Effect of \textit{Lactobacillus Plantarum} TWK10 on Improving Endurance Performance in Humans

Wen-Ching Huang\textsuperscript{1}, Yi-Ju Hsu\textsuperscript{2,\#}, HuaShuai Li\textsuperscript{1,\#}, Nai-Wen Kan\textsuperscript{3}, Yi-Ming Chen\textsuperscript{2}, Jin-Seng Lin\textsuperscript{3}, Tien-Ken Hsu\textsuperscript{4}, Tsung-Yu Tsai\textsuperscript{5}, Yen-Shuo Chiu\textsuperscript{6}, and Chi-Chang Huang\textsuperscript{2,7}

\textsuperscript{1}Department of Exercise and Health Science, National Taipei University of Nursing and Health Sciences, Taipei 11219
\textsuperscript{2}Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan 33301
\textsuperscript{3}Center for Liberal Arts, Taipei Medical University, Taipei 11031
\textsuperscript{4}Culture Collection & Research Institute, Synbio Tech Inc., Kaohsiung 82151
\textsuperscript{5}Department of Food Science, Fu Jen Catholic University, New Taipei City 24205
\textsuperscript{6}Department of Orthopedic Surgery, Taipei Medical University-Shuang Ho Hospital, New Taipei City 23561
and
\textsuperscript{7}Graduate Institute of Metabolism and Obesity Sciences, Taipei Medical University, Taipei 11031, Taiwan, ROC

Abstract

Microbiota is currently an important issue in disease and health and many studies have revealed it to play an important role in physiological homeostasis and health promotion. \textit{Lactobacillus plantarum} (\textit{L. plantarum}), isolated from Taiwan pickled vegetables, is a well-known probiotic microorganism. In a recent animal study, it was shown that supplementation of mice with \textit{L. plantarum} TWK10 (TWK10) could increase muscle mass, improve exercise performance and exert anti-fatigue effects. In order to examine the ergogenic effect of TWK10 supplementation on endurance performance in humans, we conducted a human double-blind placebo-controlled clinical study. A total of sixteen adult subjects over 20 years of age were recruited and randomly allocated to the placebo or TWK10 group (n = 8 each). The TWK10 group received 6 weeks of supplementation. Physiological assessments were conducted by exhaustive treadmill exercise measurements and related biochemical indexes. After 6 weeks of supplementation, levels of lactic acid, blood ammonia, blood glucose, free fatty acid (FFA) and creatine kinase (CK) were evaluated during exhaustive exercise. We were able to show that the TWK10 group had significantly higher endurance performance and glucose content in a maximal treadmill running test compared to the placebo group (\textit{P} < 0.05), suggesting that TWK10 supplementation may be beneficial to energy harvest. Taken together, our results suggest that TWK10 has the potential to be an aerobic exercise supplement for physiological adaptation or an ergogenic supplement with health benefits for amateur runners.

Key Words: anti-fatigue, exercise performance, lactic acid bacteria, microbiota
Introduction

Intestinal microbiota is gradually established, starting at birth, and is influenced by different factors including environment, genetics, diet, lifestyle and medication (22). The gastrointestinal (GI) tract has the most complicated ecosystem in the body because it is the natural habitat of some 10-100 trillion microorganisms, including Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, Fusobacteria, Cyanobacteria and Tenericutes (8). The microbiota is functionally considered to be like that of an invisible, but essential organ, in a symbiotic relationship with the host. It plays an important role in health maintenance and promotion (14, 17), affecting human health and disease processes such as inflammation, infections, neurodegenerative disease and tumors (10). Humans may be considered like superorganisms whose metabolism is a result of an amalgamation of the microbiome and human genome that have coevolved with physiological and metabolic adaptation (12).

Besides looking at diet, many studies have focused on the relationship between the microbiota and other lifestyle factors such as exercise (18). Recent studies have suggested that exercise can enhance/change the number or proportion of beneficial microbial and that the microbiota is responsive to the homeostatic and physiological variations from exercise (16). A previous study has demonstrated that exercise intervention could increase the diversity of Lactobacillales (5). Similarly, other studies have also shown the increase in the diversity of Lactobacillus, Blautia coccoides, and Eubacterium rectale (20) with exercise. Exercise training not only increased the number of Lactobacillus, but also showed significant correlation with biochemistry and specific microbiota (19). Estaki et al. proposed that exercise could be used as a therapeutic support in the treatment of dysbiosis-associated diseases (11), determining changes in the gut microbial composition and playing a positive role in energy homeostasis and regulation (16).

We found that most studies to date have focused on microbiota profiles caused by exercise, but few studies have investigated the relationship of the microbiota and exercise performance from the point of view of an ergogenic aid. In our previous animal study, we found that Lactobacillus plantarum (L. plantarum) TWK10 (prepared at a laboratory scale) could increase exercise performance and change the gut microbial profile for better physiological adaption (4). In the current study, the L. plantarum TWK10 used was manufactured by factory fermentation and with strain identification. Herein, we investigate the effects of supplementation of this formula on exercise performance and physiology for future applications.

Materials and Methods

Probiotics

L. plantarum TWK10 is a kind of plant Lactobacillus isolated from Taiwan pickled vegetables (4). The L. plantarum TWK10 was cultivated and produced by Synbio Tech Inc. into capsules. Each TWK10 capsule contained $1 \times 10^{11}$ CFU L. plantarum TWK10, maltodextrin, deproteinized permeate whey powder, lactose, and microcrystalline cellulose. The placebo capsule contained the same ingredients without L. plantarum TWK10. The recommended dosage for humans is one capsule per day after a meal.

Subject

The test subjects were 16 healthy male adults between 20–40 years old without professional athletic training. Subjects were excluded from this study if they had any known metabolic disorders including heart/cardiopulmonary disease, diabetes, thyroid disease, hypogonadism, hepatorenal disease, musculoskeletal disorder, neuromuscular/neurological disease, autoimmune disease, cancer, peptic ulcers or anemia. All subjects provided written informed consent before participation. The study was reviewed and approved by the Joint Institutional Review Board (IRB) of Taipei Medical Hospital (Taipei, Taiwan; TMU-JIRB no. 201302017). Subjects were asked to maintain their normal diet and not to consume any other nutritional supplements during the test period. Daily caloric intake during the experimental period was recorded by dietitians. Subjects were also instructed not to consume alcoholic drinks, yogurt, probiotic products such as Yakult, or any drugs during the week before the exercise test. The basic demographic profiles and characteristics of the subjects are shown in Table 1.

Experimental Design

The study adopted a double-blind test in which the subjects were randomly divided into two groups.

Table 1. Demographics and characteristics of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>TWK10</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Weight (kg)</td>
<td>77 ± 5</td>
<td>75 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176 ± 2</td>
<td>173 ± 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: NS, not significant.
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— placebo and TWK10, with 8 subjects in each group. Basic characteristics including height and weight were recorded as references. The basal and maximal oxygen consumption rates were assessed for exercise intensity calibration and the fatigue-related biochemical and physiological indices evaluated. The total experiment and supplementation duration was 6 weeks. All subjects were asked to record their dietary intake and to consume similar foods during the experiment. Subjects ate a standardized breakfast of 393 kcal (containing energy as 61.7% carbohydrate, 11.7% protein, and 26.6% fat) in the evening before each test day, thereby minimizing the impact of differences in food/caloric intake on physiological homeostasis.

Oxygen uptake was considered maximum when the respiratory exchange ratio (the ratio of carbon dioxide produced to oxygen consumed, $\frac{VCO_2}{VO_2}$) was above 1.10 and the maximum heart rate (HR) was achieved (maximum HR = 210 – age). Oxygen consumption was evaluated by a treadmill (Pulsar, h/p/cosmos, Germany) and an auto respiratory analyzer Vmax 29c (SensorMedics, Yorba Linda, CA, USA). HR was also monitored using a Polar HR device. Subjects were told to avoid any strenuous physical activity 3 days before the initial exercise test for $V_{O2max}$ assessment. The speed and grade of the treadmill simultaneously increased every 3 min until volitional fatigue by using the Bruce protocol (3). The three largest $V_{O2}$ values were selected and averaged to determine the $V_{O2max}$ of the individual subject. Subjects were continuously supplemented with either the placebo or TWK10 for 6 weeks.

The individual $V_{O2max}$ during pretest was used as a reference to adjust for the individual’s appropriate intensity for the exhaustive treadmill exercise. The endurance was assessed by using a warm-up step for 5 min, followed by a test on the treadmill at 85% $V_{O2max}$ workload. The gradient and speed of the treadmill were maintained until subject exhaustion. The exercise capacity and individual intensity adjustment followed previous standard protocol for evaluation of intervention effects (27). In order to predict signs of exhaustion, the oxygen consumption, HR, and Borg’s rating of perceived exertion (RPE) scale were monitored every 5 min during the sub-maximal endurance exercise. The sustained exercise duration was recorded as the endurance index. The speed was calculated for 85% $V_{O2max}$ as workload and 12% as gradient by the American College of Sports Medicine (ACSM) metabolic equivalent equation, which is $85\% \ V_{O2max} = 3.5 + 0.2 \times \text{(speed)} + [0.9 \times \text{(speed)} \times 0.12]$, simplified to $85\% \ V_{O2max} = 3.5 + 0.308 \times \text{(speed)}$. The whole experimental procedure is presented in Fig. 1.

Clinical Biochemistry

Blood serum samples were taken with an arm venous catheter at two time points after subjects had fasted for at least 8 h. The time points sampled were before and after the exhaustive exercise test. Serum levels of lactic acid, blood ammonia, blood glucose, free fatty acid (FFA) and creatine kinase (CK) were measured using an autoanalyzer (Hitachi 7060; Tokyo, Japan).

Statistical Analysis

All numerical values are expressed as mean ± standard error of the mean (SEM). The paired-sample t test and independent t test were applied for the within- and between-group analysis, respectively, using the SAS statistical software v9.0 (SAS, Cary, NC). A P-value of less than 0.05 was considered significant between the two groups.

Results

After six weeks of continuous supplement intake, the exercise performance and post-exercise fatigue-related blood biochemical indices were
Anthropometric Measurements

The general physiological characteristics of the subjects before the test procedures are shown in Table 2. The mean height was 176 ± 2 cm and 173 ± 3 cm, and the mean weight was 77 ± 5 kg and 75 ± 5 kg in the placebo and TWK10 groups, respectively. The VO$_{2\text{max}}$ of the placebo and TWK10 groups were 44.9 ± 2.4 ml/kg/min and 46.2 ± 2.0 ml/kg/min and there was no significant difference among these indexes (P > 0.05). The endurance of the placebo and TWK10 groups before supplementation were 738.1 ± 49.9 sec and 735.6 ± 97.7 sec, respectively, with no significant difference between the two groups (P > 0.05). In addition, there was no significant difference between the placebo and TWK10 groups (3318 ± 175 kcal/day and 3298 ± 182 kcal/day, respectively) in daily caloric intake with the same training programs.

Effects of L. Plantarum TWK10 on Endurance Exercise

The time-to-exhaustion measurements are shown in Fig. 2. After 6 weeks of supplementation, the exhaustive time was 817 ± 79 sec and 1292 ± 204 sec for the placebo and TWK10 groups, respectively, with 85% VO$_{2\text{max}}$ workload. The TWK10 group showed 1.58 times improvement in performance compared to that of the placebo group (t(14) = 2.19, P = 0.04).

Effects of TWK10 Supplementation on RPE before and after Endurance Exercise

In addition to change in HR, RPE can be used to evaluate physical activity or exercise intensity in humans, as well as the perceived fatigue after exercise. The mean RPE for both the placebo and TWK10 groups after 3 min of exercise was 9 ± 1, (P = 0.61). The mean RPE for the placebo and TWK10 groups at 15 min after exhaustion at 85% VO$_{2\text{max}}$ exercise were 18 ± 0 and 16 ± 1, respectively. The TWK10 group showed lower RPE than the placebo group, but the difference was not significant (P = 0.25).

Effects of TWK10 Supplementation on Fatigue-Related Biochemistry

With regards to the exercise metabolites, the average lactic acid concentration for the placebo and TWK10 groups before exercise (Fig. 3A) was 1.9 ± 0.1 mM.L$^{-1}$ and 2.3 ± 0.2 mM.L$^{-1}$, respectively (P > 0.05). After exhaustive treadmill exercise, the lactate index significantly increased to 13.6 ± 1.3 mM.L$^{-1}$ and 12.3 ± 1.2 mM.L$^{-1}$ for the placebo and TWK10 group. These increases were significant.
compared to the pre-exercise levels \((P < 0.05)\), but were not significant differently between the groups \((P = 0.49)\). For the blood ammonia index, the levels of the placebo and TWK10 groups before exercise (Fig. 3B) were \(54 \pm 4 \mu M.L^{-1}\) and \(52 \pm 9 \mu M.L^{-1}\), respectively \((P > 0.05)\). After exhaustive treadmill exercise, the lactate index significantly increased to \(137 \pm 17 \mu M.L^{-1}\) and \(134 \pm 10 \mu M.L^{-1}\) as compared to the pre-exercise measurement \((P < 0.05)\) in the placebo and TWK10 group, although there was no significant difference between the groups \((P > 0.05)\).

The blood glucose concentrations for the placebo and TWK10 groups before exercise (Fig. 4A) were \(84 \pm 1 \text{ mg.dl}^{-1}\) and \(87 \pm 2 \text{ mg.dl}^{-1}\), respectively \((P > 0.05)\). After exhaustive treadmill exercise, the glucose levels were significantly elevated in both treatment groups, as compared to the pre-exercise time \((P < 0.05)\). There was a significant difference in the glucose levels between groups \((t(14) = 2.19, P < 0.05)\) after exercise exhaustion, with the TWK10 group \((119 \pm 6 \text{ mg.dl}^{-1})\) showing significantly higher levels than the placebo group \((101 \pm 6 \text{ mg.dl}^{-1})\). The average blood FFA concentrations for the placebo and TWK10 groups before exercise (Fig. 4B) were \(1.02 \pm 0.13 \text{ mM.L}^{-1}\) and \(1.08 \pm 0.07 \text{ mM.L}^{-1}\), respectively \((P > 0.05)\). After exhaustive treadmill exercise, the FFA levels were significantly lowered to \(0.62 \pm 0.09 \text{ mM.L}^{-1}\) and \(0.63 \pm 0.08 \text{ mM.L}^{-1}\) in the placebo and TWK10 groups. Compared to the pre-exercise levels in both groups, these reductions were significant \((P < 0.05)\), although there was no significant difference between groups \((P > 0.05)\).

The CK activity, an indirect index for muscular injury, for the placebo and TWK10 groups before exercise (Fig. 5) was \(173 \pm 19 \text{ U.L}^{-1}\) and \(171 \pm 17 \text{ U.L}^{-1}\), respectively \((P > 0.05)\). After exhaustive
treadmill exercise, the CK levels significantly elevated to 277 ± 47 U.L⁻¹ and 256 ± 37 U.L⁻¹ in the placebo and TWK10 groups. This rise was significant compared to pre-exercise levels (P < 0.05) in both groups, although no significant difference was seen between groups (P > 0.05).

**Discussion**

In the current study, we applied the Taiwan endemic isolated probiotic, *L. plantarum* TWK10, from Taiwanese pickled vegetables to investigate the exercise physiological performance and adaption. Previously, this probiotic was demonstrated to improve exercise, related biochemical markers and performance in an animal model (4). The physiological-biochemical characteristics and 16s rDNA were certified by the Food Industry Research and Development Institute (Hsinchu, Taiwan). In our study, 6-week supplementation with *L. plantarum* TWK10 resulted in a significant increase in aerobic endurance performance (Fig. 2). The glucose level was also significantly elevated, possibly for energy utilization benefit. The other exercise-related biochemical indexes did not show any significant difference between the groups. However, we believe gut microbiota may play an important role in energy harvesting and physiological adaption. Study of microbiota is an emerging field, which may benefit the health food industry.

In previous bioactivity studies, *L. plantarum* was discovered to be a beneficial bacteria that is commonly found in Korean kimchi, sauerkraut and cultured vegetables. It is beneficial to GI health in different ways, such as through maintaining intestinal mucosa (9) and preventing proliferation of dominant species (13). *L. plantarum* has been shown to not only help maintain intestinal permeability, but also has significant antioxidant capacity (24). *L. plantarum* appears to be an effective therapeutic strategy for colitis, intestinal bowel syndrome (IBD) and Crohn’s disease (1). Psychotropic effects such as depression or anxiety have been ameliorated by *L. plantarum* supplementation via modulation of the hippocampal brain-derived neurotrophic factor and striatum monoamine neurotransmitter levels (15). Gut microbiota affects the muscular energy utilization preference (7) and metabolites (26) via the gut muscle axis. Previous studies showed that mice administered *L. plantarum* could regulate blood glucose levels in response to insulin (21) and prevent metabolic disturbances induced by a high-fructose diet (25). Our results suggest that *L. plantarum* TWK10 probiotics are beneficial to energy harvesting possibly due to glycogenesis regulation for exercise demand (Figs. 2 & 4A). This current study also demonstrates that *L. plantarum* TWK10 is able to ameliorate exercise fatigue-related markers and improve exercise performance. The results may be explained by the anti-inflammation effects induced by *L. plantarum* TWK10, leading to an improvement in skeletal muscle atrophy markers (4). Taken together, our findings support the view that gut microbiota promote health, improve performance and mitigate fatigue by means of exercise intervention in terms of energy balance and body composition.

Sport nutrition supplements, including high-energy supplements, constitute a $37.5 billion dollar market in 2014. The global market growth of this industry is expected to reach $66.0 billion by 2020 (2), based on a compound annual growth rate of 10.1%. According to the frequency, intensity, time, type of exercise, as well as body demand, the major consumers of sports nutrition products include athletes, recreational activities group, active groups such as homemaker, on-the-go business people and outdoor enthusiasts. Athletes are lured to such products by the promises that they will achieve a better competitive performance. The physical adaptation occurs in the recovery process in response to the exercise performed, so the appropriate type, amount and timing of intake of food, fluids and supplements could efficiently promote optimal health and performance with different training intensity (23). Bio-technologically engineered recovery powders, pills, processed foods and those products that can offer convenience are especially popular in the nutrient supplement market, but are considerably more expensive than the cost of getting the same nutrients from whole foods (28). Currently, the number of studies investigating the probiotics activities on exercise performance and physiology is still limited. Based on our current and previous studies (4), we believe that probiotics have
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the potential to not only modulate the immune-regulation and microbiota proliferation, but also affect a wide spectrum of health parameters.

Despite the encouraging results, our current study has several limitations, including the small number of sampling time-points during the exercise period. The time-to-exhaustion time point does not exactly reflect the related biochemical indicators with conditions of physiological limitation. Even though our initial study design contained multiple time-points sampling, IRB only approved a limited number of time points. We plan to conduct further investigations on the effects of the endemic novel probiotics L. plantarum TWK10 on exercise physiological adaption and metagenomics changes of the microbiome. The detailed mechanisms affected by probiotics are also interesting issues for further investigation via systemic biological tools such as proteomics and metabolomics.

Conclusions

We found that L. plantarum TWK10 supplementation for 6 weeks significantly improved the aerobic endurance capacity and energy harvest in this clinical study. In addition, the participants did not have any observable adverse reactions from the dosage range used throughout the duration of the trial. The basal biochemistry of individuals was normal and the study performed in accordance with ICH-GCP clinical standards. Our results suggest that L. plantarum TWK10 is safe for use with the potential for further development, especially in sports science.

Acknowledgments

This study was supported by the Ministry of Science and Technology of Taiwan (Grants No. MOST102-2628-B179-001-MY3 and MOST 106-2410-H-227-007).

Conflict of Interest

The authors have nothing to declare.

References


