control and probiotic groups were observed at baseline (Fig. 2). After 12 weeks of treatment, significant increases in the Lp-PLA₂ activity (P = 0.001) and ox-LDL (P < 0.001) and a reduction in the LDL particle size (P = 0.023) were observed in the placebo group; in contrast, significant decreases in the Lp-PLA₂ activity (P = 0.034) and ox-LDL (P = 0.003) and an increase in the LDL particle size (P = 0.009) were observed in the probiotic group. When we compared the Lp-PLA₂ activity, ox-LDL and LDL particle size changes between the placebo and probiotic groups, the probiotic group had greater reductions in the Lp-PLA₂ activity (P < 0.001) and ox-LDL (P < 0.001) and a greater increase in the LDL particle size (P = 0.001) than the placebo group.

### Table 2 - Effects of 12-week consumption of probiotics on blood pressure and biochemical parameters.

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 95)</th>
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<tbody>
<tr>
<td></td>
<td>Placebo group (n = 46)</td>
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<tr>
<td>Systolic BP (mmHg)</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
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<tr>
<td>Triacylglycerol (mg/dL)</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
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<tr>
<td>HDL-cholesterol (mg/dL)</td>
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<tr>
<td>LDL-cholesterol (mg/dL)</td>
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<tr>
<td>Glucose (mg/dL)</td>
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<tr>
<td>Insulin (μIU/dL)</td>
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<td>hs-CRP (mg/dL)</td>
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</table>

The data points correspond to the mean ± SE.

* Tested by logarithmic transformation.

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[Fig. 1 – Fat percentage, fat mass, and lean body mass measured via DEXA at baseline (□) and at 12-weeks’ follow-up (■) and mean changes according to treatment. The data points correspond to the mean ± SE. The data include 46 placebo group and 49 probiotic group participants. P'-values are derived from independent t-tests for changed values. P''-values are adjusted for baseline. **P < 0.01, ***P < 0.001 compared with baseline values in each group by paired t-tests. †P < 0.05 derived from independent t-test in follow-up.]

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3.5. Relationship between changes in total body fat mass and changes in body weight, abdominal fat areas and biochemical parameters

Correlations between the changes in body weight, body adiposity and biochemical markers were determined after adjusting for age and gender in all subjects (n = 95). The change (Δ) in total fat mass measured using DEXA positively correlated with Δ body weight (r = 0.601, P < 0.001), Δ waist circumference (r = 0.274, P = 0.009), Δ total body fat percentage (r = 0.916, P < 0.001), Δ L1 visceral fat area (r = 0.302, P = 0.004), Δ L1 subcutaneous fat area (r = 0.532, P < 0.001), Δ L1 total fat area (r = 0.476, P < 0.001), Δ L4 subcutaneous fat area (r = 0.374, P < 0.001), Δ L4 total fat area (r = 0.367, P < 0.001), Δ L-PPLA2 activity (r = 0.480, P < 0.001) and Δ ox-LDL (r = 0.266, P = 0.011). Additionally, Δ L-PPLA2 activity positively correlated with Δ body weight (r = 0.239, P = 0.023), Δ total body fat percentage (r = 0.472, P < 0.001), Δ L1 visceral fat area (r = 0.239, P = 0.023), Δ L1 total fat area (r = 0.270, P = 0.010), and Δ ox-LDL (r = 0.537, P < 0.001).

4. Discussion

There are several possible mechanisms for the regulation of body adiposity by probiotics. A possible effect of reduction in food intake has been reported (Bjerg et al., 2014; Yun, Park, & Kang, 2009). However, this possibility cannot account for the present results because the estimated daily calorie intake did not differ between the placebo and probiotic groups. Thus, the greater reduction in body fat composition observed in the probiotic group could result from a direct effect of the two probiotic strains or their metabolites on adipose tissue. Indeed, Park et al. (2011) reported that L. plantarum KY1032 cell extracts can directly reduce fat mass by modulating adipogenesis in maturing preadipocytes. Additionally, a recent study reported an adverse effect of two probiotic strains, L. curvatus HY7601 and L. plantarum KY1032, on adipose tissue fat accumulation in diet-induced obese mice (Yoo et al., 2013).

Another possible mechanism for the regulation of body adiposity by probiotics is modulation of gut microbial composition by probiotic consumption (Bäckhed et al., 2004; da Silva, dos Santos, & Bressan, 2013; Omar, Chana, Jonesb, Prakashb, & Jonesa, 2013). For example, Kadooka et al. (2010) reported that 12-week supplementation with milk fermented by Lactobacillus gasseri SBT2055 in overweight men and women induced significant weight loss (approximately 1 kg) and decreased abdominal visceral and subcutaneous fat mass under ad libitum conditions; these effects may be linked to decreased fat absorption. Additionally, a recent placebo-controlled, double-blind, cross-over clinical study demonstrated that the consumption of two yoghurts per day supplemented with Lactobacillus amylovorus (10^9 cfu/yoghurt) led to a decrease in total fat mass measured using DEXA, and this decreased body fat mass resulted from modulation of gut microbial composition (Omar et al., 2013). Furthermore, Yoo et al. (2013) recently reported that L. curvatus HY7601 and L. plantarum KY1032 can metabolize a broad range of carbohydrates in diet-induced obese mice and thus may increase competition for nutrients and reduce microbial diversity within the gut microbial community. Therefore, the result of the present study could partly support the view that modulation of intestinal microbes by probiotics is a novel approach for the prevention of obesity (Bäckhed et al., 2004; S. T. da Silva et al., 2013; Sanchez et al., 2014).
According to the findings of Park, Ahn, Huh et al. (2013), dual probiotic treatment with L. curvatus HY7601 and L. plantarum KY1032 isolated from Korean fermented kimchi at 10^{10} cfu/day or 10^{7} cfu/day for 3 weeks reversed the oxidative stress present in high-fructose-diet-fed rats with metabolic syndrome. In this study, we also observed a significant decrease in plasma ox-LDL, a marker of oxidative stress (Chae et al., 2011), after 12 week supplementation with a combination of L. curvatus HY7601 and L. plantarum KY1032 delivered in 2 g of powder to nondiabetic, overweight subjects, in contrast with an increase in the placebo group. Some Lactobacillus strains are reported to possess anti-oxidative activity. For example, a probiotic mix decreased plasma ox-LDL in hyperlipidaemic hamsters (Stancu, Sandra, Deleanu, & Sima, 2014). Furthermore, probiotics have been reported to reduce exercise-induced oxidative stress via increases in antioxidant activity, which helps neutralize reactive oxygen species (Martarelli et al., 2011).

Most of circulating Lp-PLA2 (approximately 80%) binds to LDL-cholesterol. Before oxidation and transformation of LDL, Lp-PLA2 is attached to LDL in a dormant state (Lerman & McConnell, 2008). This enzyme is solely responsible for the hydrolysis of oxidized phospholipid (oxPL) on the LDL particle. As the oxPL surface of LDL particles becomes oxidized, Lp-PLA2 begins to hydrolyze oxPL, thereby forming 2 molecular triggers of the inflammation cascade: oxidized fatty acids and lysophosphatidylcholine. The correlations between Lp-PLA2 activity and ox-LDL are more significant in overweight/obese subjects than in normal-weight subjects (Paik et al., 2015). In the present study, there were strong positive correlations between changes in Lp-PLA2 activity and total body fat percentage (r = 0.472, P < 0.001) and between changes in Lp-PLA2 activity and ox-LDL (r = 0.537, P < 0.001). Additionally, the change in total fat mass measured using DEXA positively correlated with the changes in Lp-PLA2 activity and ox-LDL.

Fat accumulation plays an important role in LDL atherogenicity, including increased LDL oxidation and small LDL particle size (Paik et al., 2012, 2013). Small dense LDL is preferentially associated with Lp-PLA2. For example, Gazi et al. (2005) reported that 1 in 100 small/dense LDL particles are associated with the Lp-PLA2 enzyme, but only 1 in 4000 large LDL particles are associated with the enzyme. In this study, the probiotic group exhibited significant reductions in Lp-PLA2 activity and ox-LDL and an increase in the LDL particle size, in contrast with the placebo group. Indeed, a recent study of non-diabetic subjects with borderline-to-moderate hypertriglyceridaemia indicated an increase in the LDL particle size after 12-week supplementation with L. curvatus HY7601 and L. plantarum KY1032 (Ahn et al., 2015). These results provide support for the antioxidative and anti-inflammatory effects of probiotic supplementation in overweight subjects.

The placebo group of this study showed significant increases in Lp-PLA2 activity and ox-LDL, and a decrease in LDL particle size. There are some plausible reasons to support these results. First, it may result from increase of fat area. In the present study, visceral fat area and total fat area at the L1 level were significantly increased after 12-weeks’ supplementation, and changes in subcutaneous fat area and total fat area at the L1 level were significantly different between two groups. Several studies reported that fat accumulation increases LDL oxidation and small LDL particle size (Paik et al., 2012, 2013), which are related with Lp-PLA2 activity. Paik et al. (2015) demonstrated that there was positive correlation between Lp-PLA2 activity and ox-LDL in overweight/obese postmenopausal subjects. Furthermore, they showed that LDL particle size was significantly smaller in the overweight/obese postmenopausal group than in the normal body weight postmenopausal group. Second, our study subjects were overweight individuals (25 kg/m² ≤ BMI < 30 kg/m²). Recent studies demonstrated associations between overweight/obese and Lp-PLA2 activity. Paik et al. (2015) indicated that Lp-PLA2 activity in overweight/obese postmenopausal group was higher than in the normal body weight postmenopausal group. da Silva, Timm Ade, and Damasceno (2013) found that if fat mass is higher, Lp-PLA2 activity is higher as well. The high Lp-PLA2 activity is related with ox-LDL and LDL particle size, in this reason, the present study showed higher Lp-PLA2 activity and ox-LDL, and smaller LDL particle size after 12 weeks in the placebo group. Lastly, the placebo group was not affected by beneficial effects of the probiotic supplement; thus, probiotic-induced body weight and fat reduction, anti-oxidative stress and anti-inflammatory effects were not found in the placebo group.

There are several limitations to our study design. First, the dietary intake was based on self-reports obtained from weighed food. However, measurement errors from self-reported dietary intake and lifestyle variables have been demonstrated to be relatively small (Rimm et al., 1992). Second, we specifically focused on Korean nondiabetic and overweight subjects. Therefore, our data cannot be generalized to other ethnic groups or severely obese subjects. Despite these limitations, compared with the placebo group, supplementation with two probiotic strains, L. curvatus HY7601 and L. plantarum KY1032, for 12 weeks in nondiabetic, overweight subjects led to a slight but significant reduction in body weight. Probiotic-induced weight loss was associated with reductions in body fat mass measured using DEXA, which positively correlated with changes in ox-LDL and Lp-PLA2 activities. These results suggest a beneficial effect of supplementation with L. curvatus HY7601 and L. plantarum KY1032 on body weight, body fat, and oxidative and inflammatory stresses in overweight subjects.

5. Conclusions

A 12-week supplementation with two probiotic strains, L. curvatus HY7601 and L. plantarum KY1032, reduced body weight, body fat percentage, and body fat mass and L1 subcutaneous fat area in overweight subjects. The probiotic group exhibited greater reductions in ox-LDL and Lp-PLA2 and a greater increase in LDL particle size than the placebo group. The change in total fat mass correlated with the change in Lp-PLA2, which strongly correlated with the change in ox-LDL. This study determined that probiotic-induced weight loss was associated with reductions in fat mass and decreased oxidative and inflammatory stresses in overweight subjects.

Conflict of interest

The authors declare that no conflicts of interest exist.
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